
INTERNATIONAL

ŒENOLOGICAL

CODEX



**INTERNATIONAL ORGANISATION
OF VINE AND WINE**

INTERNATIONAL ŒNOLOGICAL CODEX

EDITION 2006



INCLUDED

Resolution adopted in Paris (France)

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O.I.V. - 18, RUE D'AGUESSEAU - 75008 PARIS

Warning:

In 2000, the OIV adopted 40 monographs of products used in oenology, which make up the new edition of the **International Oenological Codex**, and are listed in this binder on white paper.

This important scientific contribution is being pursued in order to update the remaining monographs and in order to add new ones suited to the **International Code of Oenological Practices** index cards.

Moreover, the O.I.V. Sub-Commission of Methods of Analysis and Appraisal of Wines in charge with the revising of the International Oenological Codex has also undertaken the task of revising Chapter 2 « Analytical and Control techniques » and Chapter 3 « Reagents and titrated solutions ». This work thus led to the adoption in 2003 of new monographs of the chapters concerned.

Introduction

The **International Oenological Codex** gathers descriptions of the main chemical, organic and gas products used in the making and the keeping of wines.

Conditions for usage and the directions and the limits of use are set out in the **International Code of Oenological Practices**. The authorization for usage comes under national legislation.

On one hand, the identifying characteristics and the degree of purity are described in detail herein, in addition to the minimum efficiency required to be qualified as "*conforming to the International Oenological Codex*".

On the other hand, the definition or the formula, with possible synonymy, of every product is provided. Molecular weight, general characteristics, and in particular the solubilities are mentioned. To avoid any possible error, simple means of identification are indicated.

Each monograph indicates the research necessary to reveal and dose the impurities and their acceptable limit. These limits have been set for some of these including:

- selenium, arsenic, heavy metals etc., in order to prevent oenological products, given the maximum dose for its usage, to bring about even the smallest toxic effect,
- iron, copper, calcium, in order to prevent all harmful effects on wine quality and its aspect.
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In terms of holdings of other products including chlorides, sodium, sulfates, etc. the limits have been set fairly large because these products are not toxic and wines naturally contain these products in larger amounts than possibly do oenological products.

General observation: Unless otherwise indicated, solubilities are expressed at 20 °C in grams of solvent for one gram of product..

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Chapter I:

For the record in French – monographs being revised (green part)

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<i>Charbon animal purifié</i>	<i>Edition 1978</i>	<i>F-COEI-V-1-CHARAN</i>
<i>Charbon activé</i>	<i>Edition 1978</i>	<i>F-COEI-V-1-CHARAC</i>
<i>Ecorces de levures</i>	<i>OENO 4/1987</i>	<i>F-COEI-V-1-ECOLEV</i>
<i>Sodium (Alginate de)</i>	<i>Edition 1978</i>	<i>F-COEI-V-1-SODALG</i>
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Chrome - determination by AAS	OENO 18/2003	F-COEI-2-CHROME
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Chater III: Reagents and Titrated Solutions

For the record in French –being revised (green part)

Title	Adoption	Sheet name
Reagents and Titrated Solutions	OENO 19/2003	F-COEI-3-REASOL

Chapter I
Products
used in œnology

ACTIVE DRY YEASTS (A.D.Y.)
(Oeno 16/2003)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Yeasts are used for the inoculation of musts and wine.

The rate of inoculation is at the user's discretion.

Yeasts used must be isolated from grapes, musts or wine or cultures originating from the combination of these same yeasts (original mother cultures) which must be stored in genetically stable conditions. The obtaining and usage of genetically modified oenological yeast (G.M.Os) require advance authorisation from the competent authority.

2. LABELLING

The following information must be indicated on the label:

- The genus and species name in addition to the reference of the strain(s) attributed by an official body of monitoring micro-organisms or by international institutions, the breeder, the origin and strain breeder and possibly the originator that isolated it.
- Operating instructions or method and the possible reactivation additives recommended by the manufacturer.
- The number of viable cells per gram of powder (CFU as determined in the annex) guaranteed by the manufacturer, loss of viability per month of storage under defined conditions for temperature, humidity and aeration, batch number in addition to the expiration date and storage conditions.
- Where relevant, the indication that the yeasts were obtained through genetic modifications and their modified character.

3. CHARACTERISTICS

Active dry yeast is in the form of round or vermiculated pellets obtained by drying a concentrated yeast culture.

4. TEST TRIAL METHODS AND LIMITS

4.1 - Humidity

Measured by the weight loss of 5 g of product dried at 105°C until it reaches a constant weight (about 3 hours).

Maximum level should be less than 8%.

4.2 – Heavy metals

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 10 mg/kg of dry matter, expressed in lead.

4.3 - Lead

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 5 mg/kg of dry matter.

4.4 - Mercury

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 1 mg/kg of dry matter.

4.5 - Arsenic

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 3 mg/kg of dry matter.

4.6 - Cadmium

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 1 mg/kg of dry matter.

4.7 – Mycotoxins¹

4.8 – Viable yeasts

Proceed with counting according to the method in chapter II of the International Oenological Codex (method in annex of this resolution).

Content should be above or equal to 10¹⁰ CFU/g.

¹ Point to be studied at a later date by the sub-commission of methods of analysis and appraisal of wine.

4.9 – Yeasts of a different species of the indicated strain

Proceed with counting according to the method in chapter II of the International Oenological Codex (method in annex of this resolution).
Content should be less than 0.01 % of total viable yeasts.

4.10 - Moulds

Proceed with counting according to the method in chapter II of the International Oenological Codex (method in annex of this resolution).
Content should be less than 10³ CFU/g of powder.

4.11 – Lactic bacteria

Proceed with counting according to the method in chapter II of the International Oenological Codex (method in annex of this resolution).
Content should be less than 10⁴ CFU/g.

4.12 – Acetic bacteria

Proceed with counting according to the method in chapter II of the International Oenological Codex (method in annex of this resolution).
Content should be less than 10³ CFU/g.

4.13 - Salmonella

Proceed with counting according to the method in chapter II of the International Oenological Codex (method in annex of this resolution).
Absence should be checked on a 25 g sample.

4.14 - *Pseudomonas aeruginosa*²

4.15 - *Escherichia coli*

Proceed with counting according to the method in chapter II of the International Oenological Codex (method in annex of this resolution).
Absence should be checked on a 1 g sample.

4.16 - Staphylococci

Proceed with counting according to the method in chapter II of the International Oenological Codex (method in annex of this resolution).
Absence should be checked on a 1 g sample.

4.17 - Coliforms

Proceed with counting according to the method in chapter II of the International Oenological Codex (method in annex of this resolution).
Number should be less than 10 CFU/g

² Point to be studied at a later date by the experts group “Wine microbiology”.

5. ADDITIVES

They must be in conformity with regulations in force.

6. STORAGE CONDITIONS

Storage should not be in open packaging and/or at temperatures above 10 C.

Storage conditions differ according to preparation and packaging methods.

Always refer to manufacturer's recommendations.

ALGINIC ACID
Sin no. 400
C.A.S. no.: 9005-32-7
(Oeno 6/2005)

1. SUBJECT, ORIGIN AND SCOPE

Alginic acid is a colloidal polysaccharide extracted from various varieties of brown algae in particular from Laminaria. The monomers constituting the α -L-glucuronic acid and β -D-mannuronic acid are bound in pairs as connections of the type 1 \rightarrow 4



A clarifying agent, which, after being neutralized before use by potassium chloride, or potassium carbonate or potassium hydrogenocarbonate can be added to the drawn-off liquid, designed to carry out the second fermentation of sparkling wines (foam formation).

Alginic acid is made up on average of 200 basic units of uronic acids. Their molecular weight ranges between 10 000 and 600 000 U.

2. LABELING

The concentration of alginic acid must be indicated on the label, as well as the conditions of safety and conservation.

3. CHARACTERISTICS

Alginic acid exists in powder or filament form, or as amorphous granules of a yellowish white to brown color, insoluble in pure water and the various organic solvents. It can dissolve in water alkalized by sodium carbonate, sodium hydroxide or trisodium phosphate.

4. IDENTIFYING CHARACTERISTICS

4.1 pH

A suspension of 3% alginic acid in water has a pH ranging between 2 and 3.5.

4.2 Differentiation with other polysaccharides

An alginic acid solution of 5 g/l in sodium hydroxide (dissolve 4.3 g of sodium hydroxide in water and complete to 100 ml)

precipitate in gelatinous form by adding a fifth of volume of a 2.5% solution of calcium chloride.

Furthermore, an addition of a half volume of a solution saturated with ammonia sulfate to the solution previously described does not cause any turbidity.

These two tests can be used to differentiate alginic acid from other polysaccharides that may be used in foodstuffs or pharmaceuticals.

4.3 Organoleptic characteristics

Alginic acid must have no taste, or abnormal odor.

5. TESTS

All the limits described below refer to the dry weight of alginic acid.

5.1 Insoluble in a solution of sodium hydroxide

Dissolve by prolonged magnetic agitation 1 g of alginic acid weighed with precision in 100 ml of a solution of sodium hydroxide (dissolve 4.3 g of sodium hydroxide in water and complete to 100 ml), centrifuge, decant, and wash the residue 5 times with distilled water, with centrifugation and drainage of the washwater each time. Transfer all the residue using distilled water to a Gooch filter that has been tared beforehand (filter made of sintered glass of low porosity), dry for 1 hour at 105°C and weigh again.

The rate of insoluble should not exceed 2% in relation to the dry weight of the alginic acid.

5.2 Loss on desiccation

Determine until constant weight, on a test specimen of 2 g, the loss of weight, at 100-105°C, the alginic acid must be lower than 15 p. 100

5.3 Sulfuric ash

Proceed as indicated in chapter II of the international oenological Codex. The sulfuric ash content should not be higher than 8 p 100 in weight of the alginic acid.

5.4 Preparation of the solution for tests

After weighing the ashes, dissolve them in 2 ml of concentrated hydrochloric acid (R) and 10 ml of water. Heat to activate the dissolution and add water until a volume equal to 25 times the

weight of the dry alginic acid is obtained. 1 ml of this solution contains the mineral matter of 0.04 g of dry alginic acid.

5.5 Lead

On the solution prepared for tests (5.4), to carry out the dosage of lead according to the method described in chapter II of the international oenological Codex.

The lead content must be lower than 5 mg/kg.

5.6 Cadmium

On the solution prepared for the tests (5.4), determine the cadmium using the method described in chapter II of the international oenological Codex.

The cadmium content must be lower than 1 mg/kg.

5.7 Mercury

Determine the mercury using the method described in Chapter II of the international oenological Codex.

The mercury content must be lower than 1 mg/kg.

5.8 Arsenic

On the solution prepared for the tests (5.4), determine the arsenic using the method described in Chapter II of the international oenological Codex.

The arsenic content must be lower than 3 mg/kg.

5.9 Bacteriological control

Proceed as indicated in chapter II of the international Oenological Codex for each parameter.

Limit: total viable microorganisms: less than 5×10^3 CFU/g.

5.10 Coliforms

The number of coliforms must be lower than or equal to 1 per g.

5.11 Staphilococca

The number of staphilococca (β -haemolytics with positive coagulase) must be lower than or equal to 1 per g.

5.12 Salmonella

The number of salmonella must be lower than 1 per 100 g.

5.13 Yeast

Limit concentration: 5×10^2 CFU per g of preparation.

5.14 Lactic bacteria

Limit concentration: 10^2 CFU per g of preparation.

5.15 *Lactobacillus sp.*

Limit concentration: 10 CFU per g of preparation.

5.16 *Pediococcus sp.*

Limit concentration: absence in a sample of 10 g of preparation.

5.17 Acetic bacteria

Limit concentration: 10^3 CFU per g of preparation.

5.18 Moulds

Limit concentration: 5×10^2 CFU per g of preparation.

6. STORAGE

Alginic acid must be kept in sealed bags.

INTERNATIONAL CENOLOGICAL CODEX
AMMONIUM CHLORIDE

AMMONIUM CHLORIDE
Ammonia Hydrochloride
Ammonii Chloridum
NH₄Cl=53.50
SIN NO. : 510
(Oeno 13/2000)

1. OBJECTIVE, ORIGIN AND DOMAIN OF APPLICATION

This product is used as a fermentation activator and is reserved for fermentation operations. It makes available ammonium ions which can be directly assimilated by the yeast.

Statutory limits regulate the amount of ammonium added.

2. LABELING

The concentration of this product should be indicated on the label, including cases in which it is mixed. In addition, safety and storage conditions should be stipulated.

3. CENTESIMAL COMPOSITION

Cl	66.22
NH ₃	31.78
N	28.17

4. PROPERTIES

Colorless, odorless crystals with a fresh, salty and piquant taste. It sublimates without decomposing and is stable in air.

5. SOLUBILITY

Water at 20 °C	350.8 g/l
Water at 100 °C	758 g/l
Alcohol, 95% by vol.	13.3 g/l

6. IDENTIFYING CHARACTERISTICS

Aqueous solutions of ammonium chloride produce reactions of ammonium and those of chloride.

7. TESTING

7.1. Sulfur Ash

When quantified as indicated in the Annex, the sulfur ash content of the ammonium chloride should not be greater than 0.2 per 100.

7.2. Preparing the solution for tests

Prepare an aqueous solution from NH_4Cl crystals at 10 per 100 (m/v).

7.3. Sulfates

To 1 ml of solution prepared for tests under paragraph 7.2, add 2 ml of hydrochloric acid diluted to 10 pp 100 (m/v) (R), 17 ml of water and 2 ml of barium chloride solution (R). The mixture should be clear, or else the opalescence observed after 15 minutes should be less than that of the control solution prepared as indicated in the Annex. (Sulfate content expressed in terms of sulfuric acid should be less than 1 g/kg).

7.4. Nitrates

Mix 5 ml of concentrated sulfuric acid (R) and 0.5 ml of an extemporaneously prepared iron(II) sulfate solution at 5 pp 100 in a test tube. Without mixing, pour 5 ml of the solution prepared under paragraph 7.2. No coloration should be observed at the surface line separating the two solutions.

7.5. Phosphates

To 0.5 ml of the solution prepared for testing under Paragraph 7.2, add 5 ml of water and 10 ml of nitro-vanadomolybdic (R) reagent. Leave in contact for 15 minutes at 20 °C. If a yellow coloration appears, it should be less intense than that obtained by adding 0.5 ml of a solution of 0.05 g of phosphorous per liter (R), 5 ml of water and 10 ml of nitro-vanadomolybdic (R) reagent. (Phosphate content expressed in terms of phosphorous less than 500 mg/kg).

7.6. Iron

To 5 ml of solution prepared under paragraph 7.2, add 1 ml of concentrated hydrochloric acid (R), one drop of 2 pp 100 potassium permanganate and 2 ml of 5 pp 100 potassium thiocyanate (R).

If a red coloration appears, it should be less intense than that of a control prepared with 2.5 ml of an iron(III) solution containing 0.01 g of iron per liter (R), 2.5 ml of water and the same quantities of the same reagents. (Iron content should be less than 50 mg/kg).

The iron may also be quantitatively analyzed using atomic absorption spectrometry, in accordance with the method detailed in the Compendium.

7.7. Arsenic

Using the method indicated in the annex, test for arsenic in the test solution prepared in accordance with Paragraph 7.2. (Arsenic content should be less than 3 mg/kg.)

7.8. Lead

Using the method described in the Compendium, quantify the lead in the solution obtained under Paragraph 7.2. (Lead content should be less than 0.5 mg/kg.)

7.9. Mercury

Using the method described in the annex, test for mercury in the solution prepared for testing under Paragraph 7.2. (Mercury content should be less than 1 mg/kg.)

7.10. Quantitative Ammonia Analysis

Dilute the solution prepared for testing under paragraph 7.2 to one-tenth strength, then place 10 ml of this dilute solution (i.e., 0.1 g of ammonium chloride) in a steam distillation device. Add 10 ml of 30% sodium hydroxide (R) and distill 100 ml. Quantify the distilled ammonia using 0.1 M hydrochloric acid. Let n be the number of milliliters used:

100 g of ammonium chloride contains $1.7 n$ g of ammonia (NH_3).
(Ammonia content greater than 31.5 pp 100).

7.1. Quantitative Hydrochloric Acid Analysis

Take a 10 ml sample of the solution prepared for testing under paragraph 7.2, which has been diluted to one-tenth strength. Place the sample in a cylindrical flask. Add 20 ml of 0.1 M silver nitrate solution, 1 ml of concentrated nitric acid (R), 5 ml of iron(III) sulfate solution and 10 pp 100 of ammonium (R). Titrate the excess silver nitrate with a 0.1 M potassium thiocyanate solution. Let n be the number of milliliters used:

100 g of ammonium chloride contains $3.65 (20-n)$ g of hydrochloric acid (HCl). (Hydrochloric acid content greater than 67.5 pp 100).

8. STORAGE

Ammonium chloride must be stored in water-tight containers away from heat.

AMMONIUM HYDROGEN SULFITE
Ammonium bisulfite
 $\text{NH}_4\text{HSO}_3 = 99.07$
(Oeno 14/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This product falls in the category of preservatives and is used exclusively for fermentation operations. It makes available sulfur dioxide and ammonium ions, which can be directly assimilated by the yeast. There are regulatory restrictions on the amount of ammonium that can be added and on sulfur dioxide content.

2. LABELING

The concentration of this product, as well as the safety and storage conditions, should be indicated on the label.

3. CENTESIMAL COMPOSITION

NH_3	17.16
SO_2	64.67

4. PROPERTIES

Ammonium hydrogen sulfite always takes an aqueous solution form. This solution emits a piquant sulfur dioxide odor.

5. SOLUBILITY

Water at 60 °C	847 g/l
Alcohol, 95% by vol.	Slightly soluble

6. IDENTIFYING CHARACTERISTICS

Aqueous solutions of ammonium hydrogen sulfite produce reactions of ammonium (release of ammonia in the presence of sodium hydroxide when heated) and sulfur dioxide (filter paper soaked in potassium iodate and starch turns blue).

7. TESTS

7.1. Sulfur Ash

As quantified as indicated in the Annex, the proportion of ammonium hydrogen sulfite ash should not be greater than 0.2 per 100.

7.2. Preparing the Solution for Tests

Prepare a 10 pp 100 (m/v) solution.

7.3. Sulfates

To 0.5 ml of the solution prepared for testing under paragraph 2, add 2 ml of dilute hydrochloric acid (R), 17.5 ml of water and 2 ml of barium chloride solution (R). The mixture should be clear, or else the opalescence observed after 15 minutes should be less than that of the control solution, prepared as indicated in the Annex. (Sulfate content expressed in terms of sulfuric acid should be less than 2 g/kg).

7.4. Iron

To 5 ml of the solution prepared for testing under paragraph 2, add 1 ml of concentrated hydrochloric acid (R), one drop of 2 pp 100 potassium permanganate (R) and 2 ml of 5 pp 100 potassium thiocyanate (R).

If a red colorating appears, it should be less intense than that of a control prepared with 2.5 ml of an iron(III) solution of 0.01 g of iron per liter (R), 2.5 ml of water and the same quantities of the same reagents. (Iron content should be less than 50 mg/kg).

The iron may also be quantified by means of atomic absorption spectrometry, using the technique described in the Compendium.

7.5. Lead

Use the method detailed in the Compendium on the solution in a concentration of 10 pp 100 prepared for testing (under 7.2) and diluted to one one-twentieth.

7.6. Mercury

Test for mercury in the solution prepared for testing (under 7.2) using the technique detailed in the annex. (Mercury content should be less than 1 mg/kg.)

7.7. Arsenic

Using the method indicated in the Annex, test for arsenic in 2 ml of the test solution prepared for testing in accordance with paragraph 7.2. (Arsenic content should be less than 3 mg/kg).

7.8. Quantitative Ammonia Analysis

Dilute the solution prepared for testing under paragraph 7.2 to one-tenth strength, then place 10 ml of this dilute solution (0.10 g of ammonium hydrogen sulfite) in a steam distillation device (described in the annex). Add 10 ml of 30 pp 100 sodium hydroxide (R) and distill 100 ml. Quantify the distilled ammonia using 0.1 M hydrochloric acid. Let n be the number of milliliters used:

100 g of ammonium hydrogen sulfite contain 1.7 n g of ammonia (NH₃). Ammonia content should be greater than 16.5 pp 100 (m/m).

7.9. Quantitative Sulfur Dioxide Analysis

In a 200 ml conical flask, place 50 ml of cold water, then 5 ml of the freshly prepared ammonium hydrogen sulfite solution. Titrate with 0.05 M iodine in the presence of starch. Let n be the volume of iodine used.

SO₂ content per 100 g: 6.4n

Ammonium hydrogen sulfite should contain at least 62 pp 100 SO₂.

8. STORAGE

Ammonium hydrogen sulfite solutions should be stored in hermetically sealed containers away from heat and cold.

AMMONIUM SULFATE
Ammonium sulfuricum
(NH₄)₂SO₄ = 132.10
SIN NO. 517
(Oeno 16/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This product is used as a fermentation activator and is reserved for fermentation operations. It adds ammonium ions that can be directly assimilated by the yeast. The sulfates added are completely soluble in wine.

Statutory restrictions govern the addition of ammonium.

2. LABELING

The concentration of this product should be indicated on the label, including mixtures. In addition, safety and storage conditions should be noted.

3. CENTESIMAL COMPOSITION

H ₂ SO ₄	74.22
NH ₃	25.78
SO ₃	60.59
N	21.20

4. PROPERTIES

Transparent, anhydrous crystals with a bitter, pungent taste, which are similar to potassium sulfate crystals, with which this salt is isomorphous.

5. SOLUBILITY

Water at 20 °C	509 g/l
Water at 100 °C	1040 g/l
Alcohol, 90% by vol.	Insoluble
Acetone	Insoluble

6. IDENTIFYING CHARACTERISTICS

Solutions of this salt in water in a concentration of 1 pp 100 (m/v) has a pH of approximately 5.5. This solution allows reactions of ammonium and those involving sulfates.

7. TESTS

7.1. Sulfur Ash

The concentration of sulfur ash of ammonium sulfate prepared as explained in the annex in a test sample of 1 g must not exceed 5 g/kg.

7.2. Preparing the Solution for Tests

Prepare a 10 pp 100 (m/v) solution.

7.3. Chlorides

To 0.5 ml of the solution prepared for testing under paragraph 7.2, add 14.5 ml of water, 5 ml of nitric acid (R) diluted to a concentration of 10 pp 100 and 0.5 ml of 5 pp 100 silver nitrate solution (R). After 15 minutes at rest in the dark, there should be no clouding ; or else, any clouding visible should be less intense than that observed in the control prepared as indicated in the annex. (Hydrochloric acid content must be less than 1 g/kg).

7.4. Phosphates

To 0.5 ml of the solution prepared for tests under paragraph 7.2, add 5 ml of water and 10 ml of nitro-vanadomolybdic reagent (R). Leave in contact for 15 minutes at 20 °C. If a yellow coloring appears, it should be less intense than that obtained by adding, to 0.5 g of a solution containing 0.05 g phosphorous per liter, 5 ml of water and 10 ml of nitro-vanadomolybdic reagent. (Phosphate content expressed in terms of phosphorous should be less than 500 mg/kg).

7.5. Nitrates

Mix 5 ml of concentrated sulfuric acid (R) and 0.5 ml of an previously prepared iron(II) sulfate solution in a concentration of 5 pp 100 (m/v) in a test tube. Without mixing, pour 5 ml of the solution obtained by dissolving 2 g of ammonium sulfate in 10 ml of water. No coloring should be observed at the surface separating the two solutions

7.6. Iron

To 5 ml of the solution prepared for testing under paragraph 7.2, add 1 ml of concentrated hydrochloric acid (R), one drop of 2 pp 100 potassium permanganate (R) and 2 ml of 5 pp 100 potassium thiocyanate solution (R).

If a red coloring appears, it should be less intense than that of a control prepared with 2.5 ml of an iron(III) solution in a concentration of 0.01 g of iron per liter (R), 2.5 ml of water and the same quantities of the same reagents. (Iron content should be less than 50 mg/kg).

The proportion of iron may also be quantified by atomic absorption spectrometry, using the technique detailed in the Compendium.

7.7. Lead

Use the quantitative analysis technique detailed in the Compendium on the solution prepared for testing under paragraph 7.2. (Lead content should be less than 5 mg/kg.)

7.8. Mercury

Test for mercury concentration in the solution prepared for testing (7.2), using the method explained in the annex. (Mercury content should be less than 1 mg/kg.)

7.9. Arsenic

Using the method indicated in the Annex, test for arsenic concentration in the test solution prepared in accordance with paragraph 2. (Arsenic content should be less than 3 mg/kg.)

7.10. Quantitative Analysis of Ammonia

Dilute the test solution prepared under paragraph 7.2 to one-tenth strength, then place 10 ml of this dilute solution (0.10 g of ammonium sulfate) in a steam distillation device (described in the Annex). Add 20 ml of 30% sodium hydroxide (R) and distill 100 ml. Quantitatively analyze the distilled ammonia using 0.1 M hydrochloric acid. Let n be the number of milliliters used:

100 g of ammonium sulfate contains $1.7 n$ g of ammonia (NH_3).
(Ammonia concentration greater than 25 pp 100.)

7.11. Quantitative Analysis of Sulfuric Acid

Dilute the test solution prepared for testing under paragraph 7.2 to one-tenth strength, then take 25 ml of this solution and add 75 ml of water and 1 ml concentrated hydrochloric acid (R). Bring to a boil while slowly adding a small excess of barium chloride solution (R). Let the precipitate form for 30 minutes in a 100 °C water bath. Collect the precipitate, then wash, calcine in an oven at 600 °C and weigh. Let p be the weight of the barium sulfate precipitate:

100 g of ammonium sulfate contains $16.80 p$ g of sulfuric acid (H_2SO_4).
(Sulfuric acid content greater than 73.5 pp 100.)

8. STORAGE

Ammonium sulfate should be stored in a dry place in hermetically sealed containers, away from heat.

**ANTI-FOAMING AGENTS
(FATTY ACID MONO- AND DIGLYCERIDES)
SIN NO. 471
(Oeno 17/2000)**

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

The mixture of fatty acid glyceric mono- and diesters (with a small quantity of tri-esters), with fatty oils and acids and alimentary fats are termed mono- and diglycerides. The mixture of mono- and diglycerides used as anti-foaming agents are essentially constituted by oleic acid esters.

The product thus defined can contain small quantities of fatty acids and free glycerol. It is used under appropriate technological conditions and does not leave measurable traces in wine after filtering.

2. LABELING

The label must indicate the mono- and diglyceride content of the preparation, the storage and safety conditions, and the final date of use.

3. PROPERTIES

The product is usually found in the form of an oily liquid with a straw yellow color, a doughy product with an ivory color or a hard waxy solid with a white or off-white color. All of the forms have a pleasant odor and taste. The solid form can be found in flakes, powder or small granules.

The product used as an anti-foaming agent is liquid at normal temperatures, but can become cloudy at low temperatures.

4. SOLUBILITY

Insoluble in water.

Soluble in ethanol, chloroform and benzene.

5. IDENTIFYING CHARACTERISTICS

5.1. Hydrolysis of the Sample

Treat 1 g of the sample by reflux using a 0.5 M potassium hydroxide solution for 1 hour. Add 15 ml of water and acidify with hydrochloric acid diluted to 30 pp 100 (v/v) (R) (approximately 4-5 ml). Oily drops or a white/yellowish white precipitate will form. Extract the fatty acids released using 5 ml hexane, separating the solvent. Repeat the extraction with 5 ml of hexane and reunite the two extracts.

Set aside the aqueous phase.

5.2. Detection of the Fatty Acids in the Hexane Extract Using Gas Phase Chromatography

For the purpose of example, use may be made of a semi-polar column, e.g., Carbowax 20M ® measuring 25m x 0.32 mm x 0.25 µm phase thickness.

5.3. Detection of Glycerol

Place 5 ml of the aqueous phase in a test tube. Add an excess amount of powdered calcium hydroxide and place the test tube in boiling water for five minutes, stirring from time to time. Cool and filter.

Place one drop of the filtrate in a test tube and add approximately 50 mg of potassium hydrogen sulfate. At the end of the test tube, place a piece of filter paper soaked in the reagent obtained by mixing extemporaneously equal volumes of a sodium nitrosopentacyanoferrate solution (R) and piperidine (F'). Heat using a small flame. A blue coloring of the reactive paper indicates the presence of acrolein.

The color turns red by adding 1M sodium hydroxide solution.

6. TESTS

6.1. Drying Loss at 100 °C

Weigh exactly a quantity of about 5 g of the product to be analyzed in a glass crystallizing dish with a diameter of 70 mm, which has been preliminarily dried in an oven, cooled in a desiccator and calibrated. Place the crystallizing dish with the fatty material into a 103 °C oven and maintain this temperature for 30 minutes. Remove the crystallizing dish, let cool in the desiccator, then weigh. Place the sample in the oven again for 30 minutes. Weigh it again after cooling. Drying loss in the oven is completed when weight loss does not exceed 0.05% per half-hour of heating.

Drying loss at 100 °C should be less than 2 pp 100.

6.2. Sulfur Ash

Sulfur ash is quantified as indicated in the Annex using a test sample of 5 g. The sulfur ash should weigh less than 0.2 g/kg.

6.3. Arsenic

Determined as indicated in the Annex using a test sample of 5 g. The arsenic should weigh less than 3 mg/kg.

6.4. Heavy metals

Test for heavy metals either:

- After mineralization at 450 ± 5 °C of the residue left by the drying loss test. Take up the ash using 1 ml of diluted hydrochloric acid (R) and one drop of concentrated nitric acid (R) while heating in a 100 °C water bath to activate dissolution, then decant in a 25 ml volumetric flask, washing the cap with distilled water. Fill up to gauge line.

Draw off a volume of v ml of solution corresponding to 2 g of the sample to be analyzed and proceed with the test for heavy metals as indicated in the Annex.

- or, after liquid mineralization of an sample weighed with precision to about 5 g using concentrated nitric acid (R), Perhydrol and a microwave digester to accelerate the operation.

Decant the liquid obtained in a 25 ml volumetric flask and fill to the line with the wash water. Continue as indicated in the heavy metal tests.

Heavy metal content, expressed in terms of lead, should be less than 10 mg/kg.

6.5. Lead

Using the technique set forth in the Compendium, determine the quantity of lead in one of the two aforementioned preparations (6.4). The lead content should be less than 5 mg/kg.

6.6. Mercury

Using the technique described in the annex, determine the quantity of lead in one of the two aforementioned preparations (6.4). The lead content should be less than 1 mg/kg.

6.7. Cadmium

Using the technique detailed in the annex, determine the quantity of cadmium in one of the two aforementioned preparations (6.4). The lead content should be less than 1 mg/kg.

6.8. Free Fatty Acids

Prepare 125 ml of a mixture of equal volumes of isopropyl alcohol and toluene. Add 2 ml of 1 pp 100 phenolphthalein solution (m/v) in isopropyl alcohol and neutralize using an alkaline solution until a persistent but weak pink coloring appears.

Weigh with precision an amount of approximately 5 g of the sample to be analyzed in a 500 ml conical flask. Add the neutralized solvent mixture and dissolve the test sample, by heating if necessary, while stirring vigorously. Pour the 0.1 M potassium hydroxide solution until a

pink color identical to that obtained during the solvent neutralization process is obtained. Let n be the volume in ml poured:

Fatty acid content expressed in g of oleic acid pp 100 (m/m):

$2.8n$ / test sample in g

The fatty acid content in terms of oleic acid should be less than 3 pp 100 (m/m).

6.9. Soaps

Weigh precisely about 10 g of the product to be analyzed in a 250 ml conical flask. Add a mixture of 60 ml of acetone and 0.15 ml of 0.5 pp 100 (m/v) bromophenol blue solution in 95% alcohol by volume which has first been neutralized with a 0.1 M hydrochloric acid solution or a 0.1 M sodium hydroxide solution. Gently heat in a 70 °C water bath and titrate with a 0.1 M hydrochloric acid solution until the blue color disappears. Let sit for 20 minutes. Heat until the precipitate redissolves and, if the blue color reappears, continue titration.

1 ml 0.1 M hydrochloric acid solution corresponds to 0.0304 g of sodium oleate ($\text{NaC}_{18}\text{H}_{33}\text{O}_2$).

Soap content expressed in g of sodium oleate pp 100 (m/m):

$3.04n$ / test sample in g

Soap content expressed in g of sodium oleate should be less than 6 pp 100 (m/m).

6.10. Monoglycerides

6.10.1 Sample preparation

If the sample is in solid form, melt it by heating it to its melting point at a temperature of less than 10 °C. Liquid samples which are cloudy or have particles in them should also be heated. Mix vigorously.

6.10.2 Method

Weigh precisely a test sample, Q , of approximately 1 g to be analyzed in a 100 ml cylindrical flask. Dissolve using 25 ml of chloroform. Transfer this solution to a decanting glass. Wash the cylindrical flask with 25 ml of chloroform, then with 25 ml of water and add these liquids to the contents of the decanting glass.

Seal the decanting glass hermetically. Stir for 30-60 seconds. Let the two phases separate out (add 1-2 ml of crystallizable acetic acid (R)

to break the emulsion). Collect the aqueous phase in a 500 ml conical flask with an emery stopper. Extract the chloroform phase remaining in the decanting glass twice with 25 ml of water. Separate the aqueous phase and place it in the 500 ml conical flask. These aqueous extracts will be used for the free glycerol analysis.

Transfer the chloroform from the decanting glass to a 500 ml conical flask with an emery stopper. Add 50 ml of periodic acetic acid solution (R) while stirring.

In the two other 500 ml conical flasks with emery stoppers to be used as "blanks", place 50 ml of chloroform and 10 ml of water. Add 50 ml of periodic acetic acid solution (R) while stirring to each of the two flasks. Let the three flasks sit at least 30 minutes, but no more than 90 minutes.

While gently stirring, add 20 ml of potassium iodide solution (R) to each of these containers. Let sit at least 1 minute but no more than 5 minutes before volumetric analysis.

Add 100 ml water and titrate with a 0.05 M sodium thiosulfate solution using a magnetic stirrer until the brown color disappears from the aqueous phase. Add 2 ml of starch solution (R) and continue to add the reagent until the iodine disappears from the chloroform layer and the blue color disappears from the aqueous phase.

6.10.3 Calculate the percentage of monoglycerides using the formula:

$$(B-S) \cdot M \cdot 17.927 / P$$

B is the average volume in ml of the sodium thiosulfate solution used for analysis of the "blanks" containing chloroform.

S is the amount of sodium thiosulfate solution in ml used to titrate the sample.

M is the exact molarity of the sodium thiosulfate solution.

P is the weight of the sample to be analyzed in the volume of chloroform used for the analysis.

17.927 is the molar mass of glycerol monostearate, divided by 20.

The monoglyceride content expressed in terms of glycerol monostearate should be greater than 30 pp 100 (m/m).

6.11. Free glycerol

Add 50 ml of periodic acetic acid solution (R) to the aqueous extracts obtained during the monoglyceride-analysis process. Simultaneously prepare a "blank" by adding to 75 ml of water in a 500 ml conical flask 50 ml of periodic acetic acid solution (R). Continue the determination process as indicated in the method described for monoglycerides.

Calculate the percentage of glycerol using the following formula:

$$(b-S)M \cdot 2.30 / Q$$

b is the volume in ml of sodium thiosulfate solution used in the quantitative analysis the "blank" containing 75 ml of water

S is the volume in ml of sodium thiosulfate solution used in the quantitative analysis of the aqueous extracts

M is the molarity of the sodium thiosulfate solution.

Q is the weight of the first sample to to be analyzed (see monoglyceride determination).

Glycerol content should be less than 7 pp 100 (m/m).

N.B. : Glycerol can also be disclosed and identified by high performance liquid chromatography (HPLC) (5.3).

7. STORAGE

Anti-foaming agents should be kept in completely water-tight containers and away from heat.

ARGON
Ar = 40.0
N° SIN: 938
N°CAS = 7440-37-1
(OENO 31/2004)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Neutral gas, used for operations of inerting or degassing, it is used in a mixture of nitrogen and/or of carbon dioxide.

2. LABELLING

The label must mention the nature of the gas and refer to its composition and purity. The safety conditions must also be indicated on the packages.

3. CHARACTERISTICS

Colourless and odourless gas without flavour. Non flammable, it does not support combustion.

The weight of a litre of argon under the pressure of 760 mm of mercury is 1.784 g at 0°C. A volume of water dissolves 0.0336 volume of argon at 20°C.

4. TEST TRIALS

The global purity of the argon used in oenology must not be less than 99% of argon in volume.

Before any measurement it is advisable to allow any gas to escape for a few minutes in order to purge the piping.

4.1 Chromatographic dosage

The search and determination of gases: Nitrogen, carbon monoxide (less than 10 µl/l), oxygen (10 ml/l), hydrogen, carbon dioxide (less than 300 µl/l), etc., are quickly obtained by chromatography in gaseous phase according to the method in chapter II of the International Oenological Codex.

The total surface area of hydrogen chromatographic peaks, of oxygen and nitrogen must not exceed 1% of gas surfaces to be examined

The following chemical methods can also be used for oxygen.

4.2 Oxygen dosage by chemical method

Preparation of the flask for searching oxygen:

Introduce in a 24 ml flask about two fragments of copper turnings of 2 cm, 16 ml of ammoniac solution of copper sulphate (R), then 2 ml of hydrazine dihydrochloride solution (R).

Seal the flask with a rubber stopper that is easy to pierce with a needle for hypodermic injections. Crimp the neck with a metallic capsule. Then cover the capsule with wax in order to ensure perfect water tightness. Shake the flask and allow to stand away from light until complete discolouration is obtained after about eight days.

Conduct of the test trial:

Pierce the flask's stopper to search for oxygen with a needle of 8/10 millimetre for hypodermic injection (take care so as not to plunge it into the liquid) that then will be used for evacuating the gas after bubbling. Then introduce a second needle of the same diameter releasing the gas and plunging it into the liquid. After a minute of bubbling, a noticeable colouration should not be observed. In the presence of oxygen, the liquid quickly becomes blue and the colour darkens with time.

4. PACKAGING

The argon is supplied in highly resistant steel cylinders painted in white with needle valves. The resistance of these cylinders must be checked periodically.

ASCORBIC ACID
2,3-didehydro-L-threohexano-4-lactone
Acidum ascorbicum
L-Ascorbic Acid
C₆H₈O₆ = 176.1
SIN NO. 300
(Oeno 18/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Ascorbic acid is the enolic form of 3-oxo-L-gulofuranolactone (2,3-didehydro-L-threohexano-4-lactone).

This product falls into the category of antioxidants and is used as a reducing agent used to prevent oxidation.

Its use is subject to statutory regulations regarding limits.

2. LABELING

The concentration of this product should be indicated on the label, including cases in which it is used in mixtures, as should the safety and storage conditions.

3. PROPERTIES

Odorless white or very pale yellow crystalline powder with an acidic flavor. Aqueous solutions rapidly decay in air and light and have a maximum stability at pH 5.4. Melting point in a capillary tube: approximately 190 °C with decomposition.

Ascorbic acid in aqueous solution has a pH of less than or equal to 3.

4. SOLUBILITY

Water at 20 °C	290 g/l
Alcohol, 95% by vol.	320 g/l
Methanol	125 g/l
Acetone	soluble
Benzene, chloroform, ethyl ether, petroleum ether:	insoluble

5. ROTATORY POWER

In a 10 pp 100 (m/v) aqueous solution, ascorbic acid has a specific rotatory power

20°C

$[\alpha]_{\text{D}}^{20}$ is between + 20.5 and +21.5°.

D

6. ABSORPTION IN ULTRAVIOLET LIGHT

Ascorbic acid in alcohol solutions in a concentration of 10 mg/l exhibits an absorption spectrum with a maximum of approximately 244 nm.

The solution has a specific extinction of:

$$E_{\frac{1\text{ percent}}{1\text{ cm}}} \text{ approximately } 560$$

7. IDENTIFYING CHARACTERISTICS

7.1. Preparation of the Solution for Testing

Dissolve 5 g ascorbic acid in water and fill to 100 ml using the same solvent.

7.2. Add 0.5 g monosodium carbonate to 2 ml of the solution prepared for testing (Par. 7.1).

7.3. Add several drops nitric acid diluted to 10 pp 100 (R) and several drops silver nitrate in a concentration of 1 pp 100 (R) to 1 ml of the solution prepared for tests (Par. 7.1). A gray precipitate will form.

7.4. To 1 ml of the solution prepared for testing (Par. 7.1) add one drop of recently prepared sodium nitrohexacyanoferrate (III) $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}] \cdot 2\text{H}_2\text{O}$ (sodium pentacyanonitrosylferrate) in a concentration of 5 pp 100 (m.v), and 2 ml of 10 pp 100 diluted sodium hydroxide solution (R). Then, add 0.6-0.7 ml of concentrated hydrochloric acid (R) and stir. The yellow color will turn to blue.

7.5. Add drop by drop 2 ml of 2,6-dichlorophenolindophenol solution (R) to the solution prepared for testing (Par. 7.1). It will instantly become decolored.

8. TESTS

8.1. Sulfur Ash

As determined in 1.0 g ascorbic acid, the proportion of sulfur ash should not be greater than 1 g/kg.

8.2. Appearance of the Solution

The solution prepared for tests under paragraph 7.1 should be clear and colorless.

8.3. Determining pH

The pH of the solution prepared for tests under paragraph 7.1 should be between 2.4 and 2.8.

8.4. Heavy metals

10 ml of the solution prepared for tests under paragraph 7.1 should meet the heavy metal limit requirements described in the annex. (The heavy metal concentration expressed in terms of lead should be less than 10 mg/kg).

8.5. Lead

Use the technique detailed in the Compendium to analyze the solution prepared for tests (Par. 7.1). (Lead concentration should be less than 5 mg/kg).

8.6. Mercury

Use the technique described in the annex to analyze the solution prepared for tests (Par. 7.1). (Mercury concentration should be less than 1 mg/kg.)

8.7. Arsenic

Using the method indicated in the Annex, test for arsenic in the test solution prepared in accordance with paragraph 7.1. (Arsenic concentration should be less than 3 mg/kg).

8.8. Iron

Implement the atomic absorption technique described in the Compendium to analyze the solution prepared for tests (Par. 7.1). (Iron concentration should be less than 5 mg/kg.)

8.9. Copper

Implement the atomic absorption technique described in the Compendium to analyze the solution prepared for tests (Par. 7.1). (Copper concentration should be less than 2 mg/kg.)

8.10. Moisture

Dehydration loss after drying in a desiccator under a vacuum and in the presence of sulfuric acid for 24 hours must be less than 0.4%.

8.11. Quantitative Analysis

In 80 ml of recently boiled and cooled water to which 10 ml of sulfuric acid diluted to 10 pp 100 (R) has been added, dissolve a test sample weighed precisely at about 0.20 g. Add 1 ml of starch (R) and titrate using 0.05 M iodine until a persistent blue coloration appears.

INTERNATIONAL OENOLOGICAL CODEX
ASCORBIC ACID

1 ml of 0.05 M iodine corresponds to 8.81 mg ascorbic acid.
The product should contain at least 99 pp 100 ascorbic acid.

9. STORAGE

Ascorbic acid should be stored in tightly sealed non-metal containers in a dark place. Aqueous solutions decay rapidly in air and light.

ISOASCORBIC ACID

Isoascorbic acid, or D-ascorbic acid or erythorbic acid has the same antioxidant power as ascorbic acid and can be used for the same oenological purpose.

This acid exhibits the same appearance and the same solubility properties as ascorbic acid.

It is, optically, the reverse of ascorbic acid and has, under the same conditions, a specific rotatory power of:

$$[\alpha]_{20}^{\circ\text{C}} \text{ between } -20 \text{ and } -21.5^{\circ}$$

D

With the exception of rotatory power, this acid should exhibit the same properties as ascorbic acid, respond in the same way to the identifying reactions, pass the same tests and responds to the same quantitative analysis.

Note: The vitamin C efficacy of isoascorbic acid is approximately 1/20 of that of ascorbic acid.

Note : There is a preliminary draft resolution calling for registration of this product in the International Code of Oenological Practices.

BENTONITES
Bentonita
N° SIN: 558
(Oeno 11/2003)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Bentonites are hydrous aluminium silicates belonging to the montmorillonite group. The brute formula is:

$\text{Si}_4 (\text{Al}_{(2-x)} \text{R}_x) (\text{O}_{10}, \text{H}_2\text{O})(\text{Ce}_x, n\text{H}_2\text{O})$ or $\text{Si}_4(\text{Al}_{(2-x)}\text{R}_x)(\text{H}_2\text{O})_n$
where:

-R = Mg, Fe, M, Zn, Ni

-Ce (exchangeable cations) = Ca, Na, Mg.

Bentonites are used for clarification operations or protein stabilisation in musts and wine. Bentonites fix to certain unstable proteins which allows them to be eliminated.

Bentonites are capable of fixing coloured matter.

2. LABELLING

The nature of the bentonite (natural sodium, calcium, and activated calcium), batch number and the optimal expiration date for activated bentonites will be indicated on the label. The mention of risks and safety concerning the presence of crystalline silica should also be indicated.

2.1 Natural Bentonites:

Depending on the nature of the of exchangeable cations present, there are 2 naturally occurring types of bentonite:

- **Sodium bentonite**, it swells and absorbs readily where sodium is the major exchangeable cation.
- **Calcium bentonite**, where calcium is the major exchangeable cation, it is lower swelling and lower absorbent than sodium bentonites.

These two types of bentonites are simply grinded before their commercialisation after possibly being dried at 80°C to 90°C.

2.2 Activated bentonites:

In order to improve the adsorption properties of calcium bentonites, they are most often activated by sodium carbonate, then dried and grinded. This results in activated calcium bentonites with properties equal or superior to sodium bentonites.

The properties of these bentonites thus activated or permuted are less stable in time (3 to 18 months) and depend on the activation of magnesium, calcium, and sodium levels.

These different types of bentonites are in the form of powder, spherical or cylindrical granules. Colour can vary from white for the purest products to grey, beige or green for others.

3. TEST TRIALS

3.1 Odour

Bentonite should not have any undesirable odour (e.g. no mould) and should not change the taste of wine.

3.2 pH level

Shake 5 g of bentonite with 100 ml of distilled water for 5 minutes. Allow to stand for 1 hour. Measure the pH level of the supernatant liquid. Natural calcium bentonites have a neutral pH level around 6.5 to 8.5. Natural sodium or activated calcium bentonites have a much more alkaline pH level around 8.5 to 10.0.

3.3 Loss during desiccation

The desiccation of 5 g of bentonite at 105°C during 4 hours causes a weight loss of 5% to 15% of the initial weight (often around 10%).

3.4 Preparation of the test trial solution

Weigh **p** g of bentonite containing 10 g of anhydrous bentonite. In a 500 ml flask with a large opening which can be hermetically sealed, add 100 ml of tartaric acid solution to 5 g per litre until the solution has a pH level of 3 (R). Sprinkle the bentonite trial

sample in the constantly shaken solution (for example using a magnetic stirrer) and a funnel. After this addition, shake vigorously for 5 minutes. Allow to stand for 24 to 48 hours. Decant, centrifuge or filter if necessary to obtain at least 100 ml of clear liquid.

All the following set limits for bentonite are for the weight of dried bentonite.

3.5 Montmorillonite content

Minimum rate:

Manufacturer indicates that the content should not be under 80% by x-ray diffraction analysis.

3.6 Different forms of free silica content

Crystal silica content must be less than 3% (quartz N° CAS 14080-60-7, cristobalite N° CAS 14464-46-1).

Particle holdings under 10 microns must be less than 10%.

Respirable crystal silica content must be under 0.3%.

These standards must be written on the security form supplied by the manufacturer.

3.7 Lead

In the test trial solution (3.4) determine the lead content using the method described in Chapter II.

Lead content must be less than 5 mg/kg.

3.8 Mercury

Determine the mercury content according to the method described in Chapter II with the test trial solution (3.4).

Mercury content should be less than 1 mg/kg.

3.9 Arsenic

Determine the arsenic content of 5 ml of test trial solution (3.4) according to the method in Chapter II.

Soluble arsenic content should be less than 2 mg/kg.

3.10 Iron

Add 12.5 ml of water, 1 ml concentrated hydrochloric acid (R) and 2 ml of potassium thiocyanate at 5% (R) to 5 ml of the test trial solution (3.4). The red coloration should be lighter than what is obtained when using 2.5 ml citric acid at 5% at pH 3 (R), 1 ml concentrated hydrochloric acid (R), 15 ml of iron salt solution (III) at 0.010 g of iron per litre (R) and 2 ml of potassium thiocyanate solution at 5% (R).

Iron content should be less than 600 mg/kg).

Iron can also be determined by atomic absorption spectrometry according to the method in Chapter II.

3.11 Aluminium

On the test trial solution (3.4), find extractable aluminium according to the method described in Chapter II.

Extractable aluminium content should be less than 2.5 g/kg.

3.12 Calcium and magnesium

On the test trial solution (3.4), determine calcium and magnesium using the methods outlined in the Compendium of International Methods of Analysis of Wine and Musts.

Calcium and soluble magnesium combined should be less than 100 meq for 100 g.

3.13 Sodium

On the test trial solution (3.4), determine sodium using the method outlined in the Compendium of International Methods of Analysis of Wine and Musts.

Soluble sodium content should be less than 10 g/kg for natural bentonites and less than or equal to 35 g/kg for activated bentonites.

3.14 Presence of large particles

Put 1 litre of water in a 1.5 litre long stem glass. Slowly add while shaking the liquid, a quantity of bentonite corresponding to 50 g of dried bentonite. Shake vigorously 2 to 3 minutes and allow to stand for 24 hours. Shake 2 to 3 minutes and allow to stand for 2 minutes. Using a siphon, take off 9/10 of the cloudy liquid exceeding 100 ml and leave the deposits at the bottom of the

glass. Add 900 ml of water. Shake 1 minute. Allow to stand for 2 minutes and repeat to obtain 5 washings. Remove the deposit and put in a capsule. Dry and weigh. The residue must be less than 8 g for 100 g.

3.15 De-acidification tests trials

Weigh (**p**) of bentonite containing 0.2 g of dried bentonite. Put this in a 125 ml flask containing 50 ml of citric acid 0.033 M solution (**R**). Shake vigorously for 5 minutes and allow to stand for 30 minutes. Either filter or centrifuge. Take 10 ml of filtrate and titrate with an acid solution of 0.1 M of sodium hydroxide with a drop of phenolphthalein solution (**R**), that is **n** ml the volume poured to obtain a colour change in the indicator:

250 (10 – **n**) is the number of milliequivalent of acids fixed or neutralised for 100 g of bentonite.

The maximum limit is 2.5 eq/kg.

3.16 Rate of swelling

Swelling indicator: specific test is necessary.

2 g of bentonite is strewn over 100 ml of demineralised water and 100 ml of wine in a graduated test tube cylinder. After 24 hours, weigh the volume of bentonite. This will be expressed in ml/g of dried product.

3.17 Protein adsorption test trial (for bentonite to go through deproteinisation)

3.17.1- Preparation of test trial solution:

Mix 5 g of egg white with a sufficient amount of citric acid solution of 5 g per litre (pH=3) to make 1 litre. Filter. Determine total nitrogen on 100 ml of this solution by using the procedure described in Chapter II. This solution contains approximately 90 mg of total nitrogen for 575 mg of proteins per litre.

3.17.2 - For each test trial using 100 ml of this solution, mix increasingly larger doses of bentonites prepared in a 5% suspension in order to process doses of 0.1 to 0.8 g/l. Shake vigorously and maintain at 15°C–20 °C for 6 hours. Centrifuge and proceed with determinations of nitrogen or residual proteins.

A de-proteinising bentonite should eliminate at least 50% of the proteins in a synthetic solution with a 0.4 g/l dose.

3.18 Determining the specific adsorption surface (or the adsorption indicator for methylene blue)

See method described in annex.

The accepted limit should be 300 mg/100g.

4. STORAGE

Bentonites must be stored in a ventilated area in watertight containers away from volatile objects that they could adsorb.

ANNEX
DETERMINATION OF THE SPECIFIC SURFACE OF ADSORPTION
OF BENTONITE

1. GENERAL INFORMATION

1.1 Aim of the test trial

This test trial enables to measure the capacity of bentonite to adsorb methylene blue.

Clays, organic matters, and iron hydroxide preferentially adsorb methylene blue. This capacity takes into account the activity on the surface of these elements. We call, "blue value" of bentonites, the quantity expressed in grams of methylene blue adsorbed per 100 g of bentonites.

1.2 Principle of the test trial

Elemental doses of a methylene blue solution are injected successively into an aqueous solution containing the trial sample. The adsorption of blue is checked after each addition by making a spot on a paper filter (spot test, see paragraph 5).

For a simple conformity check, the specified quantity of blue is injected once.

2. EQUIPMENT AND REAGENT

2.1 A 25 ml burette graduated 1/10 ml.

2.2 Paper filter: quantitative and without ashes (< 0.010); weight: 95 g/m^2 ; thickness: 0.20 mm; filtration speed 75; retention: 8 micrometers.

2.3 A glass rod: 300 mm length; 8 mm diameter.

2.4 A magnetic stirrer and magnetic stirring bar.

2.5 Methylene blue of medicinal quality at $10\text{g/l} \pm 0.1 \text{ g/l}$.

The maximum duration for using the solution is one month. The solution must be stored away from light.

2.6 Demineralised or distilled water.

3. PREPARATION OF TEST TRIAL SAMPLES

Add 10 g of bentonite in 200 ml of distilled water, allow to swell for 2 hours, then homogenise by shaking.

4. CARRYING OUT TEST TRIAL

4.1 Definition of spot test

After each addition of blue (see paragraph 5.2), this test involves taking a drop of suspension that is placed on a paper filter using a glass rod. The spot that is formed is composed of a central deposit of matter, blue in colour surrounded by a humid colourless area.

The drop must be such that the diameter of the deposit is between 8 and 12 mm.

The test is positive if a persistent light blue ring appears around the middle deposit in the humid zone. The test is negative if the ring is colourless.

4.2 Determination

Using a burette, pour 2 ml of blue solution in a container with 200 ml of suspension of bentonite maintained in agitation. After 2 minutes, add 1 ml of blue solution. This addition is followed by the spot test on filter paper.

Allow the asorption of blue to occur which is not instantaneous. Meanwhile tests should be conducted minute by minute.

If the light blue ring disappears at the fifth spot, proceed with elemental additions of 0.2 ml of blue and then 0.1 ml.

Each addition is followed by tests conducted minute by minute.

Renew these operations until the test remains positive for 5 consecutive minutes: the determination is considered as ended.

That is V ml poured.

5. EXPRESSION OF RESULTS

5.1 Blue value

The blue value expressed in grams of blue for 100 g of bentonite is shown in the following formula:

$$V \times 10$$

V is the value of blue methylene poured in ml.

5.2 Conformity check compared to a given specification

The specification is expressed in blue value for 100 g of bentonite, or s of this value.

The volume of blue solution to be added in one time to the preparation (3) is:

$$V = \frac{s}{10}$$

The spot test is done after eight minutes of shaking. If it is negative, the bentonite complies with the specification.

BETA-GLUCANASES from *Trichoderma Sp*
(Oeno 27/2004)

(E.C. 3-2-1-58)
(C.A.S. No. 9073-49-8)

Glucan 1,3-beta-glucosidase
(exo-1,3-beta-glucosidase; beta-1,3-glucan exo-hydrolase; exo-1,3-
beta-glucanase; endo-1,3-beta-glucanase)
and glucan 1,6-beta-glucosidase

GENERAL SPECIFICATIONS

The specifications must comply with general specifications for enzymatic preparations that appear in the International Oenological Codex.

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

The degradation of beta-glucans present in wines, in particular those from grapes affected by *Botrytis cinerea* or yeast glucans. These molecules of a very high molecular weight hydrolyse the beta-1,3 and beta-1,6 bonds of 1,3 (1,6)-beta-D-glucans with glucose production.

Secondary activities: hemicellulases, cellulases.

The beta-1,3-D-glucanases are produced from *Trichoderma harzianum* and/or *Trichoderma reesei*

The preparation of the enzyme is without any harmful consequences as is production and purification.

Beta-glucanases do not contain any substances, micro-organisms nor collateral enzymatic activities that can:

- be harmful to health,
- be harmful to the quality of the products treated,
- lead to the formation of undesirable products or flavour problems.

There are regulatory limits for the use of beta-glucanases in wine.

2. LABELLING

The concentration of the product must be indicated on the label, as well as the safety conditions, storage conditions and the expiry date.

3. CHARACTERISTICS

In general, it is greyish to light brown amorphous powder or light brown to dark brown liquids or granules.

4. SOLUBILITY

Soluble in water and practically insoluble in ethanol.

5. ENZYMATIC ACTIVITY

Activity is the quantity of enzyme necessary for liberating in standardised conditions (see activity measured according to a method to be described), a quantity of reducing sugars corresponding to 1 μ mole of glucose per minute.

Remark: the enzyme produced according to paragraph 6 simultaneously has beta-1,3-glucanase and beta-1,6-glucanase activities which gives it the sought oenological properties.

6. SOURCE OF ENZYME AND PRODUCTION MEANS

The beta (-1,3-1,6) glucanases are produced by submerged culture of a selected non pathogenic, non toxic strain of *Trichoderma harzianum* and/or *Reisei* that is not genetically modified, in pure culture.

7. DILUENTS, PRESERVATIVES AND ADDITIVES

The preparation of beta-glucanase is generally in the form of granules. These products are prepared with food diluents or food additives such as maltodextrin, sodium citrate, citric acid, starch or glucose.

8. TEST TRIALS

8.1 Loss at desiccation: Less than 10%. (does not apply to liquid preparations)

8.2 Ashes/Sulphuric ashes

Determine the sulphuric cinders according to the method in Chapter II of the International Oenological Codex.

The rate of sulphuric ashes of beta-glucanases should not be more than 2% of dry matter.

8.3 Preparation of the test solution

Dissolve 5 g of beta-glucanases in 100 ml of water.

8.4 Heavy metals

Add 2 ml of buffer solution pH 3.5 (R) and 1.2 ml of thioacetamide reagent (R) to 10 ml of the test trial solution (8.3). No precipitate should form. If a brown colouration appears, it should be

lighter than the control prepared as indicated in Chapter II of the International Oenological Codex.

The heavy metal content expressed in lead should be less than 30 mg/kg.

8.5 Arsenic

In 2 ml of test trial solution (8.3), search by the method indicated in

Chapter II of the International Oenological Codex.

Arsenic content should be less than 3 mg/kg.

8.6 Lead

Using the test trial solution (8.3) determine the lead according to the method described.

Lead content should be less than 5 mg/kg.

8.7 Mercury

Using the test trial solution (8.3) determine the mercury according to the method described in Chapter II of the International Oenological Codex.

Mercury content should be less than 0.5 mg/kg.

8.8 Cadmium

Using the test trial solution (8.3) determine the cadmium according to the method described in chapter II of the International Oenological Codex.

Cadmium content should be less than 0.5 mg/kg.

8.9 Biological contaminants

Total microorganisms	less than 5 10 ⁴ CFU/g of preparation
Total bacteria	less than 10 ³ CFU/g of preparation
Total coliforms	less than 30 CFU/g of preparation
<i>Escherichia coli</i>	absence checked on a 25 g sample
<i>St. aureus</i> *	absence checked on a 1 g sample
Salmonella	absence checked on a 25 g sample
Sulfitoreducing anaerobia	less than 30 CFU/g of preparation
Yeasts	maximum content 10 ² CFU/g of preparation
Total lactic bacteria	absence checked on a 10 g sample
Acetic bacteria	maximum content 10 ² CFU/ g of preparation
Moulds	maximum content 10 ² CFU/g of preparation

INTERNATIONAL CENOLOGICAL CODEX
Beta-Glucanase

Antibiotic activity*
Mycotoxins*

not detectable
not detectable

9. STORAGE

In a solid form, the preparation can be stored for several years and in a liquid form for several months at a low temperature (+5°C).

* Method to be defined by the Sub-commission of Methods of Analysis

CALCIUM CARBONATE

Calcii carbonas

CaCO₃ = 100.1

SIN NO. 170

(Oeno 20/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This product is used for deacidification. The transport of calcium ions causes salification of free tartaric acid. The use of calcium carbonate is also authorized when using the so-called "double salt" method of deacidification. It may then contain small quantities of calcium tartromalate (double salt) and/or calcium tartrate. There are regulations governing the use of this product..

2. LABELING

The label should indicate the proportion of pure calcium carbonate and the safety and storage requirements.

3. CENTESIMAL COMPOSITION

Carbon dioxide 43.97

Calcium 40.04

4. PROPERTIES

Calcium carbonate exists as a white powder with the reaction properties of carbonates. In solution in a concentration of 5 pp 100 (m/v) in dilute acetic acid (R), it yields calcium reactions.

5. SOLUBILITY

Insoluble in water

Insoluble in alcohol at 95% by vol.

Soluble with effervescence in dilute acetic acid, hydrochloric acid and nitric acid solutions

6. TESTS

6.1. Desiccation loss

Weigh 2 g calcium carbonate in a dish. Place in an oven at 200 °C for 4 hours. Weight loss should not exceed 2 pp 100.

6.2. Substances Soluble in Water

Mix 2 g of ground calcium carbonate with 20 ml of boiled water. Filter. Collect 10 ml. The solution should be neutral. Dry evaporate. The residue should not be greater than 1 pp 100l.

6.3. Ammoniacal Ions

Place 2 g of calcium carbonate, 25 ml of distilled water and 5 ml of 30 pp 100 sodium hydroxide solution (R) in the flask of a distillation device.

Distill and collect 20 ml distillate in 40 ml 4 pp 100 boric acid (R) in the presence of methyl red (R). Two drops of 0.1 M hydrochloric acid solution should be sufficient to cause the indicator to turn color.

6.4. Barium

Dissolve 0.50 g of calcium carbonate in 10 ml of nitric acid diluted to 10 pp 100 (R). Add 10 ml of saturated calcium sulfate solution (R). The mixture should remain clear.

6.5. Preparing the Solution for Tests

Dissolve 10 g of calcium carbonate in 100 ml of 10 pp 100 dilute acetic acid (m/v) (take care as there will be effervescence due to the release of carbon dioxide).

6.6. Magnesium

Use the method described in the Compendium on the solution prepared for testing under paragraph 6.5. (Content should be less than 1 pp 100 by weight).

6.7. Iron

Use the atomic absorption spectrometry method described in the Compendium on the solution prepared under paragraph 6.5. (Iron content should be less than 300 mg/kg).

6.8. Lead

Using the technique described in the annex to quantitatively analyze the lead in the solution prepared for testing (Par. 6.5). (Lead content should be less than 5 mg/kg).

6.9. Mercury

Implement the technique described in the annex to quantitatively analyze the mercury in the solution prepared for testing (Par. 6.5). (Mercury content should be less than 1 mg/kg).

6.10. Arsenic

Using the method described in the annex, test for arsenic in the solution prepared for testing (Par. 6.5). (Arsenic content should be less than 3 mg/kg).

6.11. Sodium

In accordance with the method described in the Compendium, quantitatively determine sodium content by flame photometry in the solution prepared for testing (Par. 6.5). (Sodium content should be less than 500 mg/kg).

6.12. Quantitative Analysis

Dissolve a precisely weighed sample p of about 2 g in 50 ml of a 1 M hydrochloric acid solution. Bring to a boil. Allow to cool and titrate the excess hydrochloric acid solution using 1 M sodium hydroxide solution and methyl red (R). Let n be the amount in ml of 1 M sodium hydroxide solution used:

1 ml of 1 M hydrochloric acid corresponds to 0.05005 g calcium carbonate. Parts per 100 of calcium carbonate in the product tested:

$$(50-n) 5.005 / p$$

The wine-making product must contain a minimum of 98 pp 100 calcium carbonate.

6 STORAGE

Calcium carbonate should be stored in a dry place in hermetically sealed containers away from volatile elements it could adsorb.

CALCIUM PHYTATE
Calcium inositol hexaphosphate
Calcii phytas
 $C_6H_6Ca_6O_{24}P_6 \cdot 3H_2O = 942.11$
(Oeno 21/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Calcium phytate is the salt of the inositol hexaphosphoric ester, or inositohehexaphosphoric or phytic acid.

In its calcium and magnesium double salt forms, phytic acid composes phytin, a reserve form of phosphorous in plants.

Since it is an iron (III) complexing agent approved for removal of excess iron in wines, its use must be strictly monitored.

Any excess phytate with respect to the iron (III) content causes deposits to build up when the slightest oxidation occurs.

2. LABELING

The label should indicate product concentration even when used in mixtures, as well as its safety and storage conditions.

3. PROPERTIES

White powder with an acidulous taste, which is minimally soluble in water, soluble in dilute strong acids, and difficult to dissolve in wine, in which solubility is incomplete.

Aqueous calcium phytate solution possess an acidic nature, which is disclosed by movement of the indicator to litmus. It yields calcium reactions.

4. TESTS

4.1. Desiccation Loss

Dry a 1 g sample of calcium phytate in an oven at 105 °C until a constant weight is obtained. Weight loss should be less than 12 pp 100.

Limits indicated below are for dry product.

4.2. Ash

Incinerate a 0.250 g test sample of calcium phytate at 550 °C. The residue should not be less than 65 pp 100 nor greater than 72 pp 100 of the dry product contained in the test sample.

4.3. Insoluble Substances

Prepare a first solution containing 1 g of calcium phytate, 7 ml of 1M hydrochloric acid solution, and 93 ml of distilled water. Separately, prepare a solution of 1 g of calcium phytate with 50 ml of distilled water and 1.5 ml pure phosphoric acid (R). Filter each of the solutions separately and collect the deposit. Wash and dry the deposit at 100 °C. Each residue should be less than 1 part per 100 (10g/kg) of dried product at 105 °C.

4.4. Starch

Add several drops of iodinated water (R) to the residues obtained under Paragraph 4.3; no blue coloration should develop.

4.5. Sugars

Stir 3 g of calcium phytate with 15 ml of distilled water. Filter. The filtrate should not reduce the cupro-alkaline reagent (R) before or after the sucrose inversion.

4.6. Albumin

Dissolve 1 g of the product in a mixture of 1 ml of concentrated hydrochloric acid (R) and 3 ml of distilled water. Add 3 ml of 30% sodium hydroxide solution (R). Filter. When one drop of 4 pp 100 (m/v) copper (II) sulfate solution is added to the filtrate, no violet color should appear.

4.7. Preparing the Solution for Tests

Macerate a quantity of calcium phytate containing 5 g dry product with 100 ml of 10 g per liter citric acid (R) for 24 hours while agitating from time to time. Filter.

4.8. Iron

Add 1 ml of concentrated hydrochloric acid (R) and 2 ml of 5 pp 100 potassium thiocyanate to 10 ml of test solution prepared under paragraph 4.7. The resulting coloration should be less intense than that produced by a control tube prepared with 2.5 ml solution in a concentration of 0.010 g of iron per liter (R), 7.5 ml of distilled water, 1 ml of concentrated hydrochloric acid (R) and 2 ml of 5 pp 100 thiocyanate (R). (Iron content should be less than 50 mg/kg).

4.9. Lead

Using the method described in the Compendium, quantify lead analytically in the test solution prepared according to Par. 4.7. (Lead content should be less than 5 mg/kg).

4.10. Mercury

Using the method described in the annex, quantify mercury analytically in the test solution prepared according to Par. 4.7. (Mercury content should be less than 1 mg/kg).

4.11. Arsenic

Using the method described in the annex, quantify arsenic analytically in the test solution prepared according to Par. 4.7. (Arsenic content should be less than 3 mg/kg).

4.12. Mineral phosphates

Place 0.50 g calcium phytate in a 200 ml volumetric flask. Add 100 ml of distilled water and 5 ml of concentrated nitric acid (R). Agitate for 15 minutes at 20 °C and top off to 200 ml with distilled water. To 10 ml of this solution, add 10 ml of nitro-vanadomolybdic reagent (R). Leave in contact for 15 minutes at 20 °C. The resulting color should be less intense than that produced by adding 5 ml distilled water and 10 ml nitro-vanadomolybdic reagent (R) to 5 ml of a monopotassic phosphate solution containing 0.05 g phosphorous per liter (R). (Mineral phosphate content, expressed in terms of phosphorous, should be less than 1 pp 100).

4.13. Glycerophosphates

Heat 0.50 g of calcium phytate in the presence of monopotassic sulfate. No acrolein fumes (odor of burnt horn) should be released.

4.14. Total Phosphorous Determination

Weigh precisely a 0.25 g sample of calcium phytate which has already been dried at 105 °C. Place it in a flask which is ground and polished so it can be fitted with a tube 8 mm in diameter and 1 m long which will serve as a reflux condenser. Add 5 ml of concentrated sulfuric acid (R) and 0.5 ml concentrated nitric acid (R). Bring to boiling under reflux for approximately 15 minutes. After cooling, decant the contents of the flask diluted with water in a 1 liter volumetric flask. Wash the condenser and flask with water by pouring these liquids in the volumetric flask, and fill to gauge line after bringing the temperature to 20 °C. Agitate.

Add 10 ml of nitro-vanadomolybdic reagent (R) to 10 ml of this solution. Agitate in a 20 °C water bath and let sit in the water bath for 15 minutes. The intensity of the resulting color should be equal to or greater than that of a control prepared under the same conditions using 8 ml of monopotassic phosphate solution in a concentration of 0.05 g of

INTERNATIONAL CENOLOGICAL CODEX
CALCIUM PHYTATE

phosphorous per liter (R), 2 ml of water and 10 ml of nitro-vanadomolybdic reagent (R).

Total phosphorous analysis can also be determined using a spectrophotometer with a wavelength of 425 nm whose calibration curve was obtained based on 4-6-8-10 ml of solution in a concentration of 0.05 mg phosphorous per liter (R).

Calcium phytate should contain at least 15 parts of phosphorous per 100 , as compared with a product dried at 105 °C.

5. STORAGE

Calcium phytate should be stored in a dry place in hermetically sealed containers.

INTERNATIONAL OENOLOGICAL CODEX
CALCIUM TARTRATE

CALCIUM TARTRATE
Dextrorotatory Calcium Tartrate
Calcium tartaricum
(OOC-CHOH-CHOH-COO) Ca, 4H₂O
Tetrahydric L(+)-2,3- calcium dihydroxybutanedioate
(OOC-CHOH-CHOH-COO) Ca, H₂O
C₄H₁₂CaO₁₀ = 260.13
SIN No. 354
(Oeno 22/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

A natural wine salt primarily originating from wine residues. It is therefore typically found in L(+) form. It usually crystallizes in tetrahydrated form.

This product promotes triggering of the precipitation of the natural calcium tartrate in wine by means of a seeding technique.

2. LABELING

The label should indicate product concentration, even when used in mixtures, as well as its safety and storage conditions.

3. CENTESIMAL COMPOSITION

Tartaric acid	57.7
Calcium	15.4
Water	27.9

4. PROPERTIES

Fine, crystalline powder with a white or off-white color. Tasteless. Melting point is 270 °C.

5. SOLUBILITY

Water at 20 °C	0.525 g/l
Alcohol, 95% by vol.	0.15 g/l
Ethyl ether	0.01 g/l

6. TESTS

6.1. Rotatory Power

Dissolve 1 g of the substance in 1l of 1 M hydrochloric acid. After it has completely dissolved, it gives :

$$[\alpha]_{\text{D}}^{20^{\circ}\text{C}} = +7.2 \pm 0.2^{\circ}.$$

Rotatory power is sensitive to slight variations in pH.

6.2. pH in Saturate Solution

Add 1 g of the product to 100 ml of distilled water. After shaking for one hour and allowing the precipitate to resettle (15 minutes), an increase in pH of between 1.5 and 2.5 pH units should be observed.

6.3. Desiccation Loss

Desiccation loss is determined up to constant weight in precisely-weighed sample of about 1 g. At a temperature of between 100 and 105 °C, weight loss should be less than or equal to 2.5 pp 100.

6.4. Preparing the Solution for Tests

Dissolve a sample precisely weight to about 1 g in 100 ml of 1 M hydrochloric acid.

6.5. Sulfates

Take 10 ml of the test solution (Par. 6.4) and add to it 1 ml of 10 pp 100 barium chloride solution (R). After homogenization, let sit after 15 minutes. No clouding should occur. If clouding does occur, it should be less intense than that in a control prepared using the method indicated in the Annex. (Sulfate content, expressed in terms of sulfuric acid, should be less than 1 g/kg).

6.6. Heavy Metals

Add 0.5 ml of concentrated ammonium hydroxide (R), 2 ml of pH 3.5 buffer solution (R) and 1.2 ml of thioacetamide reagent (R) to 10 ml of the test solution prepared under paragraph 6.4. (Heavy metal content, expressed in terms of lead, should be less than 10 mg/kg).

6.7. Lead

Using the method described in the Compendium, quantify lead analytically in the test solution prepared according to Par. 6.4. (Lead content should be less than 5 mg/kg).

6.8. Mercury

Using the method described in the Annex, quantify mercury analytically in the test solution prepared according to Par. 6.4. (Mercury content should be less than 1 mg/kg).

6.9. Arsenic

Using the method described in the Annex, quantify arsenic analytically in the test solution prepared according to Par. 6.4. (Arsenic content should be less than 3 mg/kg).

6.10. Basic Residue Determination

Dissolve a sample, **p**, of tetrahydric calcium tartrate weighed precisely at about 0.5 g in 25 ml of 1 M hydrochloric acid solution (R). Bring to boiling under reflux and allow to cool. Titrate the excess acid using 1 M sodium hydroxide solution (R) and in the presence of methyl red (R). Let *n* be the quantity in millimeters of the 1 M sodium hydroxide solution used. 1 ml of 1 M hydrochloric acid corresponds to 0.05005 g of calcium carbonate. The content in parts per 100 of calcium carbonate is:

$$(25n) 5.005 / p$$

The products used in winemaking should contain a maximum of 3 pp 100 basic residues expressed in terms of calcium carbonate.

7. STORAGE

Calcium tartrate should be stored away from moisture in hermetically-sealed containers.

CARAMEL
N° SIN: 150
(Oeno 20/2004)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Caramel can be found in liquid form or solid form ranging in colour from dark brown to black. Colouring wine in the *stricto sensu* is not allowed but caramel is used as a colouring agent in certain liquor wines, spirit beverages of vitivinicultural origin and wine-based beverages.

2. DEFINITIONS

CARAMEL (OR ORDINARY CARAMEL) (Class I) (SIN: 150a)

Caramel (or ordinary caramel) is prepared by controlled heating of carbohydrates made up of glucose and fructose monomers and/or their respective polymers (for example, glucose syrup, saccharose and/or inverted sugars syrups). To favour caramelisation, acids, bases and salts excluding ammonium compounds can be used.

CAUSTIC SULPHITE CARAMEL (Class II) (SIN: 150b)

Caustic sulphite caramel is prepared by controlled heating of carbohydrates as defined for ordinary caramel, with or without acids or bases, in the presence of sulphite compounds (sulphuric acid, potassium sulphite, potassium hydrogen sulphite, sodium sulphite and sodium hydrogen sulphite). No ammonium compounds are used.

AMMONIA CARAMEL (Class III) (SIN: 150c)

Ammonia caramel is prepared by controlled heating of carbohydrates as defined for ordinary caramel, with or without acids or bases, in the presence of ammonium compounds (ammonium hydroxide, ammonium carbonate, ammonium hydrogen carbonate, and ammonium phosphate). No sulphite compounds are used.

AMMONIUM SULPHITE CARAMEL (Class IV) (SIN: 150d)

Ammonia sulphite caramel is prepared by controlled heating of carbohydrates as defined for ordinary caramel, with or without acids

or bases, in the presence of sulphite and ammonium compounds (sulphuric acid, potassium sulphite, potassium hydrogen sulphite, sodium sulphite, sodium hydrogen sulphite, ammonium hydroxide, ammonium carbonate, ammonium hydrogen carbonate, ammonium phosphate, ammonium sulphate, ammonium sulphite and ammonium hydrogen sulphite).

3. LABELLING

The concentration of the product and whether it was mixed, must be indicated on the label in addition to the storage conditions.

4. TEST TRIALS

4.1 Intensity of the colouring

The intensity of the colouring is defined as the absorbance of a liquid solution of 0.1% (m/v) concentrated caramel measured in a 1 cm space of optical pathway with light waves of 610 nm.

4.2 Total Nitrogen

Apply the method described in Chapter II of the International Oenological Codex to 2 g of exactly measured caramel.

4.3 Preparation of the solution for the test trials

Place 2 g of caramel in a capsule; put in heat chamber at 105°C for 4 hours then incinerate carefully without going beyond 550°C.

Take the cinders and put in 10 ml of 10% hydrochloric acid (R). Heat a little and transfer to a graduated 50 ml flask and rinse the capsule with water and fill up to the indicator.

4.4 Heavy metals

Take 10 ml of the solution prepared for the trial tests as in point 4.3, and add 2 ml of 3.5 pH buffer solution (R) and 1.2 ml of thioacetamide reagent (R). If the solution turns brown, it must be less brown than the control sample, as indicated in Chapter II of the International Oenological Codex.

4.5 Lead

Using the solution for test trials as prepared in the point 4.3, measure out the lead as indicated in Chapter II of the International Oenological Codex.

Please refer to point 5 for maximum contents.

4.6 Mercury

Measure out the mercury using the method described in Chapter II of the International Oenological Codex.

Please refer to point 5 for maximum contents.

4.7 Cadmium

Test solution prepared according to point 4.3; Measure out the cadmium using the method described in Chapter II of the International Oenological Codex.

Please refer to point 5 for maximum contents.

4.8 Arsenic

Test solution prepared according to point 4.3; Measure out the arsenic using the method described in Chapter II of the International Oenological Codex.

Please refer to point 5 for maximum contents.

4.9 Colouring matter retained on DEAE cellulose

See method as described by JECFA published in the Compendium of food additive specifications, FAO Food and Nutrition Paper 52 Add. 8.

4.10 Colouring matter retained on phosphorylcellulose

See method as described by JECFA published in the Compendium of food additive specifications, FAO Food and Nutrition Paper 52 Add. 8.

4.11 4-Methylimidazole

See method as described by JECFA published in the Compendium of food additive specifications, FAO Food and Nutrition Paper 52 Add. 8.

4.12 2-Acetyl-4-tetrahydroxybutylimidazole

See method as described by JECFA published in the Compendium of food additive specifications, FAO Food and Nutrition Paper 52 Add. 8.

4.13 Total sulphur

See method as described by JECFA published in the Compendium of food additive specifications, FAO Food and Nutrition Paper 52 Add. 8.

4.14 Sulphur dioxide

The method used can be found in the O.I.V. Compendium of International Methods of Analysis of Wine and Musts.

5. PARTICULAR SPECIFICATIONS

5.1 Ordinary caramel

Colouring matter retained on DEAE cellulose	Not more than 50%
Colouring matter retained on phosphorylcellulose	Not more than 50%
Colour intensity	0.01 – 0.12
Total nitrogen	Not more than 0.1%
Total sulphur	Not more than 0.3%
Arsenic	Not more than 1 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg
Cadmium	Not more than 1 mg/kg
Heavy metals (expressed in Pb)	Not more than 25 mg/kg

5.2 Caustic sulphite caramel

Colouring matter retained on DEAE cellulose	Not more than 50%
Colour intensity	0.06 – 0.10
Total Nitrogen	Not more than 0.2% (1)
Total sulphur dioxide	Not more than 0.2% (1)
Total sulphur	1.3 – 2.5% (1)
Sulphur retained on DEAE cellulose	Over 40%
Percentage of optical colour density retained on DEAE cellulose	19-34
OD 280/560 ratio	Over 50
Arsenic	Not more than 1 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg
Cadmium	Not more than 1 mg/kg
Heavy metals (expressed in lead)	Not more than 25 mg/kg

(1) Expressed by the intensity of equivalent colouring; or compared to a product with a colour intensity of 0.1 unit of absorption.

5.3 Ammonia caramel

Colouring matter retained on DEAE cellulose	Not more than 50%
Colour matter retained on phosphorylcellulose	Not more than 50%
Colour intensity	0.08 – 0.36
Ammoniac nitrogen	Not more than 0.4% (1)

4-Methylimidazole	Not more than 250 mg/kg ⁽¹⁾
2-Acetyl-4-tetrahydroxybutylimidazole	Not more than 10 mg/kg ⁽¹⁾
Total sulphur	Not more than 0.3% ⁽¹⁾
Total nitrogen	1.3 – 6.8% ⁽¹⁾
Percentage of optical colour density retained on phosphorylcellulose	13-35
Arsenic	Not more than 1 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg
Cadmium	Not more than 1 mg/kg
Heavy metals (expressed in lead)	Not more than 25 mg/kg

⁽¹⁾ Expressed by the intensity of equivalent colouring; or compared to a product with a colour intensity of 0.1 unit of absorption.

5.4 Ammonium sulphite caramel

Colouring matter retained on DEAE cellulose	Not more than 50%
Colour intensity	0.10 – 0.60
Ammoniac nitrogen	Not more than 2.6% (1)
Sulphur dioxide	Not more than 0.5% (1)
4-Methylimidazole	Not more than 250 mg/kg (1)
Total nitrogen	0.5 – 7.5% (1)
Total sulphur	1.4 – 10% (1)
Nitrogen/sulphur precipitation by alcohol ratio	0.7 – 2.7
OD precipitation by alcohol ratio (2)	8-14
OD 280/560 ratio	Not more than 50 (2)
Arsenic	Not more than 1 mg/kg
Lead	Not more than 2 mg/kg
Mercury	Not more than 1 mg/kg
Cadmium	Not more than 1 mg/kg
Heavy metals (expressed in lead)	Not more than 25 mg/kg

⁽¹⁾ Expressed by the intensity of equivalent colouring; or compared to a product with a colour intensity of 0.1 unit of absorption.

⁽²⁾ The optical densities of precipitation by alcohol is defined as the optical density of precipitation at 280 nm divided by the optical density at 560 nm (in a 1 cm space).

6. STORAGE CONDITIONS

Caramel must be stored in a closed container.

7. REFERENCES

- Directive 95/45/CE Journal officiel des Communautés européennes, L 226, 22 September 1995.

- Compendium of food additive specifications, Addendum 8, FAO Food and Nutrition Paper 52 Add.8.
Joint FAO/WHO Expert Committee on Food Additives (JECFA) ISBN 92-5-104508-9.

INTERNATIONAL OENOLOGICAL CODEX
CARBON DIOXIDE

CARBON DIOXIDE
CARBONIC ANHYDRIDE

Carbonei dioxydum

CO₂ = 44.01

SIN No. 290

(Oeno 26/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Carbon dioxide is used in gaseous form, either pure or mixed with nitrogen, in procedures designed to render inert.

2. LABELING

The label should indicate the nature and purity of the gaz, even when used in mixtures, as well as its safety and storage conditions.

3. PROPERTIES

Carbon dioxide gas is colorless and odorless. Its aqueous solution has a slightly acidic taste. At a temperature of 0 °C and under a pressure of 760 mm of mercury, 1 l of carbon dioxide weighs 1.977 g.

At a temperature of 20 °C and under a pressure of 760 mm of mercury, 1 l of water dissolves 878 ml of carbon dioxide, or 1.736 g of CO₂.

If a flame is placed in a tube of carbon dioxide, the flame is extinguished.

Fill a 50 ml test tube with carbon dioxide. Shake with 10 ml of barium hydroxide solution. A white precipitate will form, which becomes soluble with effervescence by a dilute acetic acid solution (10 pp 100) (R).

4. TESTS

Total purity of carbon dioxide should be 99 parts per 100 by volume.

Testing for and quantitative determination of gaseous impurities can be performed by gas phase chromatography. The method is described in the Annex.

Carbon dioxide determination can also be accomplished using the following chemical tests.

For the following tests, tubes containing carbon dioxide should be kept at ambient temperature for at least 6 hours prior to sampling. Volumes to be sampled are calculated by taking temperature and pressure into account, which are indicated here to be 0 °C and 760 mm of mercury.

4.1. Sulfuric Acid and Sulfur Dioxide

Let 1000 ml carbon dioxide flow, during 15 minutes at a constant speed, into 50 ml of water that has recently been boiled and cooled to room temperature. The feed tube should have an orifice whose diameter is approximately 1 mm and which is immersed to within 2 mm of the bottom of the water container which has a height of 12-14 cm. After the flow of gas is completed, pour the liquid in bucket A of a comparator and add 0.05 ml of methyl orange solution (R). To bucket B, which contains 50 ml of recently boiled and cooled water, add 1 ml of 0.01 M hydrochloric acid solution, then 0.05 ml of methyl orange solution (R). The red tint in bucket A should not be darker than that of the liquid in bucket B.

4.2. Hydrogen Sulfide, Hydrogen Phosphide, Arsine and Organic Reducing Substances

Under the same conditions as those in the preceding test, let 1000 ml of carbon dioxide flow into a mixture of 10 ml of ammoniacal silver nitrate solution (R), 3 ml of concentrated ammonium hydroxide (R) and 15 ml of distilled water. There should be no clouding or brown color as compared to an identical control solution through which no carbon dioxide gas flowed.

4.3. Oxygen

For oxygen determination tests (see « Nitrogen »), pierce the stopper of a flask with a 8/10 mm hypodermic needle (take care not to dip the needle into the liquid). This needle will allow gas to escape after bubbling. Next, insert a second hypodermic needle of the same size to feed the expanding gas into the liquid. After a minute of bubbling, there should be no significant colorating. In the presence of oxygen, the liquid will rapidly turn blue and the color become more intense over time.

4.4. Carbon Monoxide

The limiting carbon monoxide content as determined using the method described in the annex is 10µl/l/

4.5. Oil

The limiting oil content as expressed by the quantity absorbed by a suitable trap, as described in the technique described in the annex, is 0.1 mg/l.

4.6. Quantitative Analysis

Place approximately 100 ml of carbon dioxide, measured with precision, in a graduated volumetric flask turned over on a mercury

INTERNATIONAL OENOLOGICAL CODEX
CARBON DIOXIDE

tank or a graduated gas burette filled with mercury. Using a curved pipette on the mercury tank or by exerting pressure of mercury using an appropriate device, force the gas into a tube or absorber tank containing a sufficient quantity of an aqueous solution which contains 40 g of potassium hydroxide (R) per 100 ml. Shake for 5 minutes to ensure efficacious contact between the liquid and the gas. Again, feed the gas freed from the aqueous liquid to the graduated flask or the burette. Read the residual volume at the same temperature and under the same pressure as those at which the sample was measured. Once again, place the residual gas in contact with the alkaline solution and take a second residual volume reading to verify absorption was complete. There should be no more than 1 pp 100 of non-absorbable gas.

5. STORAGE

Carbon dioxide is stored in steel canisters which are painted gray. The strength of these canisters should be periodically checked.

CASEINS
(Lactic Casein or Caseina acids)
(Oeno 12/2003)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Casein, a heteroprotein containing phosphorous, is found in milk in the state of calcium salt.

It is obtained by coagulating skim milk.

It is the fining agent indicated for the treatment of oxidations in wine. It can only be used in alkaline water with potassium carbonate or potassium hydrogenocarbonate.

Casein adsorbs polyphenols, in particular oxidised polyphenols.

2. LABELLING

The concentration of casein used for the preparation must be indicated on the label including in the case of a mixture, as well as the storage conditions.

3. CHARACTERISTICS

Casein is a yellowish white coloured powder. It is amorphous, odourless and insoluble in pure water and various organic solvents. It can have a slight lactic odour. In alkaline water or in saline solutions with alkaline reactions, it swells and produces a colloidal solution: 100 ml of alkaline water for 1 g of potassium hydroxide or sodium hydroxide, dissolve 10 g of casein in a water bath at 100°C. The solution diluted 20 times its volume in water is cloudy; it should be free of lumps.

The so-called soluble caseins are mixed with pure powder and/or potassium carbonate (maximum 25%), or potassium hydrogenocarbonate).

Caseins used in oenology are fit for human consumption.

4. IDENTIFYING CHARACTERISTICS

4.1 Casein doesn't precipitate by heating its alkaline solution. This solution precipitates by acidification once the pH is less than 5.

4.2 Casein ashes contain phosphates characterised by the nitromolybdc reagent (R).

5. TEST TRIALS

Casein should have no flavour, nor abnormal odour (rotten, mouldy, putrid, etc.)

5.1 Acidity

5.1.1 Principle

Determining free acidity in casein by an acidobasic determination of an aqueous extract of the product.

5.1.2 Reagents

- Sodium hydroxide 0.1 M
- Phenolphthalein, solution at 10 g/l in ethanol

5.1.3 Procedure

Preliminary test:

- Homogenise the product by shaking vigorously;
- Put 50 g of the product on a strainer (metal mesh strainer 200 mm in diameter, nominal size of 500 µm for the opening with a receptacle (Standard ISO 3310/1);
- If 50 g of the product passes through completely, use the product as it is;
- If the 50 g of the product do not pass through, grind the product until 50 g do pass through.

During all these operations, avoid changing the water content of the product.

Preparation for the test trial solution:

- Take approximately 10 g to the nearest 10 mg of the 50 g passed through the strainer, or m of this mass.

- Put the mass m in a 250 ml conical flask.
- Pour 200 ml of recently boiled distilled water brought to 60°C into the flask.
- Shake the closed flask.
- Allow to stand for 30 minutes in a water bath at 60 °C while shaking the flask every 10 minutes.
- Filter.

The filtrate at 20°C must be clear.

Carrying out the test:

- Take 100 ml of filtrate.
- Place the test sample in a 250 ml conical flask.
- Add 0.5 ml of phenolphthalein solution to the flask.
- Titrate using 0.1 M sodium hydroxide solution.
- Let V represent the volume used.

5.1.4 Calculation

Free acidity in casein expressed in meq/l is equal to:

$$\frac{20 \cdot V \cdot T}{m}$$

- V is the volume in ml of sodium hydroxide used.
- T is the exact mole fraction of the sodium hydroxide solution.
- m is the mass density in g of the test trial sample.

Acidity expressed as lactic acid should be less than 1.6 g/l.

5.2 pH

Shake 10 g of casein in 100 ml of water for a few minutes. Decant; the pH of the solution should be less than or equal to 5 for pure casein.

5.3 Loss by dessication

Determine the weight loss of 2 g of the test trial sample by drying to constant weight at 100°C-105°C . Weight loss of casein must be less than 12%.

All the limits set below apply to dried products.

5.4 Ashes

Incinerate the residue left in the weight loss determination by dessication, without exceeding 600 °C.

The rate of the ashes should be less than 3% for casein acid and less than 11% for the casein acid and potassium carbonate or potassium hydrogenocarbonate mixture.

5.5 Preparation of test trial solution

After determining the weight of the ashes, dissolve them in 2 ml of concentrated hydrochloric acid (R) and 10 ml of water. Heat to dissolve and add water until reaching a volume equal to 25 times the weight of dried casein. 1 ml of this solution contains 0.04 g of dried casein mineral matters.

5.6 Iron

Take 10 ml of the test trial solution (5.5), and add 1 ml of concentrated hydrochloric acid (R), 3 drops of hydrogen peroxide solution at 3 volumes(R) and 2 ml of potassium thiocyanate solution at 5% (R).

If a red colouration appears, it must be lighter than the control prepared with 8 ml of iron solution (III) at 0.01 g of iron per litre (R), 2 ml of water and the same volumes of concentrated hydrochloric acid (R) and potassium thiocyanate solution at 5% (R).

Iron content should be less than 200 mg/kg.

This determination can also be carried out by atomic absorption spectrophotometry.

5.7 Lead

On the test trial solution (5.5), determine the lead according to the method described in Chapter II of the International Oenological Codex.

Lead content should be less than 5 mg/kg.

5.8 Cadmium

On the test trial solution (5.5), determine the cadmium according to the method described in Chapter II of the International Oenological Codex.

Cadmium content should be less than 1 mg/kg.

5.9 Mercury

Determine the mercury according to the method described in Chapter II of the International Oenological Codex.

Mercury content should be less than 1 mg/kg.

5.10 Arsenic

On the test trial solution (5.5), determine the arsenic according to the method described in Chapter II of the International Oenological Codex.

Arsenic content should be less than 3 mg/kg.

5.11 Total nitrogen

Introduce approximately 0.20 g of casein precisely weighed in a mineralisation flask with 15 ml of concentrated sulphuric acid (R) and 2 g of mineralisation catalyst (R) and continue the operation according to the method in chapter II of the International Oenological Codex.

Total nitrogen content must be more than 13%.

5.12 Proteins

Protein content should not be less than 82% of weight (total nitrogen 6.38).

5.13 Fat content

Determine the fat content using the gravimetric Schmid-Bondzynski-Ratslaff method (standard ISO 5543).

Fat content should be less than 2%.

5.14 Bacteriological monitoring

Proceed as indicated in chapter II of the International Oenological Codex.

Limit: total viable microorganisms: less than 3×10^4 CFU/g.

5.15 Coliforms

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Absence must be checked on a sample of 25 g.

5.16 Staphylococci

Proceed with counting according to the method in chapter II of the International Oenological Codex.

The number of staphylococci (β -hemolytiques positive coagulase) must be less than or equal to 1 per g.

5.17 *Escherichia Coli*

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Absence must be checked on a sample of 1 g.

5.18 Salmonella

Proceed with counting according to the method in chapter II of the International Oenological Codex.

The number of salmonella should be less than 1 per 100 g.

5.19 Yeasts

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Content limit: 10^3 CFU/g of preparation.

5.20 Lactic bacteria

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Content limit: 10^2 CFU/g of preparation.

5.21 *Lactobacillus sp.**

Content limit: 10 CFU/g of preparation.

5.22 *Pediococcus sp.**

Content limit: absence in a 10 g preparation sample.

5.23 Acetic bacteria

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Content limit: 10^3 CFU/g of preparation

* Method to be defined later on

5.24 Mould

Proceed with counting according to the method in chapter II of the International Œnological Codex.

Content limit: 10^3 CFU/g of preparation

6. STORAGE

Casein must be stored in watertight bags between 5°C and 20°C with relative humidity less than 65%. Its shelf life is 24 months.

7. REFERENCES

Standard ISO 5543.

CATION-EXCHANGE RESINS
(Oeno 43/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

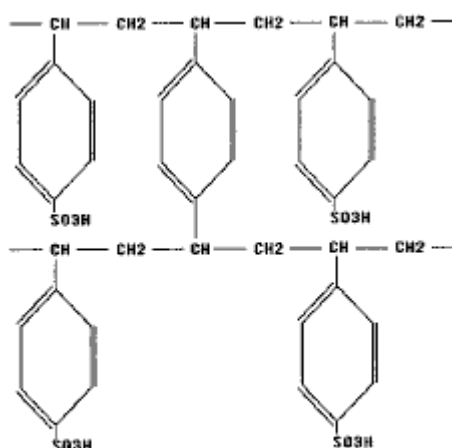
Ion exchange is the reversible exchange of ions between a liquid and a solid, during the course of which the solid does not undergo any substantial changes. When this technique is applied to wine, the solid is an insoluble, permeable synthetic resin capable of exchanging ions with the wine with which it is in contact.

These resins are used in the tartaric stabilization of wine.

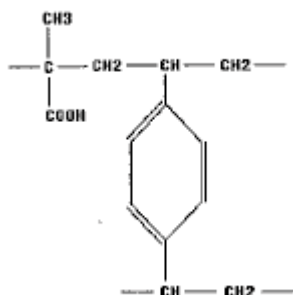
2. COMPOSITION

Cation exchange resins may be prepared in an appropriate physical form using one or more of the following formulas.

1. Sulfonated styrene-divinylbenzene copolymer:



2. Divinylbenzene-methacrylic acid copolymer:



Resin inertia must be satisfactory.

The substances which can be used in the manufacture of these resins are indicated in Annexes 1 and 2.

The resin should not contain more than 1 mg of extractable organic substance per kg. These organic extracts are obtained with each of the following solvents: a) distilled water, b) alcohol, 15% by volume, c) 3% acetic acid solution (m/m).

The resin must have been washed and conditioned in accordance with the manufacturer's instructions.

Prepare different ion exchange columns for each solvent, using 50 ml of the resin that has previously been weighed.

While maintaining the maximum temperature that may be encountered during use, pass the three solvents used in the analysis (distilled water, 15% hydroalcoholic solution and 3% acetic acid solution (m/m)) through the resins at a flow rate of 350-450 ml per hour.

The first liter of effluent from each solvent should not be considered for analytical purposes; only the following two liters of each solvent should be used to analyze the organic extracts.

Total extract : The two liter sample should be evaporated at 105 °C until a constant weight is obtained.

Ash : This dry residue derived from evaporation of the 2 liters of effluent is then burned in an oven at 850 °C until a constant weight is obtained.

Organic extract : Total extract minus total ash gives the organic extract. If the organic extract is greater than 1 ml/l of solvent used, a "blank" should be made using the solvent and a correction should be made by subtracting the organic extract found in the "blank" from that obtained during the resin test. The solvents used are prepared as follows:

Control reagents:

Distilled and/or de-ionized water.

Ethyl alcohol at 15% by volume obtained from absolute ethyl alcohol and distilled and/or de-ionized water.

3% acetic acid produced by mixing 3 parts (by mass) of acetic acid with 97 parts (by mass) of distilled and/or de-ionized water.

3. LIMITS

- The treatment must not alter the nature of the wine.
- The treatment must not reduce the color intensity of the wine.
- The treatment must not decrease the concentration of metallic cations in the wine below 300 mg/l.
- The treatment must not lower the wine's pH below 3.0. The decrease in pH should not exceed 0.3 pH units.
- The resin must not leave substances in the wine or impart to it characteristics (as a result of the resin-based treatment) that do not ordinarily exist in wine.

The winemaker may use conditioning agents and/or regenerants composed of water and inorganic acids, bases or salts, provided that the conditioned or regenerated resin is washed in water until all conditioning agents and regenerants are removed before adding the wine.

INTERNATIONAL TECHNOLOGICAL CODEX CATION-EXCHANGE RESINS

Annex 1

List of substances used in the manufacture of adsorbant ion-exchange resins
used to condition foodstuffs.

List 1

Substances assessed by an international organization

NAME	PM/REF	CASE	RESTRICTIONS
=====			
Monomers and other Starting substances			
n-butyl acrylate	10780	00141-32-2	-
Ethyl acrylate	11470	00140-88-5	-
Methyl acrylate	11710	00096-33-3	-
Acrylonitrile	12100	00107-13-1	SML = ND (DL = 0.02 mg/kg) SML = 15 mg/kg
Formaldehyde	17260	00050-00-0	-
Methyl methacrylate	21130	00080-62-6	-
Methanol	21550	00067-56-1	-
Styrene	24610	00100-42-5	-
Chemical Modifiers			
Carbonic acid, salts	42500	-	-
Hydrochloric acid	72640	07664-38-2	-
Silicic acid, salts	85980	-	-
Sulfuric acid	91920	07664-93-9	-
Acetic anhydride	10150	00108-24-7	-
tert-butyl-4-hydroxyanisole (BHA)	40720	25013-16-5	SML=30 mg/kg
Diethylene triamine	15790	00111-40-0	SML= 5 mg/kg
Dimethylamine	49225	00124-40-3	SML=0.06 mg/kg
2-(dimethylamino)ethanol	49235	00108-01-0	SML=18 mg/kg
Formaldehyde	54880	00050-00-0	SML=15 mg/kg
Hexamethylenediamine	18460	00124-09-4	SML=2.4 mg/kg
Potassium hydroxide	81600	01310-58-3	-
Sodium hydroxide	86720	01310-73-2	-
Sodium nitrite	86920	07632-00-0	SML=0.6 mg/kg
Ethylene oxide	17020	00075-21-8	MQ=1 mg/kg in FP
2-propanol	81882	00067-63-0	-
Polymerization Additives			
Alkylsulfonic acids (C ₈ -C ₂₂)	34230	-	SML=6 mg/kg
Linear, primary alkylsulfuric acids (C ₈ -C ₂₂) having an even number of carbon atoms	34281	-	-
Formic acid	55040	00064-18-6	-
Carboxymethylcellulose	42640	09000-11-7	-
Stannic chloride(IV)	93420	07646-78-8	-
Methylene chloride	66620	00075-09-2	SML=0.05 mg/kg
1,4-dihydroxybenzene	48620	00123-31-9	SML=0.6 mg/kg
Gelatin	55440	09000-70-8	-
Ammonium hydroxide	35600	01336-21-6	-
Magnesium hydroxide	64640	01309-42-8	-
Hydroxyethylcellulose	60560	09004-62-0	-
Hydroxyethylmethylcellulose	60880	09032-42-4	-
Methanol	65960	00067-56-1	-
Methylcarboxymethylcellulose	66200	37206-01-2	-
Methyl isobutyl ketone	66725	00108-10-1	SML=5 mg/kg
Toluene	93540	00108-88-3	SML=1.2 mg/kg

INTERNATIONAL TECHNOLOGICAL CODEX CATION-EXCHANGE RESINS

Annex 2

Substances that may be used provisionally to manufacture ion-exchange resins.

List 2

1. Substances not fully evaluated by an international organization

NAME	PM/REF	CASE	RESTRICTIONS
=====			
Monomers and other starting substances			
Ethylene glycol dimethacrylate	20440	00097-90-5	-
Divinylbenzene	16690	01321-74-0	-
Diallyl ether of 1,1,1-tri-methylolpropane	25645	00682-09-7	-
2,3-epoxypropyl methacrylate	20590	00106-91-2	-
2-methyl-1,3-butadiene	21640	00078-79-5	-
1,7-octadiene	22585	03710-30-3	-
1,1,1-trimethylolpropane trimethacrylate	25840	03290-92-4	-
Chemical Modifiers			
N,N-dimethyl-1,3-diamino-propane	49380	00109-55-7	-
Triethylamine	95270	00121-44-8	-
Triethylene tetramine	25520	00112-24-3	-
Polymerization Additives			
Polyvinyl alcohols	81280	09002-89-5	-
4-tert-butylcatechol	40640	00098-29-3	-
Diisobutyl ketone	49050	00108-83-8	-
Sodium hypochlorite	62110	07681-52-9	-
Isobutanol	62270	00078-83-1	-
4-methoxyphenol	66030	00150-76-5	-
Methylene bis(sodium naphthalenesulfonate)	66600	26545-58-4	-
2-methyl-2-pentanol	66860	00108-11-2	-
Dibenzoylperoxide	46440	00094-36-0	-
Partially hydrolyzed vinyl polyacetate	81260	-	-

2. Substances not evaluated by an international organization

NAME	PM/REF	CASE	RESTRICTIONS
=====			
Monomers and other starting substances			
Dimethoxymethane	-	00109-87-5	-
Diethylene glycol divinyl ether	-	00764-99-8	-
Ethyl vinyl benzene	-	28106-30-1	-
1,2,4-trivinyl cyclohexane	-	02855-27-8	-
Chemical Modifiers			
Chlorosulfonic acid	-	07790-94-5	-
Monochloroacetic acid	-	00079-11-8	-
Phosphoric acid	-	13598-36-2	-
Bromine	-	07726-95-6	-
2-chloroethanol	-	00107-07-3	-
Methyl chloride	-	00074-87-3	-
1,2-dichloroethane	-	00107-07-3	-
1,2-dichloropropane	-	00078-87-5	-
3-(dimethylamino)propane	-	03179-63-3	-

INTERNATIONAL TECHNOLOGICAL CODEX CATION-EXCHANGE RESINS

NAME	PM/REF	CASE	RESTRICTIONS
=====			
Monomers and other starting substances			
Methylic chloromethyl ether	-	00107-30-2	-
Nitrobenzene	-	00098-95-3	-
Potassium nitrite	-	07758-09-0	-
Phthalimide	-	0085-41-6	-
Sulfur trioxide	-	07446-11-9	-
Trimethylamine	-	00075-50-3	-
Polymerization additives			
Lignosulfonic acid	63940	08062-15-5	-
Peracetic acid	-	00079-21-0	-
Polyacrylic acid	76460	09003-01-4	-
Poly(styrenesulfonic) acid	-	09080-79-9	-
Acrylamide/acrylic acid copolymer	-	09003-06-9	-
Ethoxylated, propoxylated tert-alkylamines (C ₁₂ -C ₁₄)	-	68603-58-7	-
Maleic anhydride-styrene copolymer, ammonium salt	-	26022-09-3	-
Attapulgate	-	12174-11-7	-
Azobisisobutyronitrile	-	00078-67-1	-
1,1-bis(tert-butylperoxy)-3,3,5- trimethylcyclohexane	-	06731-36-8	-
n-Dodecyl mercaptan	-	25103-58-6	-
Poly(ethylene/propylene)glycol monobutyl ester	-	09038-95-3	-
Polyethylene glycol octylphenyl ether	78560	09002-93-1	-
Poly(ethylene-propylene)glycol ether with 1,1,1-trimethylol-propane	-	52624-57-4	-
tert-hexadecyl mercaptan	-	25360-09-2	-
Cumyl hydroperoxide	-	00080-15-9	-
Isododecane	62405	31807-55-3	-
Isooctane	-	26635-64-3	-
Mono- and dialkyl (C ₁₀ -C ₁₈) Sulfonamides	-	-	-
Silver nitrate	-	07761-88-8	-
n-Octane	-	00111-65-9	-
tert-Butyl peracetate	-	00107-71-1	-
tert-Butyl perbenzoate	-	00614-45-9	-
bis(4-tert-butylcyclohexyloxy) percarbonate tert-	-	15520-11-3	-
Butyl per(2-ethyl-hexanoate)	-	03006-82-6	-
tert-Butyl peroctanoate	-	13467-82-8	-
Dilauroyl peroxide	-	00105-74-8	-
Poly(diallyldimethylammonium chloride)	-	26062-79-3	-
Polyvinylpyrrolidone	81500	09003-39-8	-
=====			

CELLULOSE
(C₁₂ H₂₀ O₁₀)_n
INS N°: 460
(Oeno 8/2002)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Cellulose is obtained from mechanical processing and purification from an alpha-cellulose, which comes directly from vegetable fibres. Its molecular weight is $1.5 \cdot 10^5$ Dalton. Cellulose fibre is used for its absorbency traits, mainly for the filtration of wine.

2. LABELLING

The concentration of the product and whether it was mixed, must be indicated on the label in addition to the change.

3. CHARACTERISTICS

Cellulose is a white odourless, flavourless, fibre. It is insoluble in water.

4. TEST TRIALS

4.1 pH

Mix 5g of cellulose in 40 ml of water free of carbon dioxide, for 20 minutes. Centrifuge. The pH of the supernatant will be between 5.0 and 7.5.

4.2 Humidity and volatile matter

Put 5 g of cellulose in an incubator at 105°C for 3 hours.
Mass loss must not exceed 8%.

All of the maximum limits set below refer to the dried product.

4.3 Starch

Add 90 ml of water (R) to 10 g of microcrystalline cellulose and boil for 5 minutes. Filter when hot. Cool and add 0.1 ml of 0.05 M iodine to the filtrate. A blue colour should not appear.

4.4 Ashes

Incinerate at $600 \pm 25^{\circ}\text{C}$ the residue obtained according to point 4.2, for 4 hours. The weight of the ashes should not exceed 2%.

4.5 Preparation of the test solution

After weighing, dissolve ashes in 2 ml of concentrated hydrochloric acid (R) and 10 ml of water (R). Heat in order to dissolve and fill the water up to 50 ml. (R).

4.6 Iron

Determine iron using an atomic absorption spectrophotometer (following the method described in Chapter II on the test solution (4.5).

Iron content must be less than 100 mg/kg.

4.7 Lead

Measure out lead following the method described in Chapter II on the test solution (4.5). Lead content must be less than 5 mg/kg.

4.8 Mercury

Measure out mercury following the method described in Chapter II on the test solution (4.5).

Mercury content must be less than 1 mg/kg.

4.9 Cadmium

Measure out cadmium as described in Chapter II on the test solution (4.5).

Cadmium content must be less than 1 mg/kg.

4.10 Arsenic

Measure out arsenic following the method described in Chapter II on the test solution (4.5).

Arsenic content must be less than 2 mg/kg.

4.11 Calcium

Determine calcium using an atomic absorption spectrophotometer (see method described in Chapter II on the test solution (4.5). Calcium content must be less than 500 mg/kg.

4.12 Water soluble substances

Evaporate the aliquot part of the supernatant obtained when measuring the pH level at point 4.1, in an incubator at 105°C for 3 hours. The soluble substance content should not exceed 0.25%.

5. STORING CONDITIONS

Cellulose should be kept in a well-ventilated place in sealed packages away from volatile substances susceptible of being adsorbed.

CITRIC ACID, MONOHYDRATE
Monohydrated 3-Carboxy-3-hydroxypentanedioic acid
Acidum citricum
C₆H₈O₇·H₂O = 210.1
SIN NO. 330
(Oeno 23/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Citric acid can be used to chemically acidify wines or as a stabilizing agent to limit, in particular, the risks of iron breakdown, or again, for prewashing filter plates. Its maximum proportions in wine may be subject to statutory limits.

2. LABELING

The label should indicate product concentration, even when included in mixtures, as well as its safety and storage conditions.

3. PROPERTIES

Citric acid is found in the form of colorless, translucent crystals which are rather friable and slightly efflorescent, or in crystalline powder form.

$$\begin{array}{ccc} & 20^{\circ}\text{C} & \\ \text{D} & & = 1.542 \\ & 4^{\circ}\text{C} & \end{array}$$

4. SOLUBILITY

Water at 20 °C	very soluble
Alcohol, 95% by vol.	very soluble
Glycerol	very soluble
Ethyl ether	31.5 g/l

Aqueous citric acid is inert in polarized light.

5. IDENTIFYING CHARACTERISTICS

5.1. Verify total solubility in water. A 1 pp 100 solution (m/v) shows an acid reaction to methyl orange (R).

5.2. Place 2 ml of an aqueous 1 g/l citric acid solution and 0.5 ml of mercury (II) sulfate solution (R) in a test tube. Bring to a boil and add several drops of 2 pp 100 potassium permanganate solution (R). A white precipitate should form.

5.3. Add 1 drop of bromine water (R), 3 drops of concentrated sulfuric acid (R) and 1 drop of saturated potassium permanganate solution to 0.1 ml of 10 pp 100 (m/v) aqueous citric acid solution. Bring to a boil.

Add 2 ml of concentrated sulfuric acid (R). Heat again until completely dissolved. Let cool, then add 0.1 ml of beta-naphthol (R). A green coloring should appear. A pink coloring is obtained under the same conditions if sulforesorcin reagent (R) is used under the same conditions.

5.4. Place 5 ml of chloroform or dichloromethane in a test tube. Add 100-200 mg of citric acid. Shake. The crystals or crystalline powder should collect together at the surface of the liquid. Under these same conditions, tartaric acid collects at the very bottom of the tube.

6. TESTS

6.1. Foreign Substances

Citric acid should be soluble without residue in its weight of water and in twice its weight of 95% alcohol (by volume).

6.2. Sulfur Ash

After calcination at $600\text{ }^{\circ}\text{C} \pm 25\text{ }^{\circ}\text{C}$, the concentration of sulfur ash should not be greater than 0.5 g/kg.

6.3. Tartaric Acid Determination

Add 2 drops of sulforesorcinic reagent (R) and 2 drops of 10 pp 100 (m/v) citric acid solution to 2 ml of concentrated sulfuric acid (R). Heat to $150\text{ }^{\circ}\text{C}$. The solution should not develop a violet coloring.

6.4. Preparing the Solution for Tests

Prepare a 10 parts per 100 (m/v) solution.

6.5. Chlorides

Add 14.5 ml of water, 5 ml of nitric acid diluted to 10 pp 100 (R) and 0.5 ml of 5 pp 100 silver nitrate solution (R) to 0.5 ml of the solution prepared for tests under paragraph 6.4. After sitting for 15 minutes in the dark, there should be no clouding. If clouding does occur, it should be less intense than that observed in a control prepared as indicated in the Annex. (Chloride content expressed in terms of hydrochloric acid should be less than 1 g/kg).

6.6. Sulfates

Add 18 ml of water, 1 ml of diluted hydrochloric acid (R) and 2 ml of 10 pp 100 barium chloride solution diluted to 10 pp 100 (R) to 1 ml of the solution prepared for tests under paragraph 6.4. After 15 minutes, there should be no clouding. If clouding does occur, it should be less intense than that observed in a control prepared by replacing the test solution with 1 ml of 0.1 g/l sulfuric acid solution. (Sulfate content expressed in terms of sulfuric acid should be less than 1 g/kg).

6.7. Oxalic Acid and Barium

Neutralize 5 ml of the solution prepared for tests under paragraph 6.4 by adding concentrated ammonium hydroxide (R). Add 2 drops of acetic acid (R) and 5 ml of saturated calcium sulfate solution (R). There should be no clouding. (Oxalate content expressed in terms of oxalic acid should be less than 0.1g/kg).

6.8. Iron

Add 1 ml of concentrated hydrochloric acid (R) and 2 ml of 5 pp 100 potassium thiocyanate solution (R) to 10 ml of the solution prepared for tests under paragraph 6.4. The resulting red coloration should be less intense than that observed in a control using 1 ml of iron (III) salt solution in a concentration of 0.010 g of iron per liter, 9 ml of water and the same quantities of the same reagents. (Iron content should be less than 10 mg/kg).

Iron may also be analytically quantified by atomic absorption spectrometry in accordance with the technique detailed in the Compendium.

6.9. Cadmium

Using the method described in the Annex, quantify cadmium analytically in the test solution prepared according to Par. 6.4. (Cadmium content should be less than 1 mg/kg).

6.10. Lead

Using the method described in the Compendium, determine lead content analytically in the test solution prepared according to Par. 6.4. (Lead content should be less than 1 mg/kg).

6.11. Mercury

Using the method described in the Annex, determine the mercury content analytically in the test solution prepared according to Par. 6.4. (Mercury content should be less than 1 mg/kg).

6.12. Arsenic

Using the method described in the Annex, determine the arsenic content analytically in the test solution prepared according to Par. 6.4. (Arsenic content should be less than 1 mg/kg).

7. STORAGE

Citric acid should be stored in a dry place in air-tight bags.

COLLOIDAL SILICON DIOXIDE SOLUTION
Silica colloidalis solutio
SILICON GEL IN AQUEOUS DISPERSION
(Oeno 44/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Colloidal silicon dioxide solutions are aqueous dispersions of silicon dioxide particles which are hydroxylated on the surface and are, therefore, negatively charged.

These preparations are used to clarify wines and are associated with protein-based clarifying agents.

2. LABELING

The label should indicate silicon dioxide concentration and its safety and storage conditions.

3. PROPERTIES

Depending on the manner in which they are prepared, acidic or alkaline solutions are obtained containing sodium ions expressed as NaO₂. Alkaline solutions are most often used.

Colloidal silicon dioxide solutions are free from organic compounds.

Their concentration as determined by drying at 110 °C is always equal to or greater than 15 pp 100 (m/m) and is most often between 15 and 30.7.

The density of colloidal silicon dioxide solutions at 20 °C ($\rho_{20^{\circ}\text{C}}$) is given as a function of the concentration C (m/m) by the equation:

$$\rho_{20^{\circ}\text{C}} = \rho_{20^{\circ}\text{C}}(\text{water}) \times 1/1 - 0.0056C$$

$$\rho_{20^{\circ}\text{C}}(\text{water}) = \text{density of water at } 20^{\circ}\text{C} = 0.998203.$$

These preparations are sold in the form of opalescent or milky liquids with slightly bluish tints, or in gel form.

4. TESTS

4.1. The solution should have no disagreeable odor or taste.

4.2. pH

Depending on the preparation method and on whether acidic or alkaline solutions are employed, pH should be between 3 and 4 or between 8 and 10.5.

4.3. Silicon Dioxide Concentration (Dry Extract at 110 °C)

The weight, P, of the dry residue expressed in g per 100 g of product should correspond to within ± 0.5 g of the product's concentration.

4.4. Alkalinity

For alkaline colloidal solutions, determine the alkalinity of a 5 g sample using 0.1M hydrochloric acid (R) in the presence of 2 drops of methyl orange solution (R). Alkalinity expressed in terms of Na₂O for 100 g of product should be less than P/100.

4.5. Preparing the Solution for Tests

Place a volume of colloidal silicon dioxide solution corresponding to 10 g of dry extract in a platinum dish 7 cm in diameter and 2.5 cm high. Evaporate until dry. Take up after cooling with 5 ml fluorhydric acid. Dry evaporate. Repeat this procedure until the silicon dioxide residue is eliminated. Dry evaporate. Take up using 2 ml concentrated hydrochloric acid (R) and dry evaporate. Add 2 ml of concentrated hydrochloric acid (R). Decant in a 50 ml volumetric flask and fill to the line with distilled water.

4.6. Heavy Metals

To 5 ml of the test solution prepared under paragraph 4.5, add 5 ml of water, 2 ml of pH 3.5 buffer solution (R) and 1.2 ml of thioacetamide reagent (R).

No precipitate should form. If a color appears it should be less intense than that of a control prepared as indicated in the Annex and filled to a volume of 25 ml. (Heavy metal content, expressed in terms of lead in dry extract form, should be less than 10 mg/kg).

4.7. Lead

Using the technique described in the Compendium, determine lead content in the test solution (4.5). (Lead content to be less than 5 mg/kg.)

4.8. Mercury

Using the technique described in the annex, determine mercury content in the test solution (4.5). (Content to be less than 5 mg/kg.)

4.9. Arsenic

Using the technique described in the annex, determine arsenic content in the test solution (4.5). (Content to be less than 3 mg/kg.)

4.10. Methanol

Place 50 ml of colloidal silicon dioxide solution in a 200 ml in a balloon. Distill and collect 50 ml of distillate.

Place 1 ml of distillate in a test tube with 4 drops of 50 pp 100 (m/m) orthophosphoric acid (R) and 4 drops of 5 pp 100 (m/v) potassium permanganate solution (R). Stir and let sit 10 minutes. Decolor the permanganate with several drops (typically 8) of 2 pp 100 (m/v) of potassium anhydrous sulfite (R), while avoiding any excess. Add 5 ml of chromotropic sulfuric acid (R). Place in a 70 °C water bath for 20 minutes. No violet coloration should appear.

4.11. Formaldehyde

Place 10 ml of the distillate obtained under paragraph 4.10 in a test tube. Add 1 ml of rosaniline chlorhydrate bleach out using sulfuric acid (R). No pink coloration should appear.

5. STORAGE

Colloidal solutions of silicon dioxide should be stored in hermetically sealed containers away from contaminants and at temperatures of above 0 °C (the product freezes at 0 °C with irreversible precipitation of the silicon dioxide).

COPPER SULFATE, PENTAHYDRATE
Copper (II) Sulfate, Pentahydrate
 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O} = 249.68$
(Oeno 25/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Copper sulfate is used in processing wines possessing so-called reduction "tastes" due to the presence of hydrogen sulfide or volatile thiols.

The copper sulfides thus formed precipitate and should be removed from wine.

This product must be used in compliance with copper sulfate pentahydrate limiting quantities; furthermore, there are statutory limits restricting the copper content in wines.

2. LABELING

The label should indicate product concentration, even when used in mixtures, as well as its safety and storage conditions.

3. PROPERTIES

Blue crystals which are minimally efflorescent in dry air.

4. COMPOSITION

Minimum of 99 pp 100 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

5. IDENTIFYING CHARACTERISTICS

Melting point: 110 °C with water loss.

An aqueous solution with ammonium hydroxide (R) produces a dark blue copper tetramine compound. A solution acidified with hydrochloric acid and a barium chloride solution (R) produces a white barium sulfate precipitate.

6. SOLUBILITY

Water at 20 °C 286 g/l

Methanol 15.6 g/l

Insoluble in ethanol (95% alcohol by volume).

7. TESTS

7.1. Preparing the Solution for Tests

Dissolve 10 g of the substance in water and fill to 50 ml.

7.2. Appearance of the Test Solution

The test solution must be clear.

7.3. Iron

Place 2 ml of the solution prepared for tests under paragraph 7.1 in a decanting glass and add 8 ml of water, 10 ml of 6M hydrochloric acid (R) and 10 ml of 4-methylpentane-2-one. Shake vigorously for 3 minutes. After letting the mixture settle, decant the organic phase in a second decanting glass. Add 10 ml of water then shake vigorously again for 3 minutes. Separate out the aqueous phase and perform the test in the following manner:

Add 2 ml of citric acid solution (20 g of citric acid/100 ml), 0.10 ml of concentrated thioglycolic acid ($\text{HS-CH}_2\text{-COOH}$) and a small amount of 6M ammonium hydroxide (10-10.4 g NH_3 /100 ml) to the aqueous phase until an alkaline reaction is triggered. Dilute with water until a total volume of 20 ml is reached. After 5 minutes, the sample should not be more intensely colored than the test carried out using the comparison solution described below.

7.3.1 Preparation of the Comparison Solution

Iron (III) and ammonium sulfate solution 1

Dissolve 0.702 g of ammonium sulfate and iron (III) in 1.20 ml of 6M hydrochloric acid and fill to 100 ml with water.

Iron (III) and ammonium sulfate solution 2

Take 7 ml of ammonium sulfate and iron (III) (Par. 7.3.1.1) and fill to 100 ml with water.

1 ml of solution 2 corresponds to 10 μg of Fe(III).

7.3.2 Test Using the Comparison solution

The comparison solution should be prepared prior to use in the following manner:

Take 1 ml of ammonium sulfate and iron (III) solution (2) and process in the same way as for the substance test.

N.B. : Iron content can also be analyzed by atomic absorption spectrometry, using the method described in the Compendium.

The iron content limit is 100 mg/kg.

7.4. Nickel

Add 2 ml of concentrated hydrochloric acid (R) and 1 ml of concentrated nitric acid (R) to the aqueous phase from paragraph 7.3.

After evaporating the solution, dissolve the residue in 1 ml of 6M nitric acid (R) and 19 ml of water. Dilute 1 ml of this solution to a total volume of 10 ml. To 2.50 ml of this dilute solution, add 6 ml of water (R), 5 ml bromine solution (R), 7 ml of 6M ammonium hydroxide solution and 3 ml of dimethylgloxime solution in a concentration of 100 g in 100 ml of 96% ethanol by volume. The solution should not exhibit any change after one minute when compared to a "blank" sample.

Nickel content can also be determined using the atomic absorption photometry method described in the Annex.

7.5. Chlorides

Dilute 25 ml of the solution prepared for tests under paragraph 7.1 with 10 ml of water. After adding 8 ml of 6M sodium hydroxide, bring to a boil and heat the mixture in a 100 °C water bath until the precipitate has been completely deposited. After cooling, dilute with water to obtain a total volume of 50 ml. Add 6 ml of water to 4 ml of filtrate and conduct the following test: add 1 ml of 6M nitric acid (R) and 1 ml of 0.1M silver nitrate (R). Shake the sample after 5 minutes. There should be no more clouding than that seen in the control test conducted with the comparison solution.

(Limiting value : 100 mg/kg.)

7.5.1 Preparation of the Comparison Solution

Dilute 4 ml of 0.1M sodium chloride solution (23.4 ml/100 ml) with water to yield a total volume of 100 ml. 1 ml corresponds to 142 µg Cl⁻. Prepare the solution just before use.

7.5.2 Control Test with the Comparison Solution

Take 1 ml of the sodium chloride solution (Par. 7.5.1) and proceed in the same manner as for the test on the substance.

7.6. Lead

Using the technique explained in the Compendium, determine the lead content in the test solution (Par. 7.1). (Lead content should be less than 5 mg/kg).

7.7. Mercury

Using the technique explained in the annex, determine the mercury content in the test solution (Par. 7.1). Mercury content should be less than 1 mg/kg.

7.8. Arsenic

Using the technique explained in the annex, determine the arsenic content in the test solution (Par. 7.1). (Arsenic content should be less than 3 mg/kg).

7.9. Quantitative Analysis

Weigh exactly 0.50 g of the substance and dissolve in 20 ml of water. Add 5 ml 6M acetic acid and 2 g potassium iodide. Titrate with a 0.1M sodium thiosulfate solution in the presence of starch (R).

1 ml of a 0.1M sodium thiosulfate solution which corresponds to 6.354 mg of Cu(II), or, if the result is expressed in terms of substance, to 24.97 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

8. STORAGE

Copper sulfate should be stored in a dry place in hermetically sealed containers.

D,L-TARTARIC ACID
D,L-2,3-dihydroxybutanedioic Acid
Racemic Acid
Acidum tartaricum
COOH - CHOH - CHOH - COOH
C₄H₆O₆ = 150.1
(Oeno 48/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This product is used to eliminate excess calcium from wines and musts under certain conditions. The calcium racemate that is produced yields particularly insoluble salts. Its use is subject to certain regulations.

2. LABELING

The label should cite the purity percentage and storage requirements.

It should also clearly indicate that the product is a racemic mixture of the two isomers D and L of tartaric acid, so as to avoid suggesting that the product is the L-tartaric acid occurring naturally in grapes.

3. PROPERTIES

Colorless, transparent extremely solid crystals having a distinctly acidic taste. Instantaneous melting point is 170 °C.

4. SOLUBILITY

Water at 20 °C	245 g/l
Water at 100 °C	1428 g/l
Alcohol, 95% by vol.	26 g/l
Ethyl ether	14.9 g/l

5. IDENTIFYING CHARACTERISTICS

5.1. Verify total solubility in water. A 1% solution exhibits an acidic reaction with respect to methyl orange (R). This solution has no rotatory power.

5.2. Add 2 ml of 25% calcium acetate solution (R) to 5 ml of 1% (m/v) solution. An abundant white crystalline precipitate should form instantaneously. Under these conditions, L(+) tartaric acid (dextrorotatory tartaric acid) yields no precipitate.

5.3. Add 2 ml 5% potassium acetate solution (R) to 5 ml of 10% (m/v) solution. A crystalline precipitate will form.

6. TESTS

6.1. Foreign Substances

D,L tartaric acid should be soluble without residue in 10 times its weight of water.

6.2. Sulfur Ash

As analyzed in 2.0 g D,L tartaric acid, the sulfur ash content should not be greater than 0.2 pp 100.

6.3. Preparing the Solution for Tests

Dissolve 10 g D,L-tartaric acid in water and fill to 100 ml with the same solvent.

6.4. Citric Acid

Add 5 ml of water and 2 ml mercury (II) sulfate solution (R) to 5 ml of the solution prepared for tests under Paragraph 6.3. Bring to a boil and add several drops potassium permanganate solution (concentration: 2 pp 100) (R). No white precipitate should form.

6.5. Chlorides

Add 14.5 ml of water, 5 ml of dilute nitric acid (R) and 0.5 ml of 5 pp 100 silver nitrate solution (R) to 0.5 ml of the solution prepared for tests under Paragraph 6.3. The solution should meet the chloride limit test described in the Annex. (Chloride content expressed as hydrochloric acid should be less than 1 g/kg).

6.6. Iron

Add 1 ml of concentrated hydrochloric acid (R) and 2 ml of 5 pp 100 potassium thiocyanate solution (R) to 10 ml of the solution prepared for tests under paragraph 6.3. The resulting red coloration should be less intense than that observed in a control prepared using 1 ml of an iron (III) salt solution in a concentration of 0.010 g of iron per liter, 9 ml of water and the same quantities of the same reagents. (Iron content should be less than 10 mg/kg).

Iron content may also be determined by atomic absorption spectrometry in accordance with the method described in the Compendium.

6.7. Lead

Use the method described in the Compendium to analyze the test solution (6.3). (Content to be less than 5 mg/kg.)

6.8. Mercury

Using the technique described in the Annex, determine the proportion of mercury in the test solution (6.3). (Mercury content should be less than 1 mg/kg).

6.9. Arsenic

Using the technique described in the Annex, determine the proportion of arsenic in the test solution (6.3). (Arsenic content should be less than 3 mg/kg).

6.10. Sulfates

Add 18 ml of water, 1 ml hydrochloric acid diluted to 10 pp 100 (R) and 2 ml of 10 pp 100 barium chloride solution (R) to 1 ml of the solution prepared for tests under Paragraph 6.3. The solution should meet the sulfate limit test described in the Annex. (Concentration of sulfate expressed as sulfuric acid should be less than 1 g/kg).

6.11. Oxalate

Using the technique described in the Annex, determine the proportion of oxalate in the test solution (6.3). (Oxalate content expressed as oxalic acid should be less than 100 mg/kg after dessiccation).

7. QUANTITATIVE ANALYSIS

Dissolve a precisely-weighed sample **p** of approximately 1 g D,L-tartaric acid in 10 ml of water. Titrate with 1M sodium hydroxide solution in the presence of phenolphthalein (R). Let **n** be the number of milliliters used.

1 ml of 1M sodium hydroxide solution corresponds to 0.075 g D,L-tartaric acid.

Content, in percent, of D,L-tartaric acid of the product tested: $7.5 \text{ } n$.

Products used in wine-making must contain a minimum of 99 pp 100 D,L-tartaric acid (dry product).

8. STORAGE

D,L-tartaric acid should be stored in hermetically sealed containers.

DIAMMONIUM HYDROGEN PHOSPHATE
AMMONIUM HYDROGEN PHOSPHATE

Ammonii phosphas
(NH₄)₂HPO₄ = 132.1

SIN NO. 342

(Oeno 15/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This product is used as a fermentation activator and is reserved for fermentation operations. It makes available ammonium ions, which can be directly assimilated by the yeast. Excess phosphates can lead to iron breakdown.

Statutory provisions limit the amount of ammonium that can be added.

2. LABELING

The concentration of this product should be indicated on the label, including cases of mixtures. In addition, safety and storage conditions should also be stipulated.

3. CENTESIMAL COMPOSITION

H₃PO₄ 74.21

P₂O₅ 53.75

NH₃ 25.79

4. PROPERTIES

Colorless, monoclinic crystals. This salt slowly loses small quantities of ammonia in air.

5. SOLUBILITY

Water at 20 °C 689 g/l

Water at 100 °C 1060 g/l

Alcohol, 95% by vol. insoluble

6. IDENTIFYING CHARACTERISTICS

6.1. Prepare a 1 pp 100 (m/v) solution in water. The solution has a pH of approximately 8, and a slight pink color is produced with several drops of phenolphthalein (R). At 25 °C, the pH of this solution should be between 7.8 and 8.4.

6.2. This solution produces a yellow precipitate with a nitro-molybdic reagent (R).

6.3. When heated with several drops of 30% sodium hydroxide solution (R), this solution releases ammonia.

7. TESTS

7.1. Sulfur Ash

Quantified as indicated in the Annex, the proportion of diammonium phosphate ash should not be greater than 5 g/kg.

7.2. Preparing the solution for tests

Prepare a 10 pp 100 (m/v) solution.

7.3. Chlorides

To 0.5 ml of the solution prepared for testing under Paragraph 7.2, add 14.5 ml of water, 5 ml of nitric acid diluted to 10 pp 100 (R) and 0.5 ml of 5 pp 100 silver nitrate solution (R). After 15 minutes at rest in the dark, there should be no clouding, or any clouding visible should be less intense than that observed in the control prepared as detailed in the annex. (Hydrochloric acid content is less than 1 g/kg).

7.4. Sulfates

To 1 ml of solution prepared for tests under paragraph 7.2, add 2 ml of dilute hydrochloric acid (R), 17 ml of water and 2 ml of barium chloride solution (R). The mixture must not form any precipitate or any opalescence ; or else, any opalescence that does occur should be less intense than that observed in the control prepared as indicated in the Annex. (Sulfuric acid content should be less than 1 g/kg).

7.5. Oxalic acid

To 5 ml of solution prepared for tests under paragraph 7.2, add 20 drops of acetic acid (R) and 5 ml of solution saturated with calcium sulfate (R). The solution should remain clear.

7.6. Iron

To the 5 ml of solution prepared under paragraph 2, add 1 ml of concentrated hydrochloric acid (R) and 1 ml of 5 pp 100 potassium thiocyanate solution (R).

Coloring should be less intense than that of a control prepared with 2.5 ml of an iron solution in a concentration of 10 mg of iron per liter (R), 2.5 ml of water and the same quantities of the same reagents. (Iron content should be less than 50 mg/kg.)

Iron may also be analytically quantified by atomic absorption spectrometry, according to the method specified in the Compendium.

7.7. Lead

By implementing the method detailed in the Compendium, carry out quantitative analysis of the solution prepared for testing according to Paragraph 7.2. (Lead content should be less than 5 mg/kg).

7.8. Mercury

Test for mercury in the solution prepared for testing (Par. 7.2), in accordance with the method detailed in the Compendium. (Mercury content should be less than 1 mg/kg.)

7.9. Arsenic

Using the method indicated in the Annex, test for arsenic in 2 ml of the test solution prepared in accordance with paragraph 7.2. (Arsenic content should be less than 3 mg/kg.)

7.10. Quantitative Ammonia Analysis

Dilute the solution prepared under Paragraph 7.2 to one-tenth strength, then place 10 ml of this dilute solution (0.10 g of ammonium phosphate) in a steam distillation device (described in the Annex). Add 10 ml of water, 10 ml of 30% sodium hydroxide (R) and distill 10 ml. Analytically quantify the distilled ammonia using 0.1 M hydrochloric acid. Let n be the number of milliliters used:

100 g of ammonium phosphate contains $1.7 n$ g of ammonia (NH_3).
(Minimum content is 25 pp 100).

7.11. Quantitative Analysis of Phosphoric Acid

Place 25 ml of the solution prepared under paragraph 7.2 in a conical flask. Add 5 drops of phenolphthalein (R). The solution should have a pale pink color. If not, add just enough 0.1 M sodium hydroxide solution to cause incipient movement of the indicator. Add 10 drops of bromocresol green (R) and use a burette to pour 0.5 M sulfuric acid until the indicator turns green.

Let n be the volume in ml used:

One liter of 0.5 M solution corresponds to 71 g of phosphoric anhydride or 98 g of phosphoric acid.

Proportion of ammonium phosphate in g per 100 g :

- | | |
|---------------------------|----------|
| - in phosphoric anhydride | $2.84 n$ |
| - in phosphoric acid | $3.92 n$ |

The proportion of phosphoric anhydride must range between 51.6 and 55 pp 100, or between 71.5 and 76 pp 100 of phosphoric acid.

8. STORAGE

Ammonium phosphate must be stored away from moisture and heat, and in hermetically sealed containers.

DIATOMITE
Kieselguhr
Terra silicea
(Oeno 10/2002)

1. OBJECT, ORIGIN AND FIELD APPLICATION

Diatomite is a sedimentary rock made up of siliceous shells (tests) of diatomite fossils (unicellular microscopic algae). For enological purposes, this rock is crushed, dried, shredded, purified through cleaning, and calcinated at a high temperature of 950 to 1000°C. Melted alkali can be added during the calcination process.

It is used when pulverised with a granulometer between 5 to 40 microns and can be found in a pink powder form for calcinated products or white for calcinated activated products.

Diatomite is a filter aid for musts and wine. The usage of diatomite requires wearing a protective mask.

2. LABELLING

The label must indicate granulometry, permeability, the specifications of accompanying documents in addition to the storage and safety conditions.

3. TEST TRIALS

3.1 Odour and taste

Diatomite should not carry any odour or foreign taste to the wine. Put 2.5 g of diatomite in a litre of wine. Mix. Leave 24 hours. Compare the taste to wine not containing any diatomite.

3.2 Loss during drying

Put 5 g of diatomite in a capsule. Heat in an incubator to 103 ± 2°C. After two hours mass loss should not be more than 1%.

3.3 Loss through calcinations

Bring the dry residue obtained in point 3.2 to 550°C in a furnace. Weight loss should not be more than 3%.

3.4 Measure pH level

In a 250 ml container put approximately 10 g of diatomite, then slowly add 100 ml of water to moisten the product and to get a homogeneous suspension. Mix by hand from time to time using a magnetic mixer. After 10 minutes, let the suspension settle and measure the pH. Calcinated diatomites (pink) have a pH level between 5 to 7.5 and activated calcinated diatomites (white) have a pH level between 6 to 10.5.

3.5 Soluble products in diluted acids

Bring to a boil 10 g of dried diatomite with 20 ml of concentrated hydrochloric acid (R) and 100 ml of water. Collect the diatomite on an ashless filter paper and wash the residue with 100 ml of distilled water. After desiccation at 100-105°C and incineration, separate the filter of insoluble residue, which should weigh at least 9.8 g and constitute 98% of the dried product.

3.6 Preparation of test solution

In a 500 ml flask, which can be hermetically sealed, put 200 ml of citric acid solution at 5 g per litre bring to pH 3 (R) and 10 g of diatomite. Put this in a magnetic mixer and mix for 1 hour at a temperature of $20 \pm 2^\circ\text{C}$. Allow to settle and filter by eliminating the first 50 ml of filtrate. Collect at least 100 ml of clear liquid.

3.7 Iron

On the test solution prepared according to point 3.6, determine the iron following the procedure described in Chapter II. Iron content must be less than 300 mg/kg.

3.8 Lead

On the test solution prepared according to point 3.6, determine the lead following the procedure described in Chapter II. Lead content must be less than 5 mg/kg.

3.9 Mercury

On the test solution prepared according to point 3.6, determine the mercury following the procedure described in Chapter II. Mercury content must be less than 1 mg/kg.

3.10 Arsenic

On 4 ml of test solution prepared according to point 3.6, determine the arsenic following the procedure described in Chapter II. Arsenic content must be less than 3 mg/kg.

4. STORING CONDITIONS

Diatomite must be stored in dry well ventilated places or in vacuumed packed sealed bags in a temperate place.

DIMETHYL DICARBONATE (DMDC)
Dimethyl pyrocarbonate
(Oeno 25/2004)

N° SIN = 242

C.A.S 004-525-33-1

EINECS 224-859-8

Chemical formula: $C_4H_6O_5$
 $H_3C-O-(C=O)-O-(C=O)-O-CH_3,$

Molecular weight 134.09

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Antiseptic mainly active against yeast. Synthetic product.

2. LABELLING

The name "Dimethyl dicarbonate", the batch number, the date of expiry, storage temperature (20°C–30°C) and safety precautions must be indicated on the label.

3. CHARACTERISTICS

Colourless liquid that decomposes in an aqueous solution. Corrosive for skin and eyes. Toxic in case of inhalation and ingestion.

After dilution in water, CO_2 is formed which can be characterised.

Melting point: 17°C.

Boiling point: 172°C with decomposition.

Density at 20°C: about 1.25.

Infrared spectrum: maximum absorption at 1156 nm and 1832 nm.

4 CHARACTERISATION

4.1 Principle of the method

The sample is mixed with an excess of dibutylamine with which it reacts directly. The excess of amine is determined by back titration.

4.2 Apparatus

- 4.2.1 150 ml cylindrical vase
- 4.2.2 100 ml graduated test tube
- 4.2.3 20 ml pipette
- 4.2.4 Glass electrode/reference electrode
- 4.2.5 pH metre
- 4.2.6 20 ml plunger burette
- 4.2.7 Magnetic stirrer
- 4.2.8 2 ml disposable syringe.

4.3 Reagents

- 4.3.1 Pure acetone
- 4.3.2 Dibutylamine solution [$C_8H_{19}N$] = 1 mole/l
Weigh 128 g of dibutylamine into a 1 l volumetric flask and fill to the mark with chlorobenzene.
- 4.3.4 Molar hydrochloric acid solution [HCl] = 1 mole/l
Determine the mass concentration by titration with sodium carbonate. Titre: t
- 4.3.5 Anhydrous sodium carbonate, dried in incubator at 110°C.

4.4 Procedure

Pour about 70 ml of acetone (4.3.1) in a 150 ml cylindrical vase.
Place a cylindrical vase (4.2.1) and introduce 1.0 to 1.3 g (W) of sample by using a disposable syringe (4.2.8) (precision of ± 0.1 mg).

Add exactly 20 ml of the dibutylamine solution (4.3.2) using a pipette (4.2.3) and shake vigorously.

4.4.1 Titrate by potentiometry the excess of amine with hydrochloric acid (4.3.4).

Consumption of HCl solution = V1 ml.

4.4.2 Perform a control trial according to 4.4 but without adding the sample.

Consumption of HCl solution = V2 ml.

4.5 Result

$$\frac{(V2-V1) \cdot t \cdot 134.1 \cdot 100}{1000 \cdot W} = \frac{(V2-V1) \cdot t \cdot 13.41}{W} = \% \text{ dimethyl dicarbonate}$$

DMDC content should be more than or equal to 99.8%.

5. DMDC HEAVY METAL, CONTENT (EXPRESSED IN LEAD), MERCURY AND CHLORIDE

5.1 Buffer solution, pH= 3.5 Dissolve 6.25 of ammonium acetate in 6 ml water. Add 6.4 ml of hydrochloric acid and dilute water to 25 ml.

5.2 Solution for trials: Pour 5 ml of buffer solution in a conical flask, 25.0 g of sample and approximately 15 ml of water. Let the sample hydrolyze for 3 days, shaking from time to time. Transfer the solution to a 50 ml graduated cylinder and fill up with water to indicator.

5.3 Heavy metals

Determine the heavy metal content according to the method in chapter II of the International Oenological Codex.

The contents of heavy metals must be less than 10 mg/kg.

5.4 Mercury

Using the solution for trials (5.2) measure the mercury according to the method in chapter II of the International Oenological Codex.

The contents of mercury must be less than 1 mg/kg.

5.5 Chloride

Using the trial solution 5.2 (diluted two times compared to initial contents) measure the chloride according to the method in chapter II of the International Oenological Codex.

The contents of chloride must be less than 3 mg/kg.

6. DETERMINATION OF ARSENIC, LEAD AND CADMIUM BY ATOMIC ABSORPTION SPECTROMETRY

6.1 Preparation of the test trial solution

For the determination of arsenic, lead, and cadmium.

Weigh about 100 g of the sample with a precision of ± 0.1 g in a cylindrical vase.

Add 200 ml of water and 5 ml of pure sulphuric acid (R) and concentrate on a hot plate until the first vapours of sulphuric acid appear.

Re-dilute the solution with water and add 1 ml of pure hydrochloric acid (R). Pour while washing into a 50 ml volumetric flask and bring to mark.

6.2 Arsenic

Using the trial solution (6.1) determine the arsenic content according to the method in chapter II of the International Oenological Codex.

Arsenic content should be less than 3 mg/kg.

6.3 Lead

Using the trial solution (6.1), determine the lead content according to the method in chapter II of the International Oenological Codex.

Lead content should be less than 5 mg/kg.

6.4 Cadmium

Using the trial solution (6.1), determine the cadmium content according to the method in chapter II of the International Oenological Codex.

Cadmium content should be less than 0.5 mg/kg.

7. DETERMINATION OF DIMETHYL CARBONATE

Dimethyl carbonate content should be less than 0.2%.

7.1 Principle of the method

The concentration of dimethyl carbonate is determined by chromatography in gaseous phase. The quantitative evaluation is performed by using methyl-isobutylcetone as an internal standard.

7.2 Apparatus

- 7.2.1 Chromatograph in gaseous phase with a flame ionisation detector and capillary column (apolar type "SE 30" or other; a polar column can also be used such as the Carbowax type 20 M), 50 m x 0.3 mm.
- 7.2.2 Data acquisition system.
- 7.2.3 A 10 µl quartz needle syringe suitable for an on column injection (injection "on column" (cf. remark 7.7).
- 7.2.4 10 ml antibiotic flask with a Teflon stopper that can be sealed with a aluminium capsule with the top part that can be torn off.

7.3 Internal standard

Ultra pure methyl-isobutylcetone.

7.4 Procedure

- 7.4.1 Weigh about 1 g of the sample at ± 1 mg (W1 mg) in a flask 7.2.4.
- 7.4.2 Add a quantity of internal standard (W2 mg) of methyl-isobutylcetone (7.3) corresponding to 10 mg/kg after addition (10 µl for example).
- 7.4.3 Seal the flask, mix vigorously and inject 0.2 µl.
- 7.4.4 Determine the peak area corresponding to the internal standard (F 2) and corresponding to dimethyl carbonate (F 1).

7.5 Result

$$\frac{W2 \cdot F1 \cdot K \cdot 100}{F2 \cdot W1} = \% \text{ mass of dimethyl carbonate}$$

K = Factor for the dimethyl carbonate calculated using reference solutions of this substance preferably prepared in DMDC free from dimethyl carbonate.

7.6 Remark 1

The sample prepared with the standard should be immediately analysed.

7.7 Remark 2

A partial decomposition of DMDC can occur when in contact with the metal needles of traditional syringes.

8. STORAGE

The DMDC must be stored in perfectly watertight containers at a temperature between 20°C and 30°C. Its shelf life is 12 months.

EGG (ALBUMIN OF)
Ovalbumin
Albumen ovi
(Oeno 32/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

The albumin of an egg is obtained by desiccating fresh egg whites. It is found in the form of a fine, white, very light powder which is not completely soluble in water, but is soluble in certain alkaline solutions.

Fining agent for clarifying wines.

Egg albumin is sold in powder or spray form, or it may be used directly in the form of albumin from fresh or sterilized eggs.

Egg albumin is precipitated by tannin. Typically, 2 g of pure tannin are required to precipitate 1 g of egg albumin.

2. LABELING

The label should indicate the storage, hygienic, and safety conditions, as well as the optimal use-by date.

3. DETERMINATION OF IDENTIFYING PROPERTIES

3.1. Preparation of a 10g/l Solution and Properties

3.1.1 Prepare an egg albumin slution by weakening the power with a very small quantity of water, so as to give a homogenous paste. Next, weaken gradually in order to obtain a solution having a concentration of 10g/l. This solution must have no unpleasant taste or odor.

This solution will have a pH of between 6.5 and 7. It will foam abundantly when shaken and will coagulate when heated in the presence of neutral salts.

Ovalbumin precipitate from its solutions by ammonium sulfate dissolved at saturation, by nitric acid and by alcohol

3.1.2 The pH of albumin from fresh eggs ranges between 9 and 9.5.

3.2. Disclosure of the Presence of Gum, Dextrin, and Gelatin

To 10 ml of a solution (concentration : 10 g/l) (Par. 3.1), add 0.5 ml concentrated nitric acid (R). Heat to 50-60°. A precipitate will form. Allow to cool, then filter. The filtrate should be colorless and clear, and should not become colored when an iodo-iodized solution (R) is added. No opalescent ring should form when 5 ml filtrate and 5 ml alcohol at 95% by volume are placed one on top of the other without mixing.

3.3. Desiccation Loss

In a 70 mm diameter silica dish with cover, place 2 g egg albumin. Dry in an oven at 100-105° for 6 hours. Allow to cool in the uncovered dish in a drying apparatus. Weigh. Let **p** be the quantity of dry residue. Weight loss should not exceed 10 pp 100.

When albumin from fresh egg is used, the real dry extract must range between 11 and 12%.

All of the limiting values given above are for dry product.

3.4. Ash

Incinerate the dry residue obtained from the test (Par. 3.4) by gradually heating to 600° C in a muffle furnace, after dusting the egg albumin with 0.2 to 0.3 g paraffin without ash, in order to prevent the material mass from overflowing.

The proportion of ash must not exceed 6.5 pp 100.

3.5. Total Nitrogen

Total nitrogen is determined using the technique described in the annex. The total nitrogen content must exceed 12 pp 100.

4. TESTS

4.1. Preparation of the Test Solution

After weighing, dissolve the ash in 2 ml concentrated hydrochloric acid (R) and 10 ml water. Heat to trigger dissolution and added distilled water to obtain a volume equal to 25 times the weight of the dry egg albumin.

l of this solution contains the mineral substances from 0.04 g dry egg albumin.

4.2. Heavy metals

To 10 ml of the test solution prepared according to Par. 4.1, add 2 ml of a buffer solution (pH : 3.5 (R)) and 1.2 ml thioacetamide reagent

(R). No precipitate should form. If the mixture becomes colored, the coloration should be less intense than that of the control prepared as indicated in the annex. (heavy metals content, expressed with respect to lead, should be less than 10 mg/kg.)

4.3. Arsenic

Using the technique described in the annex, determine arsenic content in the test solution (Par. 4.1). Content I should be less than 3 mg/kg.

4.4. Lead

Using the technique described in the Compendium, determine lead content in the test solution (Par. 4.1). Content I should be less than 5 mg/kg.

4.5. Mercury

Using the technique described in the annex, determine mercury content in the test solution (Par. 4.1). Content I should be less than 1 mg/kg.

5. STORAGE

Egg albumin should be stored in packages which ensure effective protection from moisture and external contamination in places in which temperatures are moderate.

**ELECTRODIALYSIS MEMBRANES
(Oeno 29/2000)**

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

An electrodialysis membrane is a thin, dense, insoluble wall composed of a polymer material that is permeable to ions. When placed between two solutions, it allows the selective transfer of ions from one solution to the other when acted upon by an electric field.

The membrane pair consists of a cationic membrane and an anionic membrane.

The cationic membrane is a polymer which allows the preferred flow of cations, in particular the K^+ and Ca^{++} cations.

The anionic membrane is a polymer which allows the preferred flow of anions, in particular tartrate anions.

Electrodialysis membranes are used to stabilize wine in the event of tartaric precipitation.

2. COMPOSITION

The cation-exchange membrane that can be used is a styrene-divinylbenzene copolymer which carries sulfonic functional groups.

The anion-exchange membrane that can be used is either:

A styrene-divinylbenzene copolymer which carries quaternary ammonium functional groups, or

A quaternary ammonium-divinylbenzene copolymer.

Electrodialysis membranes used for tartaric stabilization in wine should meet the following requirements:

2.1. They should be manufactured in accordance with the good manufacturing practices for the substances enumerated in :

2.1.1 *Annex 1* pertaining to materials placed in contact with foodstuffs

2.1.2. *Annex 2 and Annex 3* pertaining to ion-exchange resins used in processing foodstuffs

2.2. They should be prepared to serve their intended function, in accordance with the instructions of the manufacturer or supplier.

2.3. They should not release any substance in a quantity which poses a human health threat or which alters the taste or odor of foodstuffs.

2.4. In use, there should be no interaction between the constituents of the membrane and those of the wine that could form new compounds in the product that could produce toxicological consequences.

The stability of new electrodialysis membranes shall be established using a simulator which reproduces the physicochemical properties of wine, in order to study the migration of certain substances given off by the electrodialysis membrane.

The proposed experimental method is as follows:

Composition of the simulator:

This is an hydro-alcoholic solution with the pH and conductivity of wine. It is composed of the following:

Absolute ethanol: 11 liters
Potassium hydrogen tartrate: 380 g
Potassium chloride: 60 g
Concentrated sulfuric acid: 5 ml
Distilled water: quantity sufficient for 100 liters

This solution is used to test migration in a closed circuit on a live electrodialysis stack (1 volt/cell) in a proportion of 50 liters/m² of anionic and cationic membranes until the solution is 50% demineralized. The effluent circuit is activated by a 5 g/l potassium chloride solution.

The migrating substances tested for in the simulator and in the electrodialysis effluent.

The organic molecules forming a constituent of membrane and which can migrate into the treated solution will be quantitatively analyzed.

A specific determination for each of these constituents will be carried out in an approved laboratory. The content in the simulator must be less than the total, for all compounds analyzed at 50 µg/l.

Generally, the rules governing materials used in contact with foodstuffs shall also apply to these membranes.

3. LIMITS ON USE

The membrane pair used for tartaric wine-stabilization processing using electrodialysis is specified in such a way that:

- the pH reduction in the wine is no greater than 0.3 pH units ;
- volatile acid reduction is less than 0.12 g/l (2 meq. expressed in acetic acid) ;
- electrodialysis-based processing does not affect the non-ionic constituents of the wine, in particular the polyphenols and polysaccharides ;
- the diffusion of small molecules such as ethanol is reduced and does not lead to a reduction of alcoholic content greater than 0.1%.

4. CONDITIONS OF USE

These membranes should be stored and cleaned using accepted techniques and substances whose use is authorized for the preparation of foodstuffs.

**INTERNATIONAL TECHNOLOGICAL CODEX
ELECTRODIALYSIS MEMBRANES**

Annex 1

List of monomers and other starting substances that can be used in the manufacture of plastic materials and devices designed to be placed in contact with foodstuffs, products, and beverages.

**LIST OF APPROVED MONOMERS AND OTHER STARTING
SUBSTANCES**

PM/REF No.	Case No.	Name	Restrictions
(1)	(2)	(3)	(4)
10030	000514-10-3	Abietic acid	
10060	000075-07-0	Acetaldehyde	
10090	000064-19-7	Acetic acid	
10120	000108-05-4	Vinyl acetate	SML = 12 mg/kg
10150	000108-24-7	Acetic anhydride	
10210	000074-86-2	Acetylene	
10630	000079-06-1	Acrylamide	SML = ND (DL = 0.01 mg/kg)
10660	015214-89-8	2-acrylamido-2-methylpropane-sulfonic acid	SML = 0/05 mg/kg
10690	000079-10-7	Acrylic acid	
10750	002495-35-4	Benzyl acrylate	
10780	000141-32-2	n-butyl acrylate	
10810	002998-08-5	Sec-butyl acrylate	
10840	001663-39-4	Tert-butyl acrylate	
11470	000140-88-5	Ethyl acrylate	
	000818-61-1	Hydroxyethyl acrylate	See « Ethylene glycol monoacrylate »
11590	00106-63-8	Isobutyl acrylate	
11680	00689-12-3	Isopropyl acrylate	
11710	000096-33-3	Methyl acrylate	
11830	000818-61-1	Ethylene glycol monoacrylate	
11890	002499-59-4	n-octyl acrylate	
11980	000925-60-0	Propyl acrylate	

**INTERNATIONAL ZENOLOGICAL CODEX
ELECTRODIALYSIS MEMBRANES**

PM/REF N°	Case N°	Name	Restrictions
12100	000107-13-1	Acrylonitrile	LMS = ND (LD = 0,020 mg/kg) (including analytic tolerance)
12310		Albumin	
12340		Albumin coagulated by formaldehyde	
12375		Saturate, linear, primary monhydric alcohols (C ₄ -C ₂₂)	
12670	002855-13-2	1-amino-3-aminomethyl-3,5,5-trimethylcyclohexane	SML = 6 mg/kg
12788	002432-99-7	11-aminoundecanoic acid	SML = 5 mg/kg
12789	007664-41-7	Ammonia	
12820	00123-99-9	Azelaic acid	
12970	004196-95-6	Azelaic anhydride	
13000	001477-55-0	1,3-benzene dimethanamine	SML = 0.05 mg/kg
13090	000065-85-0	Benzoic acid	
13150	000100-51-6	Benzylic acid	
	000111-46-6	Bis(2-hydroxyethyl)ether	See Diethylene glycol
	000077-99-6	2,2-bis(hydroxymethyl)-1-butanol	See 1,1,1-trimethylolpropane
13390	000105-08-8	1,4-bis(hydroxymethyl) cyclohexane	
13480	000080-05-7	2,2-bis(4-hydroxyphenyl) propane	SML = 3 mg/kg
13510	001675-54-3	Bis(2,3-epoxypropyl) ether of 2,2-bis(hydroxyphenyl) propane	MQ = 1 mg/kg PF or SML = non-detectable (DL = 0.020 mg/kg, including analytic tolerance)
	000110-98-5	Bis(hydroxypropyl) ether	See Dipropylene glycol
	005124-30-1	Bis(4-isocyanato-cyclohexyl) methane	See 4,4-Diisocyanate dicyclohexylmethane
13530	038103-06-9	Bis(phthalic anhydride) of 2,2-bis(4-hydroxyphenyl) propane	SML = 0.05 mg/kg
13600	047465-97-4	3,3-bis(3-methyl-4-hydroxyphenyl)-2-indolinone	SML = 1.8 mg/kg
	000080-05-7	Bisphenol A	See 2,2-bis(4-hydroxyphenyl) propane
	001675-54-3	Bis(2,3-epoxypropyl)ether of bisphenol A	See Bis(2,3-epoxypropyl)ether of 2,2-bis(4-hydroxyphenyl) propane
13614	038103-06-9	Bis (phthalic anhydride)of bisphenol	See 13530

INTERNATIONAL ŒNOLOGICAL CODEX
ELECTRODIALYSIS MEMBRANES

PM/REF N°	Case N°	Name	Restrictions
13630	000106-99-0	Butadiene	MQ = 1 mg/kg of PF or SML = non-detectable (DL = 0.02 mg/kg, including analytic tolerance)
3690	000107-88-0	1,3-butanediol	
13840	000071-36-3	1-butanol	
13870	000106-98-9	1-butene	
13900	000107-01-7	2-butene	
14110	000123-72-8	Butyraldehyde	
14140	000107-92-6	Butyric acid	
14170	000106-31-0	Butyric anhydride	
14200	000105-60-2	Caprolactam	SML(T) = 15 mg/kg
14230	002123-24-2	Caprolactam, sodium salt	SML(T) = 15 mg/kg (expressed in terms of caprolactam)
14320	0001207-2	Caprylic acid	
14350	00630-08-0	Carbon monoxide	
14380	000075-44-5	Carbonyl chloride	MQ = 1 mg/kg in FP
14411	008001-79-4	Castor oil	
14500	009004-34-6	Cellulose	
14530	007782-50-5	Chlorine	
	000106-89-8	1-chloro-2,3-epoxy propane	See Epichlorhydrin
14680	000077-92-9	Citric acid	
14710	000108-39-4	<i>m</i> -cresol	
14740	000095-48-7	<i>o</i> -cresol	
14770	00106-44-5	<i>p</i> -cresol	
	00105-08-8	1,4-cyclohexanedi-methanol	See 1,4-bis(hydroxymethyl) cyclohexane
14950	003173-53-3	Cyclohexyl isocyanate	MQ(T) = 1 mg/kg in FP (expressed as NCO)
15070	001647-16-1	1,9-decadiene	SML = 0.05 mg/kg
15095	000334-48-5	Decanoic acid	
15100	000112-30-1	1-decanol	

**INTERNATIONAL ZENOLOGICAL CODEX
ELECTRODIALYSIS MEMBRANES**

PM/REF N°	Case N°	Name	Restrictions
	000107-15-3	1,2-diaminoethane	See Ethylenediamine
	000124-09-4	1,6-diaminohexane	See Hexamethylene- diamine
15250	000110-61-1	1,4-diaminobutane	
15565	0000106-46-7	1,4-dichlorobenzene	SML = 12 mg/kg
15700	005124-30-1	1-cyclohexylmethane-4,4'-diisocyanate	MQ(T) = 1 mg/kg in FP (expressed as NCO)
15760	000111-46-6	Diethylene glycol	SML(T) = 30 mg/kg alone or with ethylene glycol
15790	000111-46-6	Diethylene triamine	SML = 5 mg/kg
15820	000345-92-6	4,4'-difluorobenzophenone	SML = 0.05 mg/kg
15880	000120-80-9	1,2-dihydroxybenzene	SML = 6 mg/kg
15910	000108-46-3	1,3-dihydroxybenzene	SML = 2.4 mg/kg
15940	000123-31-9	1,4-dihydroxybenzene	SML = 0.6 mg/kg
15970	000611-99-4	4,4'-dihydroxybenzophenone	SML = 6 mg/kg
16000	000092-88-6	4,4'-dihydroxydiphenyl	SML = 6 mg/kg
16150	000108-01-0	Dimethylaminoethanol	SML = 18 mg/kg
16240	000091-97-4	3,3'-dimethylbiphenyl-4,4'-diisocyanate	MQ(T) = 1 mg/kg in FP (expressed as NCO)
16480	000126-58-9	Dipentaerythritol	
16570	004128-73-8	4,4'-diisocyanate of diphenyl ether	MQ(T) = 1 mg/kg in FP (expressed as NCO)
16600	005873-54-1	Diphenylmethane-2,4'-diisocyanate	MQ(T) = 1 mg/kg in FP (expressed as NCO)
16630	000101-68-8	Diphenylmethane-4,4'-diisocyanate	MQ(T) = 1 mg/kg in FP (expressed as NCO)
16660	000110-98-5	Dipropylene glycol	
16750	000106-89-8	Epichlorohydrin	MQ = 1 mg/kg in FP
16780	000064-17-5	Ethanol	
16950	000074-85-1	Ethylene	
16960	000107-15-3	Ethylenediamine	SML = 12 mg/kg
16990	000107-21-1	Ethylene glycol	SML(T) = 30 mg/kg alone or with diethylene glycol

**INTERNATIONAL GENOTOXICOLOGICAL CODEX
ELECTRODIALYSIS MEMBRANES**

PM/REF N°	Case N°	Name	Restrictions
17005	000151-56-4	Ethyleneimine	SML = ND (DL = 01 mg/kg)
17020	000075-21-8	Ethylene oxide	MQ = 1 mg/kg in FP
17050	000104-76-7	2-ethyl-1-hexanol	SML = 30 mg/kg
17160	000097-53-0	Eugenol	SML = 0.1 mg/kg
17170	061788-47-4	Coconut fatty acids	
17200	068308-53-2	Fatty acids of soybean oil	
17230	061790-12-3	Fatty acids of tall oil	
17260	000050-00-0	Formaldehyde	SML = 15 mg/kg
17290	000110-17-8	Fumaric acid	
17530	000050-99-7	Glucose	
18010	000110-94-1	Glutaric acid	
18070	000108-55-4	Glutaric anhydride	
18100	000056-81-5	Glycerol	
18250	000115-28-6	Hexachloroendo-methyl- Enetetrahy-drophthalic acid	SML = ND (DL = 0.01 mg/kg)
18280	00115-27-5	Hexachloroendome-thyl Enetetrahydro-phthalic anhydride	SML = ND (DL = 0.01 mg/kg)
18310	036653-82-4	1-hexadecanol	
18430	00116-15-4	Hexafluoropropylene	SML = ND (DL = 0.01 mg/kg)
18460	000124-09-4	Hexamethylenediamine	SML = 2.4 mg/kg
18640	000822-06-0	Hexamethylene diisocyanate	MQ(T) = 1 mg/kg in FP (expressed as NCO)
18670	000100-97-0	Hexamethylene tetramine	SML(T) = 15 mg/kg (expressed as formaldehyde)
	00123-31-9	Hydroquinone	See 1,4-dihydroxybenzene
18880	000099-96-7	p-hydroxybenzoic acid	
19000	000115-11-7	Isobutene	
19210	001459-93-4	Dimethyl isophthalate	SML = 0.05 mg/kg
19270	000097-65-4	Itaconic acid	
19460	000050-21-5	Lactic acid	

**INTERNATIONAL ZENOLOGICAL CODEX
ELECTRODIALYSIS MEMBRANES**

PM/REF N°	Case N°	Name	Restrictions
19470	000143-07-7	Lauric acid	
19480	002146-71-6	Vinyl laurate	
19510	011132-73-3	Lignocellulose	
19540	000110-16-7	Maleic acid	SML(T) 30 mg/kg
19960	00108-31-6	Maleic anhydride	SML(T) = 30 mg/kg (expressed as maleic acid)
	000108-31-6	Melamine	See 2,4,6-triamino-1,3,5-triazine
20020	000079-41-4	Methacrylic acid	
20080	002495-37-6	Benzyl methacrylate	
20110	000097-88-1	Butyl methacrylate	
20140	002998-18-7	sec-butyl methacrylate	
20890	000097-63-2	Ethyl methacrylate	
21010	000097-86-9	Isobutyl methacrylate	
21100	004655-34-9	Isopropyl methacrylate	
21130	000080-62-6	Methyl methacrylate	
21190	000868-77-9	Ethylene glycol monomethacrylate	
21280	002177-70-0	Phenyl methacrylate	
21340	000760-93-0	Propyl methacrylate	
21460	000760-93-0	Methacrylic anhydride	
21490	000126-98-7	Methacrylonitrile	SML = not detectable (DL = 0.020 mg/kg, including analytic tolerance)
21550	000067-56-1	Methanol	
21940	000924-42-5	N-methylolacrylamide	SML = ND (DL = 0.0 mg/kg)
22150	000691-37-2	4-methyl-pentene	SML = 0.02 mg/kg
22350	000544-63-8	Myristic acid	
22390	000840-65-3	2,6-dimethyl naphthalene-dicarboxylate	SML = 0.05 mg/kg
22420	003173-72-6	1,5-naphthalene diisocyanate	MQ(T) 1 mg/kg in FP (expressed as NCO)

**INTERNATIONAL GENOTOXICOLOGICAL CODEX
ELECTRODIALYSIS MEMBRANES**

PM/REF N°	Case N°	Name	Restrictions
22450	009004-70-0	Nitrocellulose	
22480	000143-08-8	1-nonanol	
22570	000112-96-9	Octadecyl isocyanate	MQ(T) = 1 mg/kg in FP (expressed as NCO)
22600	000111-87-5	1-octanol	
22660	000111-66-0	1-octene	SML = 15 mg/kg
22763	000112-80-1	Oleic acid	
22780	000057-10-3	Palmitic acid	
22840	000115-77-5	Pentaerythritol	
22870	000071-41-0	1-pentanol	
22960	000108-95-2	Phenol	
23050	000108-45-2	1,3-phenylenediamine	MQ = 1 mg/kg in FP
	000075-44-5	Phosgene	See Carbonyl chloride
23170	007664-38-2	Phosphoric acid	
		Phthalic acid	See Terephthalic acid
23200	000088-99-3	<i>o</i> -phthalic acid	
23230	000131-17-9	Diallyl phthalate	SML = ND (DL = 0.01 mg/kg)
23380	000085-44-9	Phthalic anhydride	
23470	000080-56-8	alpha-pinene	
23500	000127-91-3	beta-pinene	
23590	025322-68-3	Polyethylene glycol	
23651	025322-69-4	Polypropylene glycol	
23740	000057-55-6	1,2-propanediol	
23800	000071-23-8	1-propanol	
23830	000067-63-0	2-propanol	
23860	000123-38-6	Propionaldehyde	
23890	000079-09-4	Propionic acid	

**INTERNATIONAL ŒNOLOGICAL CODEX
ELECTRODIALYSIS MEMBRANES**

PM/REF N°	Case N°	Name	Restrictions
23950	000123-62-6	Propionic anhydride	
23980	000115-07-1	Propylene	
24010	000075-56-9	Propylene oxide	MQ = 1 mg/kg in FP
	000120-80-9	Pyrocatechol	See 1,2-dihydroxybenzene
24057	000089-32-7	Pyromellitic anhydride	SML = 0.05 mg/kg (expressed as pyromellitic acid)
24070	073138-82-6	Resin acids	
	000108-46-3	Resorcinol	See 1,2-dihydroxybenzene
24100	008050-09-7	Rosin	
24130	008050-09-7	Rosin gum	See Rosin
24160	008052-10-6	Tall oil resin	
24190	009014-63-5	Wood resin	
24250	009006-04-6	Natural rubber	
24270	000069-72-7	Salicylic acid	
24280	000111-20-6	Sebacic acid	
24430	002561-88-8	Sebacic anhydride	
24475	001313-82-2	Sodium sulfide	
24490	000050-70-4	Sorbitol	
24520	008001-22-7	Soybean oil	
24540	009005-25-8	Food starch	
24550	000057-11-4	Stearic acid	
24610	000100-42-5	Styrene	
24820	000110-15-6	Succinic acid	
24850	000108-30-5	Succinic anhydride	
24880	000057-50-1	Saccharose	
24887	006362-79-4	5-sulfoisophthalic acid, monosodium salt	SML = 5 mg/kg

**INTERNATIONAL ZENOLOGICAL CODEX
ELECTRODIALYSIS MEMBRANES**

PM/REF N°	Case N°	Name	Restrictions
24888	003965-55-7	5-dimethylsulfo- isophthalate, monosodium salt	SML = 0.05 mg/kg
24910	000100-21-0	Terephthalic acid	SML = 7.5 mg/kg
24940	000100-20-9	Terephthalic acid dichloride	SML(T) = 7.5 mg/kg (expressed as terephthalic acid)
24970	000120-61-6	Dimethyl terephthalatae	
25090	000112-60-7	Tetraethylene glycol	
25120	000116-14-3	Tetrafluoroethylene	SML = 0.05 mg/kg
25150	000109-99-9	Tetrahydrofuran	SML = 0.6 mg/kg
25180	000102-60-3	N,N,N',N'-tetrakis(2-hydroxypropyl)-ethylene-diamine	
25210	000584-84-9	Toluene-2,4-diisocyanate	MQ(T) = 1 mg/kg in FP (expressed as nCO)
25240	000091-08-7	Toluene-2,6-diisocyanate	MQ(T) = 1 mg/kg in FP (expressed as nCO)
25270	026747-90-0	Toluene-2,4-diisocyanate, dimer	MQ(T) = 1 mg/kg in FP (expressed as nCO)
25360		2,3-epoxy trialkyl(C ₅ -C ₁₅) acetate	SML = 6 mg/kg
25420	000108-78-1	2,4,6-triamino-1,3,5-triazine	SML = 30 mg/kg
25510	000112-27-6	Triethylene glycol	
25600	000077-99-6	1,1,1-trimethylolpropane	SML = 6 mg/kg
25910	024800-44-0	Tripropylene glycol	
25960	000057-13-6	Urea	
26050	000075-01-4	Vinyl chloride	See Council Directive 78/142/EEC
26110	000075-35-4	Vinylidene chloride	MQ = 5 mg/kg in FP or SML = ND (DL = 0.05 mg/kg)
26140	000075-38-7	Vinylidene fluoride	SML = 5 mg/kg

INTERNATIONAL CENOLOGICAL CODEX ELECTRODIALYSIS MEMBRANES

A number of abbreviations or notations are given in Column 4. Their meaning is listed below :

DL = Detection limit of the analytical method.

FP = Finished material or product

NCO = isocyanate group

ND = not detectable.

For the purposes of the present directive, the expression « not detectable » means that the substance will not be detected by the approved analytical method, which is sensitive enough to detect it at the specified detection limit. If, however, a method of this kind does not currently exist, an analytical technique possessing performance characteristics suited to the specified limit may be used, while awaiting the development of an approved method.

MQ = maximum permitted quantity of the « residual » substance in the material or article.

MQ(T) = maximum permitted quantity of residual substance in the material or article, expressed as the total group or of the indicated substances(s).

For the purposes of this directive, « MQ(T) » means that the maximum permitted quantity of the « residual » substance in the material or article should be determined using an analytical method approved for the specified limit. If, however, a method of this kind does not currently exist, an analytical technique possessing performance characteristics suitable for determining the specified limit may be used, while awaiting the development of an approved method.

SML = specific migration limit in the food product or the simulated food, unless otherwise specified.

For the purposes of this directive, « SML » means that the specific migration of the substance should be determined using an analytical method approved for the specified limit. If, however, a method of this kind does not currently exist, an analytical technique possessing performance characteristics suitable for determining the specified limit may be used, while awaiting the development of an approved method.

SML(T) = specific migration limit in the food product or simulated food, expressed as the total

of the group or of the indicated substance(s).

For the purposes of this directive, « SML(T) » means that the specific migration of the substance should be determined using an analytical method approved for the specified limit. If, however, a method of this kind does not currently exist, an analytical technique possessing performance characteristics suitable for determining the specified limit may be used, while awaiting the development of an approved method.

INTERNATIONAL TECHNOLOGICAL CODEX ELECTRODIALYSIS MEMBRANES

Annex 2

List of substances used in the manufacture of adsorbant ion-exchange resins
used to condition foodstuffs. (Resolution AP (97)1 EC)

List 1

Substances assessed by an international organization

NAME	PM/REF	CASE	RESTRICTIONS
=====			
Monomers and other Starting substances			
n-butyl acrylate	10780	00141-32-2	-
Ethyl acrylate	11470	00140-88-5	-
Methyl acrylate	11710	00096-33-3	-
Acrylonitrile	12100	00107-13-1	SML = ND (DL = 0.02 mg/kg) SML = 15 mg/kg
Formaldehyde	17260	00050-00-0	-
Methyl methacrylate	21130	00080-62-6	-
Methanol	21550	00067-56-1	-
Styrene	24610	00100-42-5	-
Chemical Modifiers			
Carbonic acid, salts	42500	-	-
Hydrochloric acid	72640	07664-38-2	-
Silicic acid, salts	85980	-	-
Sulfuric acid	91920	07664-93-9	-
Acetic anhydride	10150	00108-24-7	-
tert-butyl-4-hydroxyanisole (BHA)	40720	25013-16-5	SML=30 mg/kg
Diethylene triamine	15790	00111-40-0	SML= 5 mg/kg
Dimethylamine	49225	00124-40-3	SML=0.06 mg/kg
2-(dimethylamino)ethanol	49235	00108-01-0	SML=18 mg/kg
Formaldehyde	54880	00050-00-0	SML=15 mg/kg
Hexamethylenediamine	18460	00124-09-4	SML=2.4 mg/kg
Potassium hydroxide	81600	01310-58-3	-
Sodium hydroxide	86720	01310-73-2	-
Sodium nitrite	86920	07632-00-0	SML=0.6 mg/kg
Ethylene oxide	17020	00075-21-8	MQ=1 mg/kg in FP
2-propanol	81882	00067-63-0	-
Polymerization Additives			
Alkylsulfonic acids (C ₈ -C ₂₂)	34230	-	SML=6 mg/kg
Linear, primary alkylsulfuric acids (C ₈ -C ₂₂) having an even number of carbon atoms	34281	-	-
Formic acid	55040	00064-18-6	-
Carboxymethylcellulose	42640	09000-11-7	-
Stannic chloride(IV)	93420	07646-78-8	-
Methylene chloride	66620	00075-09-2	SML=0.05 mg/kg
1,4-dihydroxybenzene	48620	00123-31-9	SML=0.6 mg/kg
Gelatin	55440	09000-70-8	-
Ammonium hydroxide	35600	01336-21-6	-
Magnesium hydroxide	64640	01309-42-8	-
Hydroxyethylcellulose	60560	09004-62-0	-
Hydroxyethylmethylcellulose	60880	09032-42-4	-
Methanol	65960	00067-56-1	-
Methylcarboxymethylcellulose	66200	37206-01-2	-
Methyl isobutyl ketone	66725	00108-10-1	SML=5 mg/kg
Toluene	93540	00108-88-3	SML=1.2 mg/kg

INTERNATIONAL TECHNOLOGICAL CODEX ELECTRODIALYSIS MEMBRANES

Annex 3

Substances that may be used provisionally to manufacture ion-exchange resins.

List 2

1. Substances not fully evaluated by an international organization

NAME	PM/REF	CASE	RESTRICTIONS
=====			
Monomers and other starting substances			
Ethylene glycol dimethacrylate	20440	00097-90-5	-
Divinylbenzene	16690	01321-74-0	-
Diallyl ether of 1,1,1-tri-methylolpropane	25645	00682-09-7	-
2,3-epoxypropyl methacrylate	20590	00106-91-2	-
2-methyl-1,3-butadiene	21640	00078-79-5	-
1,7-octadiene	22585	03710-30-3	-
1,1,1-trimethylolpropane trimethacrylate	25840	03290-92-4	-
Chemical Modifiers			
N,N-dimethyl-1,3-diamino-propane	49380	00109-55-7	-
Triethylamine	95270	00121-44-8	-
Triethylene tetramine	25520	00112-24-3	-
Polymerization Additives			
Polyvinyl alcohols	81280	09002-89-5	-
4-tert-butylcatechol	40640	00098-29-3	-
Diisobutyl ketone	49050	00108-83-8	-
Sodium hypochlorite	62110	07681-52-9	-
Isobutanol	62270	00078-83-1	-
4-methoxyphenol	66030	00150-76-5	-
Methylene bis(sodium naphthalenesulfonate)	66600	26545-58-4	-
2-methyl-2-pentanol	66860	00108-11-2	-
Dibenzoylperoxide	46440	00094-36-0	-
Partially hydrolyzed vinyl polyacetate	81260	-	-

2. Substances not evaluated by an international organization

NAME	PM/REF	CASE	RESTRICTIONS
=====			
Monomers and other starting substances			
Dimethoxymethane	-	00109-87-5	-
Diethylene glycol divinyl ether	-	00764-99-8	-
Ethyl vinyl benzene	-	28106-30-1	-
1,2,4-trivinyl cyclohexane	-	02855-27-8	-
Chemical Modifiers			
Chlorosulfonic acid	-	07790-94-5	-
Monochloroacetic acid	-	00079-11-8	-
Phosphoric acid	-	13598-36-2	-
Bromine	-	07726-95-6	-
2-chloroethanol	-	00107-07-3	-
Methyl chloride	-	00074-87-3	-
1,2-dichloroethane	-	00107-07-3	-
1,2-dichloropropane	-	00078-87-5	-
3-(dimethylamino)propane	-	03179-63-3	-

INTERNATIONAL CENOLOGICAL CODEX ELECTRODIALYSIS MEMBRANES

NAME	PM/REF	CASE	RESTRICTIONS
=====			
Monomers and other starting substances			
Methylic chloromethyl ether	-	00107-30-2	-
Nitrobenzene	-	00098-95-3	-
Potassium nitrite	-	07758-09-0	-
Phthalimide	-	0085-41-6	-
Sulfur trioxide	-	07446-11-9	-
Trimethylamine	-	00075-50-3	-
Polymerization additives			
Lignosulfonic acid	63940	08062-15-5	-
Peracetic acid	-	00079-21-0	-
Polyacrylic acid	76460	09003-01-4	-
Poly(styrenesulfonic) acid	-	09080-79-9	-
Acrylamide/acrylic acid copolymer	-	09003-06-9	-
Ethoxylated, propoxylated tert-alkylamines (C ₁₂ -C ₁₄)	-	68603-58-7	-
Maleic anhydride-styrene copolymer, ammonium salt	-	26022-09-3	-
Attapulgate	-	12174-11-7	-
Azobisisobutyronitrile	-	00078-67-1	-
1,1-bis(tert-butylperoxy)-3,3,5- trimethylcyclohexane	-	06731-36-8	-
n-Dodecyl mercaptan	-	25103-58-6	-
Poly(ethylene/propylene)glycol monobutyl ester	-	09038-95-3	-
Polyethylene glycol octylphenyl ether	78560	09002-93-1	-
Poly(ethylene-propylene)glycol ether with 1,1,1-trimethylol-propane	-	52624-57-4	-
tert-hexadecyl mercaptan	-	25360-09-2	-
Cumyl hydroperoxide	-	00080-15-9	-
Isododecane	62405	31807-55-3	-
Isooctane	-	26635-64-3	-
Mono- and dialkyl (C ₁₀ -C ₁₈) Sulfonamides	-	-	-
Silver nitrate	-	07761-88-8	-
n-Octane	-	00111-65-9	-
tert-Butyl peracetate	-	00107-71-1	-
tert-Butyl perbenzoate	-	00614-45-9	-
bis(4-tert-butylcyclohexyloxy) percarbonate tert-	-	15520-11-3	-
Butyl per(2-ethyl-hexanoate)	-	03006-82-6	-
tert-Butyl peroctanoate	-	13467-82-8	-
Dilauroyl peroxide	-	00105-74-8	-
Poly(diallyldimethylammonium chloride)	-	26062-79-3	-
Polyvinylpyrrolidone	81500	09003-39-8	-
=====			

ENZYMATIC PREPARATIONS
(Oeno 14/2003)

The prescriptions described below concern all enzymatic preparations susceptible of being used during various operations that can be applied to grapes and their derivatives.

The prescriptions are based on the recommendations from the *"Joint FAO/WHO Expert Committee on Food Additives (JECFA), 35th Session, Rome 29 May – 7 June 1989"* and published in 1990 in the *FAO Food and Nutrition Paper n° 49 "Specifications for identity and purity of certain food additives. General specifications for enzyme preparations used in Food Processing"*.

1. GENERAL CONSIDERATIONS

Enzymatic preparations can be made from micro-organisms or animal tissue or vegetable tissue.

When looking for synergies between various enzymatic activities including pectinase, cellulase and hemicellulase, mixtures of preparations made from different strains can be carried out. These preparations can contain one or more active compounds, in addition to supports, diluents, preservatives, antioxidants and other substances compatible with correct manufacturing rules and in accordance with the regulation. In certain cases, the preparations can contain cells or cell fragments. Furthermore they can be in either liquid or solid form. The active substances can also be immobilised on a support admitted for oenological products. The use of glycerol is not admitted in certain countries.

2. LABELLING

The labelling of admitted enzymatic preparations must specify the storage conditions, additives, the nature of the enzymatic activities, batch number and the expiration date. Also the indication that the enzymatic preparations were obtained by genetic modification where relevant.

3. ADMITTED ENZYMATIC PREPARATIONS

All enzymatic preparations presenting a technological interest duly proven in practice and meeting the conditions and criteria mentioned above, are accepted for the treatment of grapes and their by-products.

Enzymatic preparations used must not contain any substance, microorganism, nor enzymatic activity that:

- is harmful to health,
- is harmful to the quality of the products manufactured,
- can lead to the formation of undesirable products,
- or that will give rise or facilitate fraud.

4. ENZYMATIC ACTIVITIES

4.1 General considerations

Enzymatic preparations contain many enzymatic activities. Other than the main enzymatic activities, whose technological interest has been duly proven, secondary enzymatic activities are only tolerated if they are set within the technological constraint limits for manufacturing of enzymatic preparations. They must be as limited as possible. Generally speaking, the sum of all secondary activities must not be superior to 50% of the sum of the activities necessary for the desired function. The activities are expressed in nkat. (nKat= 1 nmol of transformed substrate or product formed per second per gram of the preparation).

The secondary activities more than 10% of the main activity must be declared within the technical characteristics of the commercial product.

Enzymatic activity in a preparation and corresponding to the expressed technological need is indicated in units of activities by preparation mass units. These units represent enzymatic activity for which the preparation is standardised.

4.2 Activity measurement

The enzymatic activities presented are measured in wine conditions. The incubations are carried out at 25°C for 20 minutes. Activity measurements are carried out by measuring the initial speed of the reaction.

For each measurement, the values obtained with each preparation inactivated by boiling, (the value of white enzymatic preparations) are to be deducted from the measurements made with active enzymes.

Perform the measurements in duplicate.
The results are expressed in nanokatal.

When the sought out technological transformation results from the action of different enzymes within the same preparation, it is important to specifically measure each enzymatic activity. These activities will require special sheets, where the details of the type of measurement will be specified.

The partial undesirable activities must be sought out and identified.

5. SOURCES OF ENZYMES AND PRODUCTION ENVIRONMENT

The microbial sources of enzymes must be non-pathogenic, non-toxic and genetically stable, and the fermentation environments should not leave harmful residues in enzymatic preparations. In the case of microorganisms, a safety study must be conducted in order to ensure that enzymatic preparation produced by a microorganism species (*e.g. Aspergillus niger*) does not pose a health risk. This study can be based on principles brought forth on food enzyme guidelines published by the Scientific Committee for Food (SCF), or other equivalent organisations.

The techniques implemented must be compatible with the good practices of manufacturing and the prescriptions of the International Oenological Codex if yeast and/or lactic bacteria are used.

Animal tissues used in enzymatic preparations must be compatible with demands set by the official monitoring authorities. These tissues must be treated in compliance with good hygiene and manufacturing standards.

6. SUPPORTS, DILUENTS, PRESERVATIVES AND OTHER ADDITIVES

The enzymatic preparations can only be diluted in substances which comply with the regulations in force in different countries for the treatment of grapes and by-products.

In the case of immobilised enzymes, the supports used must comply to standards on material in contact with foodstuffs. For this type of preparation, the content of compounds of the supports used, susceptible to spread out in musts and wine, should be determined and indicated on the label of the enzymatic preparation.

The presence of preservatives will only be tolerated for commercialised preparations in liquid form. Only preservatives authorised in wines are accepted and their contents must be clearly indicated on the label of the enzymatic preparation.

7. HYGIENE

Enzymatic preparations must be produced in accordance with good practices of manufacturing and must not provoke a significant increase in germs in the treated products.

8. LIMITS AND TEST TRIAL METHODS

8.1 Heavy metals

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content less than 30 mg/kg.

8.2 Lead

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content less than 5 mg/kg.

8.3 Mercury

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content less than 0.5 mg/kg.

8.4 Arsenic

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content less than 3 mg/kg.

8.5 Cadmium

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content less than 0.5 mg/kg.

8.6 Salmonella

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Absence checked on a 25 g sample.

8.7 Coliforms

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Content less than 30 CFU/g of preparation.

8.8 *Escherichia coli*

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Absence checked on a 1 g sample.

8.9 Total germs

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Content less than 10^4 CFU/g of preparation.

8.10 Yeasts

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Content less than 10^3 CFU/g of preparation.

8.11 Lactic bacteria

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Content less than 10^3 CFU/g of preparation.

8.12 Acetic bacteria

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Content less than 10^2 CFU/g of commercial product.

The enzymatic preparations should not present antibiotic activity, nor detectable levels of aflatoxins * (4 µg/kg), ochratoxin A* (3 µg/kg), sterigmacycline*, T-2 toxins* (to be set) or zearalenones* (10 µg/kg).

* According to methods to be defined at a later date.

9. OBLIGATORY TECHNICAL SHEET TO BE SUPPLIED BY MANUFACTURER

Each type of enzymatic preparation must be defined using a technical sheet.

It must contain at least the following information:

- Nature of the preparation (e.g. pectolytic enzymes),
- Origin (*e.g. Aspergillus niger*),
- Fields and the application procedure,
- Activity and stability of the preparation with the expiration date guaranteeing the activity and storage conditions (temperature),
- Types of reactions catalysed by the main enzymatic activities,
- Main enzymatic activities with n° IUB (for example Tannase 3.1.1.20),
- Secondary enzymatic activities with, if possible, IUB number, and their activity as a percentage of the main activity,
- Types of supports, diluents, preservatives and additives used and their respective contents,
- Whether the enzymatic preparation is genetically modified or not,
- A statement identifying the batch.

FURTHER INFORMATION FOR EACH ENZYME WILL BE MADE AVAILABLE.

FISH GLUE
Isinglass
(Oeno 24/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Fish glue is made from the swim bladder, gills and ears of certain fish, notably sturgeon.

It is produced in the form of colorless or slightly yellowish transparent sheets or, most frequently, in ribbons which have the appearance of dry parchment, or in vermiculated or powder form.

Fish glue expands in cold water and becomes opaque. It dissolves in hot water acidified with tartaric acid, leaving at most 3 pp 100 of residue composed of membranes. With 30-50 parts of hot water and after cooling, it forms a colorless, translucent jelly.

After partial hydrolysis, fish glue is often found in ready-to-use colloidal solution form stabilized by SO₂. In this case, it should be stored cool in a closed container.

Fish glue is used to clarify white and rose wines.

2. LABELING

The label should indicate product concentration, even when used in mixtures, as well as its safety and storage conditions. The expiration date and the SO₂ content should be indicated on the label.

3. TESTS

3.1. A hot water solution should be odorless and have no disagreeable taste. Reaction should be neutral or slightly alkaline. It precipitates with tannin.

Fish glue pH ranges between 3.5 and 4 when tartaric acid has been used to facilitate dissolution.

3.2. Fish glue processed with a potassium hydroxide solution (R) should remain transparent and, after several hours, yield a colorless liquid which will produce a light, frothy precipitate over time. Under the same conditions, the gelatin becomes opaque and difficult to make soluble. It produces an abundant, white precipitate.

3.3. Test for albuminoid substances. Aqueous solutions should not form a precipitate when iron (III) sulfate solution (R) is added.

3.4. Desiccation Loss

3.4.1 Fish Glue in Solid Form

In a silica dish with cover and measuring 70 mm in diameter, place 2g fish glue. Oven dry at 100-105° for six hours. Allow to cool in the uncovered dish in a desiccator. Weigh. Let **p** be the quantity of dry residue. Weight loss should not exceed 18 pp 100.

3.4.2 Fish Glue in a Liquid State

In a silica dish 70 mm in diameter place approximately 10 g fish glue colloidal solution, weight this amount with precision in the uncovered dish, dry in a water bath at 100° C for 4 hours and complete the drying process in the oven at 100-105° C for 3 hours. Allow to cool in the uncovered dish in the drying apparatus. Weigh the dry residue produced. Let **p** be the quantity added to 100 g colloidal solution. The dry residue should reach a minimum level of 1 pp 100.

All of the limiting values set forth above are stipulated for the dry product.

3.5. Ash

Burn the dry residue in test 3.4 by gradually heating to 600° in a muffle furnace after dusting the fish glue with 0.1 to 0.3 g paraffin without ash, in order to prevent the mass of material from overflowing. Ash content should be less than 2 pp 100.

3.6. Preparation of Test Solution

After eighing, dissolve the ash in 2 ml concentrated hydrochloric acid (R) and 10 ml water. Heat to stimulate dissolution and add distilled water until a volume equal to 25 times the weight of the dry fish glue is obtained. 1 ml of this solution contains the mineral substances derived from 0.04 g dry fish glue.

3.7. Total Nitrogen

Refer to the technique described in the Annex.

Total nitrogen content should be greater than 14 pp 100.

3.8. Iron

1 ml concentrated hydrochloric acid (R), one drop potassium permangante in concentration of 1 pp 100 (R) and 2 ml potassium thiocyanate in a concentration of 5 pp 100 (R) are added to 10 ml of the test solution prepared according to Par. 3.6).

If a red coloring appears, it must be less intense that that of a control prepared from 4.2 ml of iron (III) solution in a concentration of

0.010 g per liter, 5.8 ml water, and the same quantities of concentrated hydrochloric acid (R) and of potassium thiocyanate in a concentration of 5 pp 100.

Iron content should be less than 100 mg/kg.

The quantitative analysis of iron can also be implemented by atomic absorption spectrometry, using the technique reported in the Compendium.

3.9. Arsenic

Using the method described in the Annex, determine the arsenic content in the test solution prepared according to Par. 3.6. Arsenic content should be less than 3 mg/kg..

3.10. Lead

Using the method described in the Compendium, determine the lead content in the test solution prepared according to Par. 3.6. (Lead content should be less than 5 mg/kg).

3.11. Mercury

Using the method described in the annex, quantify the mercury content in the test solution prepared according to Par. 3.6. (Mercury content should be less than 1 mg/kg).

4. STORAGE

Fish glue should be stored in hermetic flasks. An expiration date should be specified.

Store colloidal solutions at temperatures of less than 10 °C to avoid rapid hydrolysis of the product during storage.

GELATINE
Proteinum ossii
Gelatina
(Oeno 13/2003)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Gelatine is the result of the partial hydrolysis of collagen contained in the skins, connective tissue and bones of animals. Gelatine comes in the form of roll sheets, flexible sheets, sprinkles, grains or colourless or slightly yellowish brown powder.

Certain gelatines are intentionally hydrolysed more than usual edible gelatines so as to be presented in ready-to-use colloidal solutions or in the form of atomised powder, soluble when cold. These products do not have the characteristic of becoming gel with water.

The structure and the iso-electric point of bovine skin gelatine proteins are different from gelatine from pork bones and rind.

Taking into account available scientific data, international standards and directives, gelatine must come from animals sources in compliance with recommendations from the International Office of Epizootics (IOE).

Gelatines are used as fining and clarification agents for wine. Gelatines react with wine tannins or additions and certain cations depending on their origin, the extraction process and their final degree of hydrolysis at the time of use in wine.

For the same quality of gelatine, the hydrolysis quality and the different phases of hydrolysis will produce products with very different behaviour concerning fining.

There is no single parameter to characterise the different types of gelatine due to their diversity.

2. LABELLING

The origin of basic edible gelatine must be indicated as well as the optimal storage conditions, expiration date and the concentration of SO₂.

3. SOLUBILITY

Basic edible gelatine swells in cold water. It dissolves in hot water (80°C to 90°C) and the solution jellifies upon cooling.

4. TEST TRIALS

4.1 Taste test

The solution in warm water should not have an unpleasant odour nor taste.

4.2 pH

Evaluate the pH on a 1% solution at 40°C,

The colloidal solution pH level is between 3 to 4,

The solutions prepared from powder or grain products have a pH level between 5 to 7.

4.3 Loss through dessication

4.3.1 Solid form gelatine:

Place 2 g of gelatine in a 70 mm diameter silica capsule with a lid. Dry in an incubator at 100°C–105°C for 6 hours. Allow to cool in a covered capsule and a desiccator. Weigh. Let the quantity of dry residue be **p** g. Weight loss should not exceed 15%.

4.3.2 Liquid form gelatine:

Put about 10 g of colloidal gelatine solution in a 70 mm diameter silica capsule. Weigh exactly this quantity in a covered capsule and dry over a water bath at 100°C for 4 hours. Then proceed by drying in an incubator at 100°C–105°C for 3 hours. Allow to cool in a covered capsule and a dessicator. Weigh the amount of dry residue. Given **p** g of this quantity. In relation to 100 g of the colloidal solution, the dry residue must reach a minimum of 5%.

All the limits set above are for the dry product.

4.4 Ashes

Incinerate the dry residue from point 4.3 by slowly heating to 600°C in a muffle furnace after sprinkling gelatine with 0.2 to 0.3g of paraffin without ashes to avoid over spilling. Total ash content should not exceed 2.0%.

4.5 Preparation of test trial solution

After being weighed, dissolve ashes in 2 ml of concentrated hydrochloric acid (R) and 10 ml of water. Heat to activate the dissolving and add distilled water until a volume equal to 25 times the weight of dried gelatine is reached. 1 ml of this solution contains mineral matter of 0.04 g of dried gelatine.

4.6 Iron

Add 1 ml of concentrated hydrochloric acid (R), one drop of concentrate potassium permanganate at 1% (R), 2 ml of potassium thiocyanate at 5% (R) to 10 ml of the test trial solution (4.5).

If a red colouration appears, it must be lighter than the control sample prepared with 2 ml of iron solution (III) at 0.010 g per litre (R), 5.2 ml of water and the same amounts of concentrated hydrochloric acid (R) and potassium thiocyanate at 5% (R).

Iron content should be less than 50 mg/kg.

It is also possible to determine iron by atomic absorption spectrophotometry. (See method described in Chapter II of the International Oenological Codex).

4.7 Chromium

Put 10 ml of test trial solution (4.5), 1 ml of ammonia persulfate solution at 15% (R), 0.5 ml of silver nitrate solution at 1% into a 50 ml conical flask. Heat and add potassium permanganate solution at 3% (R) drop by drop until the solution reaches a stable pink colour. Add a couple more drops and simmer 10 minutes. If the solution changes colour while boiling, add more potassium permanganate. After 10 minutes, add 1/10 diluted hydrochloric acid (R) until the solution is completely discoloured.

After cooling, transfer to a 20 ml graduated flask and add 2 ml of newly made 0.05% diphenylcarbazide solution in alcohol (R). Bring to 20 ml.

If a purplish red colouration appears, it must be lighter than the colour obtained when treating 4 ml of potassium dichromate solution at 0.001g of chrome per litre with 2 ml of sulphuric acid at 5% (R), 5 ml of distilled water, and after mixing add 2 ml of 0.05% diphenylcarbazide solution in alcohol (R) and bringing it up to 20 ml.

Chromium content should be less than 10 mg/kg.

It is also possible to determine chrome by atomic absorption spectrophotometry. (See method described in Chapter II of the International Oenological Codex).

4.8 Copper

Put 2.5 ml of test trial solution (4.5) in a test tube and add 7.5 ml of water, 0.5 ml of hydrochloric citric solution (R), 1 ml 5M ammonia hydroxide (R), 0.5 ml of sodium diethyldithiocarbamate reagent (R). If a yellow colouration appears, it must not be darker than the solution obtained when adding the same volumes of the same reagents to 3.5 ml of a copper solution at 1 mg per litre (R) brought to 10 ml.

Copper content should be below 30 mg/kg.

It is also possible to determine copper by atomic absorption spectrophotometry (See method described in Chapter II of the International Oenological Codex).

4.9 Zinc

Put 3.75 ml of distilled water, 5 ml of buffer acetate solution (R), 1 ml of sodium thiosulfate solution at 25% (m/v) (R), 5 ml of dithizone solution at 25 mg per litre in the dichloromethane (R) in 1.25 ml of test trial solution (4.5). Shake for 2 minutes. Separate the organic phase. The colouration must be lighter than the colour obtained when treating the same volumes of the same reagents, 2.5 ml of zinc solution at 1 mg per litre (R).

Zinc content should be less than 50 mg/kg.

It is also possible to determine zinc by atomic absorption spectrophotometry. (See method described in Chapter II of the International Oenological Codex).

4.10 Lead

Using the test trial solution (4.5), determine the lead according to the method in described in Chapter II of the International Oenological Codex by atomic absorption spectrophotometry.

Lead content should be less than 5 mg/kg.

4.11 Mercury

Determine the mercury according to the method described in Chapter II of the International Oenological Codex by atomic absorption spectrophotometry.

Mercury content should be less than 0.15 mg/kg.

4.12 Arsenic

Determine the arsenic according to the method in Chapter II of the International Oenological Codex by atomic absorption spectrophotometry.

Arsenic content should be less than 1 mg/kg.

4.13 Cadmium

Determine the cadmium according to the method described in Chapter II of the International Oenological Codex by atomic absorption spectrophotometry.

Cadmium content should be less than 0.5 mg/kg.

4.14 Determining total nitrogen

Determine the total nitrogen according to the method in Chapter II of the International Oenological Codex. Total nitrogen must be more than 14% of the weight of dry gelatine.

4.15 Sulphur dioxide

Gelatine in dried form

Sulphur dioxide, freed by a little excess of phosphoric acid, starts to boil under the reflux of a flow of nitrogen. Which is oxidised and set by a hydrogen peroxide solution and measured by an acid meter in the presence of bromophenol blue, according to the reference method in the Compendium of International Methods of Analysis of Wines and Musts. This is done with a sample of 2 g of solid gelatine and on 10 ml of diluted solution at 10% of gelatine. Sulphur dioxide content should not exceed 50 mg/kg.

Gelatine in colloidal solution form

Liquid forms are stabilised with SO₂ and should not contain benzylic alcohol; sulphur dioxide content should not exceed 4 g/litre.

4.16 Urea

Determine urea using the Boehringer enzymatic method.

Content should be less than 2.5 g/kg.

4.17 Bacteria monitoring

Proceed as is indicated in Chapter II of the International Oenological Codex.

Limit: total viable micro-organisms: less than 10^4 CFU/g

4.18 *Escherichia coli*

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence checked on a sample of 1 g.

4.19 *Salmonella*

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence of salmonella is checked on a 25 g sample.

4.20 Coliforms

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence of coliform bacteria is checked on a 1 g sample.

4.21 Spores of anaerobic sulphite-reducing micro-organisms *

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence is checked on a 1 g sample.

4.22 *Clostridium perfringens* spores *

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence is checked on a 1 g sample.

4.23 Staphylococci (*Staphylococcus aureus*)

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Absence is checked on a 1 g sample.

* Method to be defined later on by the experts' group "Wine microbiology".

4.24 Yeasts

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Content limit: 10^3 CFU/g of preparation.

4.25 Total lactic bacteria

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Content limit: 10^3 CFU/ g of preparation.

4.26 Acetic bacteria

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Content limit: 10^3 CFU/ g of preparation.

4.27 Mould

Proceed with counting according to the method in Chapter II of the International Oenological Codex.

Content limit: 10^3 CFU/g of preparation.

5. STORAGE

Solid gelatine must be stored in closed containers or in a humidity-proof bag under temperate conditions.

Gelatine in ready-to-use colloidal solutions may contain preservatives authorised in wines and their concentrations must be indicated on the label.

GRAPE SUGAR
(Oeno 47/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Grape sugar is obtained exclusively from grape musts. The addition of grape sugar to wine is subject to regulation.

The label, or, when this is absent, the documentation accompanying the containers of grape sugar, must cite the sugar percentage.

2. PROPERTIES

Syrupy, milk-white or slightly yellowish liquid with a sugary flavor.

Refraction index at 20 °C	1.42410-1.46663
Total sugar in terms of invert sugar	63% (m/m) minimum
Absorbance at 425 nm under 1 cm at 25° Brix	maximum 0.100
pH at 25° Brix	maximum 5 *
Titration acidity in mEq/kg of sugar	maximum 15 *
Sucrose	negative
	by recommended method
Sulfur dioxide in mg/kg of sugar	maximum 25
Folin-Ciocalteu index at 25° Brix	maximum 6
Total cations in mEq/kg of sugar	maximum 8
Conductivity at 25° Brix in Micro-Siemens/cm (μScm^{-1})	maximum 120
5-(hydroxymethyl)furfural in mg/kg sugar	maximum 25
Residual ethanol in g/kg sugar	maximum 8
Heavy metals in mg/kg grape sugar expressed in terms of lead	less than 10
No antiseptics and anti-fermenting agents	

1° Brix = 1 g of sugar in 100 g of solution

* after vacuum removal of the carbon dioxide

3. TESTS

3.1. Preparing the Sample

Drawing samples for the various different analyses is difficult; therefore, the following two dilutions are recommended:

3.1.1 Principal Solution I - for the following tests: titration acidity, total sulfur dioxide and total cations

Weigh exactly 200 g of grape sugar. Fill to 500 ml with water.

3.1.2 Principal Solution II - necessary for the following tests: Folin-Ciocalteu index, pH, conductivity, sucrose test and absorbance at 425 nm.

Dilute the grape sugar with water until it has a concentration of $25^{\circ} \pm 0.5^{\circ}$ Brix (25 g of sugar in 100 g of solution).

3.2. Refraction Index at 20 °C (total sugars)

3.2.1. Equipment:

The refractometer used gives the following, based on type of graduation:

- 0.1% by mass of sucrose (or dry matter or Brix degrees)
- the 5th decimal of the index of refraction

The refractometer used should be equipped with a thermometer (+ 10 °C at + 30 °C).

3.2.2. Procedure Method:

Place two drops of grape sugar on the surface of the fixed prism. Lower the moving prism and point the instrument toward a light source that illuminates the graduated scale. Observe the line of separation on this scale between a lower clear zone and an upper dark. Read the graduation line at which this line of separation occurs and record the temperature in °C.

3.2.3. Calculation:

If the device is graduated in percentage (m/m) of sucrose (or dry matter or Brix degrees), the measurement converted to 20 °C using Table 2 is recorded in Table 1 which provides (Column 3) total sugar content in percent (m/m) expressed in terms of sugar.

If the device is graduated by refraction index, the index measured at t °C is used to obtain the corresponding value in percent of sucrose (m/m) at t °C in Table 1 (Column 1). This value as expressed at 20 °C using the temperature correction table N° 2, transferred to Table 1, which, in Column 3, gives the total sugar number in percent (m/m) of invert sugar.

To obtain the refraction index at 20 °C, refer to the total sugar content expressed in terms of invert sugar in Table 1.

3.2.4. Recording the Findings:

Total sugar content is expressed parts per 100 by mass of sucrose and is recorded with a decimal.

The refraction index at 20 °C is expressed to 5 decimal places.

3.3. Absorbance of a 25° Brix Solution at 425 nm

Prior to taking the measurements, filter Principal Solution II using a membrane with a porosity of 0.45 µm.

The absorbance of Principal Solution II (25° Brix) is measured in a 1 cm vessel at 425 nm.

3.4. Measuring pH

Measure the pH of Principal Solution II (25° Brix) at 20 °C. Take at least two measurements on the same sample. Take as the finding the arithmetic mean of the two measurements, which should not differ by more than 0.05.

3.5. Titration Acidity

Place 10 ml of Principal Solution I in a cylindrical vessel (3.1.1). Add 0.1M (or 0.01M) sodium hydroxide solution until the pH, as measured with a glass electrode, equals 7.0 at 20 °C.

The sodium hydroxide solution should be added slowly and the solution should be stirred constantly.

1 ml 0.1M NaOH = 7.5 mg of tartaric acid or 0.1 mEq

Expressing the findings:

In mEq per kilogram of total sugar, with one decimal place.

3.6. Sucrose Test by Thin Layer Chromatography

Equipment and reagents (R) (see Annex).

3.6.1. Preparing the Sample

Dilute Principal Solution II (25° Brix) to 1/4: 25 ml are topped off to 100 ml with water in a volumetric flask.

3.6.2. Obtaining the Chromatogram

Deposit 10 µl of the sample and 10 µl of the reference solution on the start line of the plate (R), this line being 2 cm wide.

After positioning the substances, place the plate in the developing chamber containing the solvent (R).

Let the solvent migrate to a height of 16 cm from the starting line.

Dry the plate in an air current after removing it from the vessel. Place it in an oven at 105 °C for 15 minutes, parallel to the air current, after grinding the developer reagent.

An orange-yellow colored stain appears in the presence of sucrose. Its R_f is identical to that of sucrose in the reference solution. Glucose and fructose produce yellow-orange stains whose R_f is greater than that of the sucrose stain.

If an orange-yellow stain appears, it should not be more intense than that obtained by the sucrose benchmark solution.

It is also possible to use high-performance liquid or gas chromatography techniques. The method thus implemented should allow detection of at least 2g sucrose per kg of grape sugar.

3.7. Sulfur Dioxide

Place 25.0 ml of Principal Solution I and 5 ml of orthophosphoric acid (25%) (R) in the distillation device and proceed as indicated in the reference method detailed in the Compendium.

3.8. Folin-Ciocalteu Index of the 25° Brix Solution

Place the following, in order, in a 100 ml volumetric flask:

- 5 ml of Principal Solution II
- 50 ml water
- 5 ml Folin-Ciocalteu reagent (R)
- 20 ml of sodium carbonate solution (R)

Fill to the 100 ml level with water. Stir to homogenize. Wait 30 minutes for the reaction to stabilize.

Determine absorbance at 750 nm in 1 cm as compared with a control prepared with water instead of Principal Solution II.

Expressing the results:

Express the results in the form of an index obtained by multiplying the absorbance by 16 in order to obtain a scale comparable to that used for wines.

3.9. Total Cations

Place approximately 10 ml of cation-exchange resin in acid form in a column with an inner diameter of 1 cm. Wash with water until the acidity disappears from the wash water, as indicated by pH test paper.

Pour 100 ml of Principal Solution I (3.1.1) (rate: one drop/second). Wash with 50 ml of water and titrate the acidity in the effluent using 0.1M NaOH solution until a pH of 7.0 is reached. Let n be the volume of sodium hydroxide, in ml, that has been poured.

Calculation:

$$Q \text{ mEq/kg of grape sugar} = 2.5n$$

Total cations in mEq/kg of grape sugar = Q - titration acidity in mEq/kg of grape sugar.

Expressing the results:

Total cations are expressed in mEq/kg of total sugar to one decimal place.

3.10. Conductivity of the Solution at 25° Brix

Bring Principal Solution II to a temperature of 20 °C by immersing it in a water bath and proceed as indicated in Annex II (sugars).

Expressing the results:

Conductivity is expressed in micro-Siemens per cm ($\mu\text{S}\cdot\text{cm}^{-1}$) at 20 °C without a decimal place and is for a 25° Brix solution of grape sugar.

3.11. 5-(Hydroxymethyl)furfural(HMF)

Principle :

HMF is determined by HPLC (high-performance liquid chromatography).

Equipment (cited as an example)

Instrument specifications :

- chromatograph equipped with an isocratic pump
- UV/visible light detection apparatus
- Column : grafted silicon dioxide C 18 (20 cm ; 4.6 mm ; 5 μm)
- Liquid phase : ultrafiltered demineralized water/methanol/acetic acid (80, 10, 3: v/v/v)
- Flow rate : 0.5 ml/mn
- Detection wavelength : 280 nm
- Volume injected : 20 μl

Preparation of the Reference Solution

In a 100 ml volumetric flask, add 20 mg HMF preliminarily weighed at approximately 0.1 mg, and fill to the gauge line with ultrafiltered demineralized water.

Place 10 of this solution in a 100 ml volumetric flask and fill with ultrafiltered demineralized water (or the equivalent having a resistivity of 18 M Ω , for example). The solution will have a concentration of 20 mg/l and must be prepared daily.

Preparation of the samples :

The samples and the reference solution are injected after filtration on a membrane (pore diameter : 0.45 μm).

Working Method :

The chromatographic column is filled with the liquid phase for approximately 30 minutes before injecting the samples.

3.12. Heavy Metals

Dissolve 12 g of grape sugar in 15 ml of water. Place 10 ml of this solution in a test tube with 2 ml of pH 3.5 buffer solution and 1.2 ml of thioacetamide reagent (R). No precipitate should form. If a brown coloration appears, it should be less intense than that obtained in a control prepared as indicated in the Annex.

(Heavy metal content, expressed in terms of lead, should be less than 10 mg/kg).

3.13. Lead

Using the method set forth in the Compendium, quantitatively analyze lead in the Principal Solution I (3.1.1). (Lead content should be less than 1 mg/kg.)

3.14. Mercury

Using the method set forth in the annex, quantitatively analyze mercury in the Principal Solution I (3.1.1). (Mercury content should be less than 0.3 mg/kg.)

3.15. Arsenic

Using the method described in the annex, quantitatively analyze arsenic in the Principal Solution I (3.1.1). (Arsenic concentration should be less than 0.5 mg/kg.)

3.16. Ethanol

3.16.1. Principle

Simple distillation of the alkalized sample. The alcohol is oxidized in an acidic medium using a potassium dichromate solution in acetic acid.

The dichromate excess is titrated using an iron (III) solution and ammonium.

3.16.2. Method

Distillation: Separate out the alcohol by distillation using a device which does not result in a loss of alcohol greater than 0.02% by volume during distillation.

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Place 100 g of grape sugar and 100 ml of water in the distillation vessel. Collect the distillate in a 100 ml volumetric flask and fill to the gauge line with water.

Oxidation: Take a 300 ml flask with an emery stopper with a wide neck ending in a flared portion allowing the neck to be washed without loss. Place 20.0 ml of titrated potassium dichromate solution (33.79 g/l), 20 ml of sulfuric acid diluted to 1/2 (v/v) in the flask and agitate. Add 20 ml of distillate. Place the stopper in the flask, shake, and wait at least 30 minutes while shaking from time to time.

Volumetric analysis: Add 4 drops of orthophenanthroline reagent (0.695 g iron (II) sulfate dissolved in 100 ml of water, to which 1.485 g of monohydrated orthophenanthroline is added, then heat and agitate).

Titrate the excess dichromate by pouring the iron (II) sulfate and ammonium solution (R). Stop adding iron (II) solution as soon as the indicator turns from blue-green to chestnut brown.

If the indicator turns too far, return to the exact color by using a potassium permanganate solution (1.083 g/l). One-tenth of the volume of the solution used is subtracted from the volume of the iron (II) solution, where n is this difference.

Perform the same operation in a similar flask containing the same volumes of the same reagents, but in which the 20.0 ml of distillate are replaced with 20.0 ml of water. Let n be the volume of iron (II) solution used.

3.16.3. Calculations

A quantity of n' ml of iron (II) solution reduces 20 ml of dichromate solution, which oxidize 157.85 mg ethanol.

One milliliter of iron (II) solution has the same reducing power as

$$157.85/n' \text{ mg ethanol}$$

$n' - n$ ml of iron (II) solution have the same reducing power as

$$157.85 \cdot n' - n/n' \text{ mg ethanol}$$

If 100 g of grape sugar have been distilled to 100 ml and 20 ml have been treated, the concentration of pure alcohol is:

Ethanol in g/kg grape sugar = $7.892 \frac{n-n'}{n'}$

3.16.4. *Expressing the results:* in g per kilogram of grape sugar to 1 decimal place.

3.17. Meso-Inositol

Gas phase chromatography of a silyl-containing derivative.

N.B. : The information given above is provided for informational purposes. There are other techniques for deriving sugars and polyhydroxy alcohols, and chromatographic methods for determining meso-inositol concentrations

3.17.1. Preparing the sample:

Dilute 5 g of grape sugar in 50 ml of water. Dry 50 µl of the dilution and 50 µl of a methyl D-glucopyranoside solution in a concentration of 1 g/liter, (internal standard) under a vacuum in a small 2 ml flask.

Dissolve the residue with 100 µl of pyridine. Add 100 µl of trimethylchlorosilane. Seal the small flask with a teflon stopper and heat at 80 °C for 1 hour. Inject 1 µl with division of the injected volume to 1/60.

3.17.2. Separation

Column: apolar capillary type of fused silica 25 m long and inner diameter of 0.2 mm.

Supporting Gas: helium, 1 ml/minute

Injector and detector: 280 °C

Column temperature: 60-250 °C, at 4 °C per minute, then isothermal at 250 °C.

3.17.3. Expressing the results: g per kg of sugar

4. STORAGE

Grape sugar must be stored in impermeable containers and at ambient temperature from the time it is made.

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ANNEX 1 (sugars)

TABLE 1

Sugar Content in Musts Using Refractometry

Sucrose % (m/m)	Index of refraction at 20°C	Density at 20°C	Sugars in g/l	Sugars in g/kg
50.0	1.42008	1.2342	627.6	508.5
50.1	1.42029	1.2348	629.3	509.6
50.2	1.42050	1.2355	630.9	510.6
50.3	1.42071	1.2362	632.4	511.6
50.4	1.42092	1.2367	634.1	512.7
50.5	1.42113	1.2374	635.7	513.7
50.6	1.42135	1.2381	637.3	514.7
50.7	1.42156	1.2386	638.7	515.7
50.8	1.42177	1.2391	640.4	516.8
50.9	1.42198	1.2396	641.9	517.8
51.0	1.42219	1.2401	643.4	518.8
51.1	1.42240	1.2406	645.0	519.9
51.2	1.42261	1.2411	646.5	520.9
51.3	1.42282	1.2416	648.1	522.0
51.4	1.42304	1.2421	649.6	523.0
51.5	1.42325	1.2427	651.2	524.0
51.6	1.42347	1.2434	652.9	525.1
51.7	1.42368	1.2441	654.5	526.1
51.8	1.42389	1.2447	656.1	527.1
51.9	1.42410	1.2454	657.8	528.2
52.0	1.42432	1.2461	659.4	529.2
52.1	1.42453	1.2466	661.0	530.2
52.2	1.42475	1.2470	662.5	531.3
52.3	1.42496	1.2475	664.1	532.3
52.4	1.42517	1.2480	665.6	533.3
52.5	1.42538	1.2486	667.2	534.4
52.6	1.42560	1.2493	668.9	535.4
52.7	1.42581	1.2500	670.5	536.4
52.8	1.42603	1.2506	672.2	537.5
52.9	1.42624	1.2513	673.8	538.5
53.0	1.42645	1.2520	675.5	539.5
53.1	1.42667	1.2525	677.1	540.6
53.2	1.42689	1.2530	678.5	541.5
53.3	1.42711	1.2535	680.2	542.6
53.4	1.42733	1.2540	681.8	543.7
53.5	1.42754	1.2546	683.4	544.7
53.6	1.42776	1.2553	685.1	545.8
53.7	1.42797	1.2560	686.7	546.7
53.8	1.42819	1.2566	688.4	547.8
53.9	1.42840	1.2573	690.1	548.9
54.0	1.42861	1.2580	691.7	549.8
54.1	1.42884	1.2585	693.3	550.9
54.2	1.42906	1.2590	694.9	551.9
54.3	1.42927	1.2595	696.5	553.0
54.4	1.42949	1.2600	698.1	554.0
54.5	1.42971	1.2606	699.7	555.1
54.6	1.42993	1.2613	701.4	556.1
54.7	1.43014	1.2620	703.1	557.1
54.8	1.43036	1.2625	704.7	558.2
54.9	1.43058	1.2630	706.2	559.1

INTERNATIONAL OENOLOGICAL CODEX
GRAPE SUGAR

TABLE 1 (Cont'd)

Sucrose % (m/m)	Index of refraction at 20°C	Density at 20°C	Sugars in g/l	Sugars in g/kg
55.0	1.43079	1.2635	707.8	560.2
55.1	1.43102	1.2639	709.4	561.3
55.2	1.43124	1.2645	711.0	562.3
55.3	1.43146	1.2652	712.7	563.3
55.4	1.43168	1.2659	714.4	564.3
55.5	1.43189	1.2665	716.1	565.4
55.6	1.43211	1.2672	717.8	566.4
55.7	1.43233	1.2679	719.5	567.5
55.8	1.43255	1.2685	721.1	568.5
55.9	1.43277	1.2692	722.8	569.5
56.0	1.43298	1.2699	724.5	570.5
56.1	1.43321	1.2703	726.1	571.6
56.2	1.43343	1.2708	727.7	572.6
56.3	1.43365	1.2713	729.3	573.7
56.4	1.43387	1.2718	730.9	574.7
56.5	1.43409	1.2724	732.6	575.8
56.6	1.43431	1.2731	734.3	576.8
56.7	1.43454	1.2738	736.0	577.8
56.8	1.43476	1.2744	737.6	578.8
56.9	1.43498	1.2751	739.4	579.9
57.0	1.43519	1.2758	741.1	580.9
57.1	1.43542	1.2763	742.8	582.0
57.2	1.43564	1.2768	744.4	583.0
57.3	1.43586	1.2773	745.9	584.0
57.4	1.43609	1.2778	747.6	585.1
57.5	1.43631	1.2784	749.3	586.1
57.6	1.43653	1.2791	751.0	587.1
57.7	1.43675	1.2798	752.7	588.1
57.8	1.43698	1.2804	754.4	589.2
57.9	1.43720	1.2810	756.1	590.2
58.0	1.43741	1.2818	757.8	591.2
58.1	1.43764	1.2822	759.5	592.3
58.2	1.43784	1.2827	761.1	593.4
58.3	1.43809	1.2832	762.6	594.3
58.4	1.43832	1.2837	764.3	595.4
58.5	1.43854	1.2843	766.0	596.4
58.6	1.43877	1.2850	767.8	597.5
58.7	1.43899	1.2857	769.5	598.5
58.8	1.43922	1.2863	771.1	599.5
58.9	1.43944	1.2869	772.9	600.6
59.0	1.43966	1.2876	774.6	601.6
59.1	1.43988	1.2882	776.3	602.6
59.2	1.44011	1.2889	778.1	603.7
59.3	1.44034	1.2896	779.8	604.7
59.4	1.44057	1.2902	781.6	605.8
59.5	1.44079	1.2909	783.3	606.8
59.6	1.44102	1.2916	785.2	607.9
59.7	1.44124	1.2921	786.8	608.9
59.8	1.44147	1.2926	788.4	609.9
59.9	1.44169	1.2931	790.0	610.9

INTERNATIONAL OENOLOGICAL CODEX
GRAPE SUGAR

TABLE 1 (Cont'd)

Sucrose % (m/m)	Index of refraction at 20°C	Density at 20°C	Sugars in g/l	Sugars in g/kg
60.0	1.44192	1.2936	791.7	612.0
60.1	1.44215	1.2942	793.3	613.0
60.2	1.44238	1.2949	795.2	614.1
60.3	1.44260	1.2956	796.9	615.1
60.4	1.44283	1.2962	798.6	616.1
60.5	1.44305	1.2969	800.5	617.2
60.6	1.44328	1.2976	802.2	618.2
60.7	1.44351	1.2981	803.9	619.3
60.8	1.44374	1.2986	805.5	620.3
60.9	1.44397	1.2991	807.1	621.3
61.0	1.44419	1.2996	808.7	622.3
61.1	1.44442	1.3002	810.5	623.4
61.2	1.44465	1.3009	812.3	624.4
61.3	1.44488	1.3016	814.2	625.5
61.4	1.44511	1.3022	815.8	626.5
61.5	1.44534	1.3029	817.7	627.6
61.6	1.44557	1.3036	819.4	628.6
61.7	1.44580	1.3042	821.3	629.7
61.8	1.44603	1.3049	823.0	630.7
61.9	1.44626	1.3056	824.8	631.7
62.0	1.44648	1.3062	826.6	632.8
62.1	1.44672	1.3068	828.3	633.8
62.2	1.44695	1.3075	830.0	634.8
62.3	1.44718	1.3080	831.8	635.9
62.4	1.44741	1.3085	833.4	636.9
62.5	1.44764	1.3090	835.1	638.0
62.6	1.44787	1.3095	836.8	639.0
62.7	1.44810	1.3101	838.5	640.0
62.8	1.44833	1.3108	840.2	641.0
62.9	1.44856	1.3115	842.1	642.1
63.0	1.44879	1.3121	843.8	643.1
63.1	1.44902	1.3128	845.7	644.2
63.2	1.44926	1.3135	847.5	645.2
63.3	1.44949	1.3141	849.3	646.3
63.4	1.44972	1.3148	851.1	647.3
63.5	1.44955	1.3155	853.0	648.4
63.6	1.45019	1.3161	854.7	649.4
63.7	1.45042	1.3168	856.5	650.4
63.8	1.45065	1.3175	858.4	651.5
63.9	1.45088	1.3180	860.0	652.5
64.0	1.45112	1.3185	861.6	653.5
64.1	1.45135	1.3190	863.4	654.6
64.2	1.45158	1.3195	865.1	655.6
64.3	1.45181	1.3201	866.9	656.7
64.4	1.45205	1.3208	868.7	657.7
64.5	1.45228	1.3215	870.6	658.8
64.6	1.45252	1.3221	872.3	659.8
64.7	1.45275	1.3228	874.1	660.8
64.8	1.45299	1.3235	876.0	661.9
64.9	1.45322	1.3241	877.8	662.9

INTERNATIONAL OENOLOGICAL CODEX
GRAPE SUGAR

TABLE 1 (Cont'd)

Sucrose % (m/m)	Index of refraction at 20°C	Density at 20°C	Sugars in g/l	Sugars in g/kg
65.0	1.45347	1.3248	879.7	664.0
65.1	1.45369	1.3255	881.5	665.0
65.2	1.45393	1.3261	883.2	666.0
65.3	1.45416	1.3268	885.0	667.0
65.4	1.45440	1.3275	886.9	668.1
65.5	1.45463	1.3281	888.8	669.2
65.6	1.45487	1.3288	890.6	670.2
65.7	1.45510	1.3295	892.4	671.2
65.8	1.45534	1.3301	894.2	672.3
65.9	1.45557	1.3308	896.0	673.3
66.0	1.45583	1.3315	898.0	674.4
66.1	1.45605	1.3320	899.6	675.4
66.2	1.45629	1.3325	901.3	676.4
66.3	1.45652	1.3330	903.1	677.5
66.4	1.45676	1.3335	904.8	678.5
66.5	1.45700	1.3341	906.7	679.6
66.6	1.45724	1.3348	908.5	680.6
66.7	1.45747	1.3355	910.4	681.7
66.8	1.45771	1.3361	912.2	682.7
66.9	1.45795	1.3367	913.9	683.7
67.0	1.45820	1.3374	915.9	684.8
67.1	1.45843	1.3380	917.6	685.8
67.2	1.45867	1.3387	919.6	686.9
67.3	1.45890	1.3395	921.4	687.9
67.4	1.45914	1.3400	923.1	688.9
67.5	1.45938	1.3407	925.1	690.0
67.6	1.45962	1.3415	927.0	691.0
67.7	1.45986	1.3420	928.8	692.1
67.8	1.46010	1.3427	930.6	693.1
67.9	1.46034	1.3434	932.6	694.2
68.0	1.46060	1.3440	934.4	695.2
68.1	1.46082	1.3447	936.2	696.2
68.2	1.46106	1.3454	938.0	697.2
68.3	1.46130	1.3460	939.9	698.3
68.4	1.46154	1.3466	941.8	699.4
68.5	1.46178	1.3473	943.7	700.4
68.6	1.46202	1.3479	945.4	701.4
68.7	1.46226	1.3486	947.4	702.5
68.8	1.46251	1.3493	949.2	703.5
68.9	1.46275	1.3499	951.1	704.6
69.0	1.46301	1.3506	953.0	705.6
69.1	1.46323	1.3513	954.8	706.6
69.2	1.46347	1.3519	956.7	707.7
69.3	1.46371	1.3526	958.6	708.7
69.4	1.46396	1.3533	960.6	709.8
69.5	1.46420	1.3539	962.4	710.8
69.6	1.46444	1.3546	964.3	711.9
69.7	1.46468	1.3553	966.2	712.9
69.8	1.46493	1.3560	968.2	714.0
69.9	1.46517	1.3566	970.0	715.0

INTERNATIONAL OENOLOGICAL CODEX
GRAPE SUGAR

TABLE 1 (end)

Sucrose % (m/m)	Index of refraction at 20°C	Density at 20°C	Sugars in g/l	Sugars in g/kg
70.0	1.46544	1.3573	971.8	716.0
70.1	1.46565	1.3579	973.8	717.1
70.2	1.46590	1.3586	975.6	718.1
70.3	1.46614	1.3593	977.6	719.2
70.4	1.46639	1.3599	979.4	720.2
70.5	1.46663	1.3606	981.3	721.2
70.6	1.46688	1.3613	983.3	722.3
70.7	1.46712	1.3619	985.2	723.4
70.8	1.46737	1.3626	987.1	724.4
70.9	1.46761	1.3633	988.9	725.4
71.0	1.46789	1.3639	990.9	726.5
71.1	1.46810	1.3646	992.8	727.5
71.2	1.46835	1.3653	994.8	728.6
71.3	1.46859	1.3659	996.6	729.6
71.4	1.46884	1.3665	998.5	730.7
71.5	1.46908	1.3672	1000.4	731.7
71.6	1.46933	1.3678	1002.2	732.7
71.7	1.46957	1.3685	1004.2	733.8
71.8	1.46982	1.3692	1006.1	734.8
71.9	1.47007	1.3698	1008.0	735.9
72.0	1.47036	1.3705	1009.9	736.9
72.1	1.47056	1.3712	1012.0	738.0
72.2	1.47081	1.3718	1013.8	739.0
72.3	1.47106	1.3725	1015.7	740.0
72.4	1.47131	1.3732	1017.7	741.1
72.5	1.47155	1.3738	1019.5	742.1
72.6	1.47180	1.3745	1021.5	743.2
72.7	1.47205	1.3752	1023.4	744.2
72.8	1.47230	1.3758	1025.4	745.3
72.9	1.47254	1.3765	1027.3	746.3
73.0	1.47284	1.3772	1029.3	747.4
73.1	1.47304	1.3778	1031.2	748.4
73.2	1.47329	1.3785	1033.2	749.5
73.3	1.47354	1.3792	1035.1	750.5
73.4	1.47379	1.3798	1037.1	751.6
73.5	1.47404	1.3805	1039.0	752.6
73.6	1.47429	1.3812	1040.9	753.6
73.7	1.47454	1.3818	1042.8	754.7
73.8	1.47479	1.3825	1044.8	755.7
73.9	1.47504	1.3832	1046.8	756.8
74.0	1.47534	1.3838	1048.6	757.8
74.1	1.47554	1.3845	1050.7	758.9
74.2	1.47579	1.3852	1052.6	759.9
74.3	1.47604	1.3858	1054.6	761.0
74.4	1.47629	1.3865	1056.5	762.0
74.5	1.47654	1.3871	1058.5	763.1
74.6	1.47679	1.3878	1060.4	764.1
74.7	1.47704	1.3885	1062.3	765.1
74.8	1.47730	1.3892	1064.4	766.2
74.9	1.47755	1.3898	1066.3	767.2
75.0	1.47785	1.3905	1068.3	768.3

INTERNATIONAL OENOLOGICAL CODEX
GRAPE SUGAR

TABLE 2
Correction of the Conventional Sugar Mass Titer as a Function of
Temperature
Mass Titer Measured in %

Température °C	10	15	20	25	30	35	40	45	50	55	60	65	70	75	
5	-0,82	-0,87	-0,92	-0,95	-0,99										
6	-0,80	-0,82	-0,87	-0,90	-0,94										
7	-0,74	-0,78	-0,82	-0,84	-0,88										
8	-0,69	-0,73	-0,76	-0,79	-0,82										
9	-0,64	-0,67	-0,71	-0,73	-0,75										
10	-0,59	-0,62	-0,65	-0,67	-0,69	-0,71	-0,72	-0,73	-0,74	-0,75	-0,75	-0,75	-0,75	-0,75	
11	-0,54	-0,57	-0,59	-0,61	-0,63	-0,64	-0,65	-0,66	-0,67	-0,68	-0,68	-0,68	-0,68	-0,67	
12	-0,49	-0,51	-0,53	-0,55	-0,56	-0,57	-0,58	-0,59	-0,60	-0,60	-0,61	-0,61	-0,60	-0,60	
13	-0,43	-0,45	-0,47	-0,48	-0,50	-0,51	-0,52	-0,52	-0,53	-0,53	-0,53	-0,53	-0,53	-0,53	
14	-0,38	-0,39	-0,40	-0,42	-0,43	-0,44	-0,44	-0,45	-0,45	-0,46	-0,46	-0,46	-0,46	-0,45	
15	-0,32	-0,33	-0,34	-0,35	-0,36	-0,37	-0,37	-0,38	-0,38	-0,38	-0,38	-0,38	-0,38	-0,38	
16	-0,26	-0,27	-0,28	-0,28	-0,29	-0,30	-0,30	-0,30	-0,31	-0,31	-0,31	-0,31	-0,31	-0,30	
17	-0,20	-0,20	-0,21	-0,21	-0,22	-0,22	-0,23	-0,23	-0,23	-0,23	-0,23	-0,23	-0,23	-0,23	
18	-0,13	-0,14	-0,14	-0,14	-0,15	-0,15	-0,15	-0,15	-0,15	-0,15	-0,15	-0,15	-0,15	-0,15	
19	-0,07	-0,07	-0,07	-0,07	-0,07	-0,08	-0,08	-0,08	-0,08	-0,08	-0,08	-0,08	-0,08	-0,08	
20	0	R É F É R E N C E													0
21	+0,07	+0,07	+0,07	+0,07	+0,08	+0,08	+0,08	+0,08	+0,08	+0,08	+0,08	+0,08	+0,08	+0,08	
22	+0,14	+0,14	+0,15	+0,15	+0,15	+0,15	+0,16	+0,16	+0,16	+0,16	+0,16	+0,16	+0,15	+0,15	
23	+0,21	+0,22	+0,22	+0,23	+0,23	+0,23	+0,23	+0,24	+0,24	+0,24	+0,24	+0,23	+0,23	+0,23	
24	+0,29	+0,29	+0,30	+0,30	+0,31	+0,31	+0,31	+0,32	+0,32	+0,32	+0,32	+0,31	+0,31	+0,31	
25	+0,36	+0,37	+0,38	+0,38	+0,39	+0,39	+0,40	+0,40	+0,40	+0,40	+0,40	+0,39	+0,39	+0,39	
26	+0,44	+0,45	+0,46	+0,46	+0,47	+0,47	+0,48	+0,48	+0,48	+0,48	+0,48	+0,47	+0,47	+0,46	
27	+0,52	+0,53	+0,54	+0,55	+0,55	+0,56	+0,56	+0,56	+0,56	+0,56	+0,56	+0,55	+0,55	+0,54	
28	+0,60	+0,61	+0,62	+0,63	+0,64	+0,64	+0,64	+0,65	+0,65	+0,64	+0,64	+0,64	+0,63	+0,62	
29	+0,68	+0,69	+0,70	+0,71	+0,72	+0,73	+0,73	+0,73	+0,73	+0,73	+0,72	+0,72	+0,71	+0,70	
30	+0,77	+0,78	+0,79	+0,80	+0,81	+0,81	+0,81	+0,82	+0,81	+0,81	+0,81	+0,80	+0,79	+0,78	
31	+0,85	+0,87	+0,88	+0,89	+0,89	+0,90	+0,90	+0,90	+0,90	+0,90	+0,89	+0,88	+0,87	+0,86	
32	+0,94	+0,95	+0,96	+0,97	+0,98	+0,99	+0,99	+0,99	+0,99	+0,98	+0,97	+0,96	+0,95	+0,94	
33	+1,03	+1,04	+1,05	+1,06	+1,07	+1,08	+1,08	+1,08	+1,07	+1,07	+1,06	+1,05	+1,03	+1,02	
34	+1,12	+1,13	+1,15	+1,15	+1,16	+1,17	+1,17	+1,17	+1,16	+1,15	+1,14	+1,13	+1,12	+1,10	
35	+1,22	+1,23	+1,24	+1,25	+1,25	+1,26	+1,26	+1,25	+1,25	+1,24	+1,23	+1,21	+1,20	+1,18	
36	+1,31	+1,32	+1,33	+1,34	+1,35	+1,35	+1,35	+1,35	+1,34	+1,33	+1,32	+1,30	+1,28	+1,26	
37	+1,41	+1,42	+1,43	+1,44	+1,44	+1,44	+1,44	+1,44	+1,43	+1,42	+1,40	+1,38	+1,36	+1,34	
38	+1,51	+1,52	+1,53	+1,53	+1,54	+1,54	+1,53	+1,53	+1,52	+1,51	+1,49	+1,47	+1,45	+1,42	
39	+1,61	+1,62	+1,62	+1,63	+1,63	+1,63	+1,63	+1,62	+1,61	+1,60	+1,58	+1,56	+1,53	+1,50	
40	+1,71	+1,72	+1,72	+1,73	+1,73	+1,73	+1,72	+1,71	+1,70	+1,69	+1,67	+1,64	+1,62	+1,59	

(N.B. : In the French original reproduced here, commas should be replaced with decimal points)

TABLE 3

**Conductivity Corrections for Temperatures Other Than
20°C in μ siemens/cm⁻¹**

	Temperatures									
	20,2	20,4	20,26	20,8	21,0	21,2	21,4	21,6	21,8	22,0(1)
Conductivity	19,8	19,6	19,4	19,2	19,0	18,8	18,6	18,4	18,2	18,0(2)
0	0	0	0	0	0	0	0	0	0	0
50	0	0	1	1	1	1	1	2	2	2
100	0	1	1	2	2	3	3	3	4	4
150	1	1	2	3	3	4	5	5	6	7
200	1	2	3	3	4	5	6	7	8	9
250	1	2	3	4	6	7	8	9	10	11
300	1	3	4	5	7	8	9	11	12	13
350	1	3	5	6	8	9	11	12	14	15
400	2	3	5	7	9	11	12	14	16	18
450	2	3	6	8	10	12	14	16	18	20
500	2	4	7	9	11	13	15	18	20	22
550	2	5	7	10	12	14	17	19	22	24
600	3	5	8	11	13	16	18	21	24	26

(1) Subtract the correction

(2) Add the correction

(N.B. : In the French original reproduced here, commas should be read as decimal points.

ANNEX II (sugars)

CONDUCTIVITY

1. APPARATUS

Conductometer allowing measurement in the range of 1-1000 micro-Siemens per cm.

2. EQUIPMENT

200 ml volumetric flasks

Water bath to bring samples to a temperature of 20 °C

3. REAGENTS

Water: specific conductivity should be less than 2 micro-Siemens per cm

KCl reference solution (R)

4. WORKING METHOD

Bring the solution to be analyzed to a temperature of 20 °C by immersing it in the water bath. Wash the measurement cells of the conductometer twice with the solution to be tested. Measure conductivity expressed in micro-Siemens per cm at 20 ± 0.1 °C.

5. CALCULATION

5.1. Temperature Correction

If the apparatus is not equipped with a temperature compensator, correct conductivity using Table 3 (**Annex 1 (sugars)**).

If the temperature is below 20 °C, add the correction.

If the temperature is above 20 °C, subtract the correction.

5.2. Water Correction

Subtract one-half of the conductivity, as measured in the water used for the dilution at a temperature of 20°C, from the conductivity of the prepared sample.

ANNEX III (sugars)

**SUCROSE DETERMINATION IN GRAPE SUGAR
USING THIN LAYER CHROMATOGRAPHY**

1. PRINCIPLE UNDERLYING THE METHOD

Sucrose is separated out from the other sugars using thin layer chromatography and is detected using urea/hydrochloric acid reagent.

2. EQUIPMENT AND REAGENTS

- Chromatographic plates covered with a thin layer of cellulose (thickness of 0.1 mm)
- Liquid phase: methylene chloride + acetic acid + ethanol + methanol + water (50 + 25 + 9 + 6 + 10)
- Developing agent: urea: 5 g + 20 ml of 2M HCl + 100 ml ethanol
- Reference solution which contains the following per liter:

Glucose	160 g
Fructose	160 g
Sucrose	0.8 g

3. PREPARING THE SAMPLE

Use Principal Solution I (3.1.1) (200 g of grape increased to a volume of 500 ml with water).

4. CHROMATOGRAPHY

Place the following 2.5 cm from the lower edge the plate:

0.5 µl of the sample

0.5 µl of the reference solution

Place the plate in the chromatography tank saturated by steam from the liquid phase. Let the liquid travel to a level 1 cm from the upper edge. Remove the plate and dry under hot air. Repeat this migration procedure twice, drying the plate each time.

Uniformly spray 15 ml of the developing agent over the plate and maintain at 100 °C in the oven for 5 minutes.

The sucrose and fructose will appear as orange stains.

GUM ARABIC
Gumme arabicum
Acaciae gummi
SIN No. 414
(Oeno 27/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Gum arabic is a gummy exudation which hardens in air and flows naturally or through cuts made in tree trunks and branches of the L. *Acacia senegal* L. Willdenow and other African *Acacia* species. It is composed of spherical tear drop-shaped globules, or sometimes irregular oval shapes with a diameter of 1-3 cm.

Gum arabic exists in powder form or in colloidal solution.

The product is used to improve the stability of bottled wine.

Gum arabic is composed of a polysaccharide rich in galactose and arabinose along with a small protein fraction which gives its stabilizing power with respect to the precipitation of coloring substances and iron or copper breakdown.

There are limits imposed on the quantity of gum arabic used in wine.

2. LABELING

The label should indicate the gum arabic solution concentration and sulfur dioxide content (there are limits imposed on the sulfur dioxide content in wine), as well as its safety and storage conditions.

3. PROPERTIES

Gum arabic tear drops are relatively friable and break cleanly into fragments. Whole tear drops often have a small cavity in the center.

Powdered gum arabic is odorless, tasteless, and has a white or yellow transparent color and glassy luster. It dissolves slowly in twice its weight and leaves only a slight residue of vegetable debris. It is insoluble in alcohol.

Gum arabic in solution is a yellowish-white viscous, translucent liquid which is slightly acidic. It precipitates abundantly when an equal volume of ethanol is added.

4. TESTS

4.1. Desiccation loss

4.1.1. Powdered gum arabic

Place 5 g of gum arabic in a silica dish with a diameter of 70 mm. Place in a n oven at 100-105 °C for 5 hours. Weight loss should be no greater than 15 pp 100.

4.1.2. Gum arabic in solution

Place 10 g of gum arabic solution in a silica dish with a diameter of 70 mm. Place in a water bath at 100 °C for 4 hours, then in an oven set to 100-105° C for 3 hours. The quantity of dry residue should be at least 10 pp 100.

The limiting values indicated below are for dry product.

4.2. Ash

Incinerate the dry residue at 550-600 °C. Ash content should not be greater than 4 pp 100.

4.3. Preparing the Solution for Tests

The ash from 5 g of powdered gum arabic or from a weight of solution corresponding to 5 g of solid gum arabic are taken up by 2 ml of concentrated hydrochloric acid (R). Place in a 100 °C water bath with a stirring apparatus to ensure solubilizing. Decant in a 50 ml volumetric flask and bring the volume to 50 ml using wash water from the dish used during incineration.

4.4. Iron

Add 1 drop of 1 pp 100 potassium permanganate (R), 1 ml of concentrated hydrochloric acid (R) and 2 ml 2 ml of 5 pp 100 potassium thiocyanate to 10 ml of the solution prepared for tests under paragraph 4.3. The resulting coloration should be less intense than that of a control prepared with 6 ml of an iron (III) solution with 10 mg of iron per liter (R), 4 ml of water, 1 ml of concentrated hydrochloric acid (R) and 2 ml of 5 pp 100 potassium thiocyanate (R). (Iron content should be less than 60 mg/kg).

Iron content can also be quantified by atomic absorption spectrometry using the technique explained in the Compendium.

4.5. Cadmium

Using the techniques described in the annex, determine the cadmium content in the test solution prepared in accordance with Par. 4.3. (Content should be less than 1 mg/kg.)

4.6. Lead

Using the techniques described in the Compendium, determine the lead content in the test solution prepared in accordance with Par. 4.3. (Content should be less than 5 mg/kg.)

4.7. Mercury

Using the techniques described in the annex, determine the mercury content in the test solution prepared in accordance with Par. 4.3. (Content should be less than 1 mg/kg.)

4.8. Arsenic

Mineralize 0.5 g of dry gum arabic using the nitrosulfuric method and test for arsenic using the method described in the Annex. (Arsenic content should be less than 3 mg/kg).

4.9. Total nitrogen

Place 5 g of gum arabic in a 300 ml mineralization cucurbit with 15 ml of concentrated sulfuric acid (R) and 2 g of mineralization catalyst. Proceed with the quantitative analysis as indicated in the annex.

For gum arabic in solution, weigh an amount corresponding to 5 g dry residue. Evaporate until almost dry, then proceed as described before.

(Nitrogen content should be less than 4 g/kg).

Nitrogen content should be between:

0.25% and 0.4% (m/m) for Senegal Acacia gum and

0.10% and 0.20% (m/m) for Seyal Acacia gum.

4.10. Starch and Dextrin

Bring 20 ml of solution containing 2 g of dry gum arabic to a boil. Cool. Add 0.2 ml of 0.05M iodine. No blue or red-brown coloration should appear.

4.11. Tannin

Add 0.1 ml of iron (III) sulfate (R) to 10 ml of solution containing 1 g of dry gum arabic. A gelatinous precipitate will form, but neither the precipitate nor the liquid should become dark blue.

4.12. Rotatory power

Specific rotatory power is measured at 589 nm (sodium line) and for a solution with 1 g/ml of gum and a length of 1 dm.

$$\begin{array}{lll} - 26^{\circ} \leq & [\alpha]_D^{20^{\circ}\text{C}} & \leq - 34^{\circ}, \text{ for Senegal Acacia gum} \\ 40 \leq & [\alpha]_D^{20^{\circ}\text{C}} & \leq 50^{\circ}, \text{ for Senegal seya gum.} \end{array}$$

4.13. Salmonella

A 1 g sample must be free from salmonellosis (determination procedure described in the annex).

4.14. *Escherichia coli*

A 1 g sample must be free from *Escherichia coli* (determination procedure described in the annex).

4.15. Hydrolytic Products

Mannose, xylose, and galacturonic acid should not be present (as determined by chromatography).

4.16. Efficacy Test for Gum Arabic

4.16.1 Principle

Determine the quantity of gum arabic required to prevent flocculation of a colloidal iron (III) hexacyanoferrate (II) solution in an aqueous-alcoholic medium by calcium salt.

4.16.2. Products

Crystallized tartaric acid: Molar weight = 150.05

Purified potassium sulfate (K_2SO_4): Molar weight = 174.25

Dihydrate calcium chloride ($CaCl_2 \cdot 2H_2O$): Molar weight = 143.03

Crystallized iron (III) chloride ($FeCl_3 \cdot 6H_2O$): Molecular weight = 270.32

Potassium hexacyanoferrate (II) ($K_4[Fe(CN)_6]$): Molecular weight = 422.4

Metatartaric acid

1M sodium hydroxide solution

Ethanol, 95% by volume

20 volumes hydrogen peroxide solution

4.16.3. Protocol

Gum arabic solution in a concentration of 5 g/l (A)

Dissolve 5 g of gum arabic in 100 ml of distilled water, then dilute this solution to 1/10 strength using distilled water.

Iron (III) solution in a concentration of 2.5 g iron/l (B)

Weigh exactly 1.21 g of iron (III) chloride and place it in a 100 ml volumetric flask. Fill to 3/4 with distilled water and add 0.1 ml of hydrogen peroxide solution at 20 volumes. Adjust to the flask mark with distilled water.

Calcium chloride solution in a concentration of 27 g/l (C)

Dissolve exactly 2.7 g of dihydrous calcium chloride in 100 ml of distilled water.

Hydro-alcoholic Matrix (D)

Fill a 1 liter volumetric flask half way with distilled water, then dissolve the following in order:

Tartaric acid: 2.5 g

K₂SO₄: 1 g (complete dissolution before proceeding to the following)

Metatartaric acid: 50 mg

Ethanol, 95% by volume: 120 ml

1M NaOH: 10 ml

Adjust the pH of the matrix to 3.5 by adding 1M NaOH (1-2 ml). Homogenize and top off with distilled water.

Potassium hexacyanoferrate (II) solution in a concentration of 12.5 g/l (E)

Weigh exactly 0.25 g of potassium hexacyanoferrate and place it in a 20 ml volumetric flask. Top off with distilled water.

This preparation should be made extemporaneously.

4.16.4. Test

Place the liter of matrix (D) in a flask and add exactly 2 ml of potassium hexacyanoferrate (II) solution (E). Place a stopper in the flask and shake. Next, add 1 ml of iron (III) chloride solution (B). Shake and let sit one-half hour. Solution S (blue in color).

In a series of test tubes (capacity > 50 ml), pour increasing volumes of gum solution in concentrations of 5 g/l (A): 0 - 0.25 - 0.5 - 0.75 - 1.0 - 1.25 - 1.5 - 1.75 - 2.0 - 2.5 - 3.0 ml. These volumes correspond to final gum concentrations of 0 - 25 - 50 - 75 - 100 - 125 - 150 - 175 - 200 - 250 and 300 mg/l.

Place 50 ml of solution S to each test tube. Shake and let sit 5 minutes.

Next, pour 1 ml of calcium chloride solution (C) into each tube. Place a stopper in the tube and agitate.

Store the tubes at ambient temperature ($\approx 25^{\circ}\text{C}$) and out of the light.

After 3 days, read:

The control tube should have a deep blue deposit with a nearly colorless surfactant. This deposit will be more or less significant in the other tubes depending on the efficacy and dose of gum added.

In one tube, a solution with a homogeneous color and no blue deposit at the bottom will be seen. This corresponds to the quantity in mg/l of efficacious gum arabic to use in the wine.

5. STORAGE

Solid gum arabic has a very long shelf life if stored in a dry, temperate place in sealed packages. Solutions have a limited shelf life due to the presence of sulfur dioxide.

KAOLIN
Kaolinum
(Oeno 28/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Kaolin is a natural hydrated aluminum silicate.
It is used as a clarification agent in wines.

2. LABELING

The label should indicate purity and safety and storage conditions.

3. PROPERTIES

Fine white or yellowish-white powder which is oily to the touch. When weakened in hot water it releases a clay-like odor. It is insoluble in water and dilute acids.

The product of the alkaline liquefaction of kaolin taken up by water exhibits the reaction properties of alkaline aluminates and alkaline silicates.

4. TESTS

4.1. Consistency

Mix 1 g of kaolin with 1ml of water. The resulting paste should not be runny.

4.2. Water Loss at 700 °C

Burn a precisely-weighed sample of about 1 g of kaolin at 700 °C. Weight loss should not be greater than 15 pp 100.

4.3. Products Soluble in Dilute Acids

Weaken 1 g of kaolin in 50 ml of 0.2M hydrochloric acid. Bring to a boil under reflux for 15 minutes. Filter. The filtrate, when evaporated then incinerated, should not leave a residue of more than 2 pp 100.

4.4. Preparing the Solution for Tests

Macerate 5 g of kaolin with 100 ml of citric acid in a concentration of 5 g per liter with a pH of 3.5 for 24 hours, stirring from time to time. Filter.

4.5. Soluble Iron

Add 1 ml of concentrated hydrochloric acid (R) and 5 ml of 5 pp 100 potassium thiocyanate to 10 ml of the solution prepared for tests under paragraph 4.4. The resulting coloration should be less intense than that

of a control prepared with 5 ml of an iron solution in a concentration of 0.010 g of iron per liter (R), 5 ml of citric acid in a concentration of 20 g per liter (R), 1 ml of concentrated hydrochloric acid (R) and 5 ml of 5 pp 100 potassium thiocyanate (R). (Soluble iron content should be less than 100 mg/kg).

It is also possible to determine iron content using the atomic absorption photometry method described in the Compendium.

4.6. Calcium

To 5 ml of the solution prepared for tests under paragraph 4.4, add 5 ml of ammonium oxalate in a 4 pp 100 solution (R), 5 drops of bromophenol blue (R) and a sufficient quantity of concentrated ammonium hydroxide (R) to turn the indicator blue. There should be no clouding.

4.7. Soluble Magnesium and Aluminum

To 5 ml of the solution prepared for tests under paragraph 4.4, add 5 ml of 10 pp 100 sodium phosphate solution (R), 1 drop of phenolphthalein in a concentration of 1 g per 100 ml alcohol at 90% by volume (R) and a sufficient quantity of diluted ammonium hydroxide (R) to obtain a pink coloration. No precipitate should form in less than one hour.

4.8. Lead

Using the technique described in the Compendium, determine the lead content in the test solution prepared in accordance with Par. 4.4. (Lead content should be less than 5 mg/kg.)

4.9. Mercury

Using the technique described in the annex, determine the mercury content in the test solution prepared in accordance with Par. 4.4. (Content should be less than 1 mg/kg.)

4.10. Arsenic

Using the technique described in the annex, determine the mercury content in the test solution prepared in accordance with Par. 4.4. (Content should be less than 3 mg/kg.)

4.11. Evaluation of Coarse Particles

Place a suspension of 5 g of kaolin in 60 ml of a 1 pp 100 tetrasodic pyrophosphate solution (R) in a 250 ml test tube (diameter of approximately 40 mm) with an emery stopper. Shake vigorously for 1-2 minutes. Let sit for 5 minutes then use a siphon to draw off 50 ml of the suspension. The siphon should have two tubes whose length ratio is

2:5. It should consist of a glass tube with a diameter of 5 mm. The tip of the small tube, which should be suitably tapered, is then placed and maintained below the surface of the liquid so the siphon is drained once 50 ml of the suspension have been drawn.

Add 50 ml of water to the remaining liquid. Stir and let sit 5 minutes, then take another 50 ml sample with the siphon. Repeat this procedure until 400 ml of water have been taken up. Finally, decant the residue remaining in the test tube into a calibrated crucible.

Dry evaporate, then ddry at 100 °C for 15 minutes. Weigh. The residue should not be greater than 2 pp 100.

4.12. Adsorption Power

Place 1 g kaolin and 10 ml 0.01M methylene blue in a test tube with a stopper and let the deposit form. Centrifuge the solution and dilute 100 times. The solution should be no more intensely colored than a 0.08 mM methylene blue solution.

5. STORAGE

Kaolin should be stored in well-ventilated, places at moderate temperatures in airtight containers and away from volatile substances it can adsorb.

L(+) TARTARIC ACID
L-2,3-dihydroxybutanedioic acid
Dextrorotatory tartaric acid
Acidum tartaricum
COOH - CHOH - CHOH - COOH
 $C_4H_6O_6 = 150.1$
SIN NO. 334
(Oeno 49/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This is a natural acid extracted from grapes. It is used to acidify musts and wines under conditions stipulated by regulation.

2. LABELING

The label should indicate in a clear manner that the product is L-tartaric acid, sometimes written L(+)tartaric acid, since its rotatory power is positive. It must also indicate the purity percentage (greater than 99.5%) and storage requirements.

3. PROPERTIES

Very solid colorless, transparent crystals which have a distinctly acidic flavor and containing no water of crystallization.

Melting point is 170 °C.

4. SOLUBILITY

Water at 20 °C	highly soluble
Alcohol, 95% by vol.	379 g/l
Glycerol	soluble
Ethyl ether	very slightly soluble

5. ROTATORY POWER

In an aqueous solution of 20 g per 100 ml
20°C

$[\alpha]_D$ is between +11.5 and +13.5°.

D

Specific rotatory power varies greatly with temperature and pH.

6. IDENTIFYING CHARACTERISTICS

6.1. Verify total solubility in water. A 1% solution exhibits an acidic reaction in the presence of methyl orange (R)

6.2. Place 2 ml of concentrated sulfuric acid (R), 2 drops of sulforesorcinic reagent (R) and a very small crystal of tartaric acid (1-5 mg) in a test tube. Heat to 150 °C. An intense violet coloration should appear.

6.3. Add 2 ml of 5 pp 100 potassium acetate solution (R) to 5 ml of 10 pp 100 solution (m/v). A crystallized precipitate should form immediately.

6.4. Place 5 ml chloroform or dichloromethane in a test tube. Add 100-200 mg tartaric acid. Shake. Crystals should gather at the bottom of the tube. Under these conditions, citric acid crystals will collect at the liquid surface.

7. TESTS

7.1. Foreign Matter

Tartaric acid should be soluble without residue in its weight of water and in 4 times its weight of alcohol at 95% by volume.

7.2. Sulfur Ash

As explained in the annex, use a precisely-weighed sample of approximately 2 g to determine sulfur ash concentration in the tartaric acid. This sulfuric ash concentration must not be greater than 1 g/kg.

7.3. Preparing the Solution for Tests

Dissolve 10 g of tartaric acid in a quantity of water sufficient to produce 100 ml of solution.

7.4. Chlorides

Add 14.5 ml of water, 5 ml of nitric acid diluted to 10 pp 100 (R), and 0.5 ml of 5 pp 100 silver nitrate solution (R) to 0.5 ml of the solution prepared for tests under Paragraph 7.3. After 15 minutes of sitting in the dark, there should be no clouding ; or else, any clouding that does appear should be less intense than that observed in a control prepared as indicated in the annex. (Chloride content expressed in terms of hydrochloric acid should be less than 1 g/kg).

7.5. Sulfates

Add 18 ml of water, 1 ml of hydrochloric acid diluted to 10 pp 100 (R), and 2 ml of 10 pp 100 barium chloride solution (R) to 1 ml of the solution prepared for tests under Paragraph 7.3. After 15 minutes of sitting in the dark, there should be no clouding, ;or else, any clouding that does appear should be less intense than that observed in a control prepared as indicated in the annex. (Sulfate content expressed in terms of sulfuric acid should be less than 1 g/kg).

7.6. Citric Acid

Add 5 ml of water and 2 ml of mercury (II) sulfate solution (R) to 5 ml of the solution prepared for tests under paragraph 7.3. Bring to a boil and add several drops of 2 pp 100 potassium permanganate solution (R). No white precipitate should form.

7.7. Oxalic Acid and Barium (test)

Neutralize 5 ml of test solution prepared under Paragraph 7.3 by adding ammonium hydroxide. Add 2 drops of acetic acid (R) and 5 ml of a saturate calcium sulfate solution (R).The solution should remain clear. (Opalescence may appear by virtue of the precipitation of calcium oxalate or barium sulfate.)

7.8. Oxalic Acid (quantitative analysis)

If the test conducted under 7.7 is positive, perform quantitative analysis of the oxalic acid.

Using the method described in the annex, determine oxalic acid content in the test solution (6.3). (Content expressed for oxalic acid should be less than 100 mg/kg after dessiccation).

7.9. Iron

Add 1 ml of concentrated hydrochloric acid (R) and 2 ml of 5 pp 100 potassium thiocyanate solution (R) to 10 ml of the solution prepared for tests under Paragraph 7.3. The resulting red coloration should be less intense than that observed in a control prepared using 1 ml of an iron (III) salt solution having a concentration of 0.010 g of iron per liter, 9 ml of water and the same quantities of the same reagents. (Iron content should be less than 10 mg/kg).

It is also possible to determine iron content by atomic absorption spectrometry, using the technique detailed in the Compendium.

7.10. Lead

Using the technique in the Appendix, determine the lead content in the test solution (7.3). (Lead content should be less than 5 mg/kg).

7.11. Mercury

Using the technique described in the Annex, determine the proportion of mercury in the test solution (7.3). (Mercury content should be less than 1 mg/kg).

7.12. Arsenic

Using the technique described in the Annex, determine the proportion of arsenic in the test solution (7.3). (Arsenic content should be less than 3 mg/kg).

8. QUANTITATIVE ANALYSIS

In 10 ml water, dissolve a precisely-weighed test sample **p** weighing about 1 g L-tartaric acid. Titrate with a 1 M sodium hydroxide solution (R) in the presence of phenolphthalein (R). Let **n** be the number of milliliters used.

1 ml 1 M sodium hydroxide solution corresponds to 0.075 g L-tartaric acid.

Content in percent of L-tartaric acid of the product assayed :

$$7.5 \text{ } n.$$

The product used for wine-making must contain at least 99.5 pp 100 L-tartaric acid (dry product).

9. STORAGE

L-tartaric acid should be stored in hermetically sealed containers.

L-LACTIC ACID, D-LACTIC ACID, D,L-LACTIC ACID
2-hydroxypropanoic acid
N° SIN : 270
C.A.S. number 50-21-5
(L-: 79-33-4; D-: 10326-41-7; DL-: 598-82-3)
chemical formula C₃H₆O₃
Molecular mass: 90.08, density 1.20-1.21.
(OENO 29/2004)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

An acid of natural origin obtained by lactic fermentation of sugars or synthetically made; it may contain condensation products such as lactate from lactic acid and dilactide.

It is used for the acidification of musts and wines in the conditions set by the regulation.

2. LABELLING

The label must mention particularly clearly that it concerns L-lactic or D-Lactic acids obtained by fermentation or D,L-Lactic obtained by a chemical process, the storage conditions and expiration date.

The common commercial products are solutions at 50%-90%.

Solid products containing about 100%-125% of titrable lactic acid also exist. (Note: Lactic acid is hygroscopic and once concentrated by boiling or distillation, it forms condensation products that hydrolyse into lactic acid by dilution and by heating in water).

Purity level: not less than 95.0% and not more than 105.0% of the concentration marked.

3. CHARACTERISTICS

Colourless or slightly yellow and syrupy liquid with a clearly acid flavour to a slightly lactic taste.

4. SOLUBILITY

Water at 20°C: very soluble

Alcohol at 95% vol.: Very soluble

Ether: very soluble

Insoluble in chloroform.

5. OPTICAL ROTATION

For L-lactic acid aqueous solution at 2.5 g for 100 ml.

$\alpha_{21-22^{\circ}C}^D$ is 2.6°

For D-lactic acid in aqueous solution at 8 g for 100 ml.

$\alpha_D^{21-22^\circ\text{C}}$ is -2.6°

6. IDENTITY CHARACTERS

6.1 Characterisation of lactic acid

In a 100 ml conical flask, weigh 10 g of lactic acid, add 5 ml of sulphuric acid 0.5 M, shake, add 25 ml of potassium permanganate at 0.33% and place on a hot plate. Collect the vapour released on a filter paper soaked with a solution at 50% vol/vol of morpholine at 20% and potassium nitrocyanoferate (II) at 5%.

The filter paper becomes blue.

6.2 Determination of total lactic acid

Titrate the free lactic acid with sodium hydroxide 1 M then hydrolyse the polymerised lactic acid using an excess of sodium hydroxide and then determined by sulphuric acid 0.5 M.

6.3 Colour

Compare the colour with the standards of the alpha scale (colour standards of platinum-cobalt).

6.4 Stereochemical purity

The method is based on the separation by HPLC using a chiral phase of two enantiomers of lactic acid. The product is diluted in water beforehand. Enzymatic determinations can also be performed according to the methods in the Compendium of international methods of analysis of wines and musts.

7. TEST TRIALS

7.1 Preparation of the test trial solution

For the purity test trials, prepare a solution containing 10% m/v of lactic acid by using the concentration marked.

7.2 Sulphuric ashes

From a 2 g sample of lactic acid, determine the sulphuric ashes as indicated in chapter II of the International Oenological Codex.

The content must be less than or equal to 1 g/kg.

7.3 Chlorides

To 0.5 ml of the test trial solution (7.1), add 14.5 ml of water, 5 ml of diluted nitric acid (R) and 0.5 ml of silver nitrate solution at 5% (R). The solution should satisfy for the test trial, the determination limit of chlorides described in chapter II of the International Oenological Codex.

The chloride content must be less than 1 g/kg expressed in hydrochloric acid.

7.4 Iron

To 10 ml of the test trial solution (7.1), add 1 ml of concentrated hydrochloric acid (R) and 2 ml of the potassium thiocyanate solution at 5% (R). The red colouration obtained must not be darker than the control prepared with 1 ml of the iron salt solution (III) at 0.010 g of iron per litre (R), 9 ml of water and the same quantities of the same reagents.

The iron content must be less than 10 mg/kg.

Iron can also be determined by atomic absorption spectrometry according to the method described in chapter II of the International Oenological Codex.

7.5 Lead

Using the test trial solution (7.1), apply the method described in chapter II of the International Oenological Codex.

Lead content should be less than 5 mg/kg.

7.6 Mercury

Using the test trial solution (7.1), determine the mercury according to the method described in chapter II of the International Oenological Codex.

Mercury content should be less than 1 mg/kg.

7.7 Cadmium

Using the test trial solution (7.1), determine the cadmium according to the method described in chapter II of the International Oenological Codex.

Cadmium content should be less than 1mg/kg.

7.8 Arsenic

Using the test trial solution (7.1), determine the arsenic according to the method described in chapter II of the International Oenological Codex.

Arsenic content should be less than 3 mg/kg.

7.9 Sulphates

To 1 ml of the test trial solution (7.1), add 18 ml of water, 1 ml of diluted hydrochloric acid at 10% (R) and 2 ml of barium chloride solution at 10% (R). The solution should satisfy for the test trial, the determination limit of sulphates described in chapter II of the International Oenological Codex.

Sulphate content should be less than 1 g/kg, expressed in sulphuric acid.

7.10 Cyanides

In a 40 ml volumetric flask containing 25 ml of distilled water and 2.5 ml of buffer solution at pH 7.5 (R), introduce 0.4 ml of the test trial solution (7.1), add 0.3 ml of chloramine T solution at 0.1% (R). Wait 90

seconds and add 6 ml of pyridine-pyrazolone reagent (R). Complete to 40 ml with distilled water and mix. The colouration obtained must not be darker than that obtained by treating the same way 4 ml of a freshly prepared potassium cyanide solution titrating 1 mg of hydrocyanic acid per litre (R).

Free cyanide content expressed in hydrocyanic acid should be less than 1 mg/kg.

7.11 Citric acid

To 5 ml of the test trial solution (7.1), add 5 ml of water, 2 ml of mercury sulphate solution (II) (R), bring to the boil and add a few drops to the potassium permanganate solution at 2% (R). No white precipitate should form.

7.12 Citric, oxalic, tartaric and phosphoric acids

Dilute 1 ml of the test trial solution (7.1) in 10 ml of water, add 40 ml of the calcium hydroxide solution (R) bring to the boil for 2 minutes. No turbidity should form.

7.13 Sugars

Add 2 ml of the test trial solution (7.1) to 10 ml of cupro-alkaline reagent (R). No red precipitate should form.

8. STORAGE

Lactic acid should be stored in hermetically sealed containers away from heat and light.

LACTIC BACTERIA
(Oeno 15/2003)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Lactic bacteria are used in oenology to perform malolactic fermentation. The lactic bacteria must belong to the *Oenococcus* (*Leuconostoc*), *Lactobacillus* and *Pediococcus* genus and must be isolated from grapes, musts, wine or cultures originating from the crossing of these same bacteria (original mother culture) which must be stored in genetically stable conditions.

Obtaining and using genetically modified bacteria (GMO's) require advance authorisation from a competent authority.

Lactic bacteria used in oenology must transform the malic acid in must and wine into lactic acid and carbon dioxide. This must produce biogenous aminos in the smallest possible quantities, and must neither produce an off taste nor produce substances harmful to health.

2. LABELLING

The following information must be indicated on the label:

- The genus and species name in addition to the reference of the strain(s) attributed by an official body of monitoring micro-organisms or by international institutions, the breeder, the origin, the strain breeder and possibly the originator that isolated it.
- Operating instructions or the reactivation method and possible additives recommended by the manufacturer.
- The number of viable cells per gram of preparation that is guaranteed by the manufacturer, the loss of viability per month of storage under defined conditions for temperature, humidity and aeration, the batch number, in addition to the expiration date and storage conditions.
- Where relevant, the indication that lactic bacteria were obtained by genetic modifications and their modified character.

3. CHARACTERISTICS

Lactic bacteria are used in liquid, frozen or powder form obtained by lyophilisation or drying, in pure culture or in association with pure cultures.

4. TEST TRIALS

4.1 – Humidity

Measured by the weight loss of 5 g of the product, dried at 105°C until constant weight (about 3 hours).

Maximum content should not exceed 8%.

4.2 – Heavy metals

Proceed with the dosage according to the method in chapter II of the International Oenological Codex.

Content should be less than 10 mg/kg of dry matter, expressed in lead.

4.3 - Lead

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 5 mg/kg of dry matter.

4.4 - Mercury

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 1 mg/kg of dry matter.

4.5 - Arsenic

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 3 mg/kg of dry matter.

4.6 - Cadmium

Proceed with the determination according to the method in chapter II of the International Oenological Codex.

Content should be less than 1 mg/kg of dry matter.

4.7 - Mycotoxins¹

4.8 – Viable lactic bacteria

Proceed with counting according to the method in chapter II of the International Oenological Codex.

The number should be more or equal to 10⁸ CFU/g or 10⁷ CFU/ml.

¹ Point to be studied at a later date by the sub-commission of methods of analysis and appraisal of wine.

4.9 – Content of viable cells of lactic bacteria of a different species of an indicated strain²

4.10 - Mould

Proceed with counting according to the method in chapter II of the International Oenological Codex.

The number should be less than 10³ CFU/g of powder.

4.11 – Contaminant acetic bacteria

Proceed with counting according to the method in chapter II of the International Oenological Codex.

The number should be less than 10³ CFU/g of powder or 10³ CFU/ml.

The sum of *Acetobacter* + *Gluconobacter* should be less than 10³ CFU/g of powder or 10³ CFU/ml millilitre.

4.12 - Yeasts

Proceed with counting according to the method in chapter II of the International Oenological Codex.

The number of viable cells of total contaminant yeasts (for example *Shizosaccharomyces* or *Brettanomyces*) must be less than 10³ CFU/g of powder or 10³ CFU/ml.

4.13 - Salmonella

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Absence should be checked on a 25 g sample.

4.14 - *Pseudomonas aeruginosa*³

4.15 - *Escherichia coli*

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Absence should be checked on 1 g sample.

4.16 - Staphylococci

Proceed with counting according to the method in chapter II of the International Oenological Codex.

Absence should be checked on 1 g sample.

² Point to be studied at a later date by the expert group "Wine microbiology".

³ Point to be studied at a later date by the expert group "Wine microbiology".

4.17 - Coliforms

Proceed with counting according to the method in chapter II of the International Œnological Codex.

The number of coliforms should be less than 10 CFU/g.

5. ADDITIVES

They must be in conformity with regulations in force.

6. STORAGE CONDITIONS

Storage should not be in open packaging and/or at temperatures above 10°C.

Storage conditions differ according to preparation and packaging methods.

Always refer to manufacturer's recommendations.

LIQUID SULFUR DIOXIDE
Liquid sulfurous anhydride
Sulfuris dioxydum solutum
SO₂ = 64.07
SIN NO. 220
(Oeno 46/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Sulfur dioxide is a colorless, non-flammable gas with a sharp, suffocating odor. It is stored and transported in liquid form in hardened steel tanks. These solutions are not stable and should not contain less than 50 g/l SO₂.

At a temperature of 20 °C, it is liquid under a pressure of 3.36 kg per square centimeter, or 3.30 bars.

Under standard pressure, its boiling point is -10 °C. Its density is $\rho_{20} = 1.383$.

This product falls under the category of antiseptic and antioxidant preservatives. Its content level in wine is subject to the regulatory limits in force concerning quantities.

2. LABELING

The label should indicate product SO₂ content at the time of sale and its safety and storage conditions.

3. SOLUBILITY

Water at 0 °C 79.79 l of sulfur dioxide per liter of water at standard pressure

Water at 20 °C 39.37 l of sulfur dioxide per liter of water at standard pressure

Alcohol, 95% by vol. at 20 °C : 114.48 l of sulfur dioxide per liter of water
Hydrocarbons, fatty substances and other organic compounds : soluble

4. IDENTIFYING PROPERTIES

4.1. Sulfur dioxide blackens a filter paper which is impregnated with mercury (I) nitrate.

4.2. Sulfur dioxide turns a filter paper impregnated with potassium iodine and starch solution blue. Then, the blue color disappears because of reduction of the iodine initially released.

4.3. Sulfur dioxide has a strong, characteristic odor.

5. TESTS

5.1. Non-volatile Substances

In a 500 ml container that has already been calibrated, collect 200 ml of liquid sulfur dioxide. Weigh the container immediately afterward. Let **p** be the mass in g sampled. Let the sulfur dioxide spontaneously evaporate. After reheating the container and removing any gaseous sulfur dioxide it still contains, weigh the container which holds the residue from evaporation. The mass of this residue should be less than 0.01 pp 100.

5.2. Preparing the Solution for Tests

Add 2 ml of concentrated nitric acid (R) and 5 ml of water to the residue left by evaporating 200 ml of sulfur dioxide (5.1). Place in a 100 °C water bath for 5 minutes. The remaining volume should be topped off with water to 200 ml.

5.3. Copper

Take a sample which corresponds to 1 g of liquid sulfur dioxide from the test solution prepared under paragraph 5.2. Top off to 10 ml with distilled water and add 0.5 ml of 10 pp 100 (v/v) hydrochloric acid solution, 15 pp 100 (m/v) citric acid (R), 1 ml of 5M ammonium hydroxide solution (R) and 0.5 ml of 1 pp 100 sodium diethyldithiocarbamate solution (concentration : 1 pp 100) in alcohol at 40% by volume (R). If a yellow coloration appears, it should be less intense than that obtained by adding 1 ml of copper solution in a concentration of 0.01 g per liter (R), 9 ml of water, 0.5 ml of 10 pp 100 (v/v) hydrochloric acid solution, 15 pp 100 (m/v) citric acid (R), 1 ml of 5M ammonium hydroxide solution (R) and 0.5 ml of 1 pp 100 sodium diethyldithiocarbamate solution (concentration : 1 pp 100) in alcohol at 40% by volume (R). (Copper content should be less than 10 mg/kg).

5.4. Iron

Take a sample of the solution prepared for tests under paragraph 5.2 corresponding to 1 g of liquid sulfur dioxide. Top off to 5 ml with water. Add 1 ml of concentrated hydrochloric acid (R), one drop of 1 pp 100 potassium permanganate (R) and 5 ml of 5 pp 100 potassium thiocyanate solution. If a red coloration appears, it should be less intense than that obtained by a control prepared with 5 ml of iron solution in a concentration of 0.010 g of iron per liter (R) and the same

quantities of hydrochloric acid and thiocyanate. (Iron content should be less than 50 mg/kg).

Iron may also be quantitatively analyzed using the atomic absorption spectrophotometry method detailed in the Compendium.

5.5. Lead

In the test solution prepared under paragraph 5.2, determine the lead content using the method described in the Compendium. (Lead content should be less than 5 mg/kg). It is also possible to dose iron using the atomic photometry method described in the Annex.

Lead may also be quantitatively analyzed using the atomic absorption spectrophotometry method detailed in the Compendium.

5.6. Mercury

Using the technique described in the annex, determine mercury concentration in the test solution (5.2). (Content should be less than 1 mg/kg.)

5.7. Selenium

In a test tube, take a volume of the solution prepared for tests under paragraph 5.2 corresponding to 1.5 g of sulfur dioxide and top off to 2 ml with water. Add 8 ml of hydrochloric acid diluted to 30 pp 100 (R) and 50 mg of powdered anhydrous potassium sulfite (R) which has been verified to be selenium free. After dissolving, place the test tube in a 100 °C water bath. Examine the color in the tube after 15 minutes.

If a pink coloration appears, it should not be more intense than that obtained in a control prepared by adding 0.15 ml selenium dioxide solution in a concentration of 100 mg selenium per liter (R), 1.85 ml of water, 8 ml of 30 pp 100 hydrochloric acid (R) and 50 mg of powdered, selenium-free anhydrous potassium sulfite and, after dissolving, by placing the test tube in a 100 °C water bath for 15 minutes. (Selenium content should be less than 10 mg/kg).

5.8. Arsenic

Using the technique described in the annex, determine arsenic concentration in the test solution (5.2). Concentration should be less than 3 mg/kg.

6. STORAGE

Sulfur dioxide should be stored and delivered in a liquid state in metal cylinders equipped with a needle valve tap or slide valve and whose strength is checked regularly. Keep the containers in a cool place.

LYSOZYME
Muramidase
N°SIN: 1105 (enzyme 3.2.1.17)
(Oeno 15/2001)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Lysozyme (Chlorhydrate and Lysozyme) is an edible egg white extract from hens. It is used to inhibit bacterial growth and can be used in musts and wine. Doses are limited in level.

Lysozymes contain no substances, micro-organisms or collateral enzyme activities, which are:

- harmful to the health,
- harmful for the quality of the products treated,
- lead to the formation of undesirable products or favour acts of fraud.

2. LABELLING

The concentration of the product must be indicated on the label, in addition to the security conditions, the preservation and the expiration date.

3. COMPOSITION

Lysozyme is a natural polypeptide made up of 129 amino acids, out of which there are 21 aspartic acids, 5 glutamic acids, 12 alanines, 11 arginines, 8 cystines, 3 phenylalanines, 12 glycines, 6 isoleucines, 1 histidine, 8 leucines, 6 lysines, 2 prolines, 2 methionines, 10 serines, 3 tyrosines, 7 threonines, 6 tryptophanes and 6 valines.

The molecular mass of lysozyme is 14,700 Daltons.

The water content must be less than or equal to 6%.

4. CHARACTERISTICS

Lysozyme can be in crystal powder form, white, odourless with a mild taste.

5. SOLUBILITY

Lysozyme is soluble in water and insoluble in organic solvents.

6. IDENTIFYING CHARACTERISTICS

A 2% aqueous solution must have a pH between 3.0 and 3.6. An aqueous solution containing 25-mg/100 ml has a maximum absorption of 281 nm and a minimum of 252 nm.

7. ENZYME ACTIVITY

Enzyme activity is capable of hydrolysing a link between N-acetylmuramic acid and N-acetylglucosamine of gram positive bacteria cell walls. The minimum concentration for lysozyme is 95%. There is no secondary enzyme activity.

8. ENZYME ORIGIN AND MEANS OF PRODUCTION

Enzyme is extracted from edible hen egg white by a procedure of separating ion-exchange resin.

The microbiological purity guarantees the security for its usage in food. The egg white used in the preparation of enzymes are compatible with parameters established by inspection agencies and is treated in compliance with hygienic manufacturing procedures.

9. SUBSTANCES USED AS DILUENTS, PRESERVATIVES, AND ADDITIVES

There are no substances used as preservatives as the crystalline form guarantees the stability.

10. TRIAL TESTS

10.1 Sulphuric ashes

As indicated in the appendix, the sulphuric ash content of lysozyme should not exceed 1.5%.

10.2 Total nitrogen

Evaluated according to the procedure outlined in the appendix, nitrogen content should be between 16.8 and 17.8% on dry matter.

10.3 Preparation of test trials solution

Dissolve 5 g of lysozyme in 100 ml of water.

10.4 Heavy Metals

Add 2 ml of pH 3.5 solution (R) and 1.2 ml of reactive thioacetamide (R) to 10 ml of prepared test trial solution (10.3). There should be no precipitate. If a brown colour is produced, it should be less than the sample produced as indicated in the appendix. (Heavy metal content measured in lead should be under 10 mg/kg).

10.5 Arsenic

Look for arsenic using the procedure in the appendix on 2 ml of test trial solution (10.3). (Arsenic content under 1 mg/kg).

10.6 Lead

Measure out lead following the procedure in the Compendium on the test trial solution (10.3). (Lead content under 5mg/kg.)

10.7 Mercury

Measure out mercury following the procedure in the appendix on the test trial solution (10.3). (Mercury content under 1 mg/kg).

10.8 Biological Contaminants

Evaluation carried out according to procedure in the appendix.

Total bacteria	under 10^3 CFU per g of preparation
Coliforms	maximum 10 per g of preparation
Escherichia coli	absence checked on 1 g sample
St. aureus	absence checked on 1 g sample
<i>Salmonella</i>	absence checked on 25 g sample
Yeasts	content limit 10^2 CFU per g of preparation
Total lactic bacteria	content limit : absence checked on a 10 g sample preparation
Acetic bacteria	content limit 10^2 CFU per g of preparation
Mould	content limit 10^2 CFU per g of preparation.

11. MEASURING TURBIDITY OF LYSOZYME ACTIVITY IN WINE

(Turbidimetric measuring)

11.1 Principle

The analytical procedure was established by FIP (1997) with some modifications made by FORDRAS. The procedure is based on changes in turbidity changes in *Micrococcus luteus* ATCC 4678 induced by a lytic lysozyme activity.

Under normal test conditions, the above-mentioned changes are in proportion to the quantity of lysozyme.

11.2 Substrate

Do not use an electromagnetic mixer when suspending between 40 – 60 mg of *Micrococcus luteus* ATCC 4698 (Boehringer) in powder form in phosphate solution M/15 pH 6.6 (\pm 0.1), when obtaining a homogeneous suspension and complete it with 100 ml with the same buffer. Use a hand mixer or an ultrasound bath.

The exact quantity of *Micrococcus luteus* to be taken depends on the spectrophotometer used.

Prepare a control sample with 5 ml of buffer and 5 ml of *Micrococcus luteus* and measure the absorbency with the aid of a 540 nm spectrophotometers compared to control sample of phosphate buffer. Absorbency should not be under 0.800.

If reading the measurement doesn't correspond, the content of *Micrococcus luteus* must be adapted in the suspension and then measure the desired absorbency.

Note: With a sensitive spectrophotometer, the absorbency levels of above-mentioned solutions are 0.800 to 0.900. Equipment that are not as sensitive may give readings for the absorbency for this same suspension of 0.500 to 0.600.

In this case, we should not increase the amount of substrate to obtain initial absorbency rates of 0.800 to 0.900, because reproducing the measurement linearity are not very dependable.

11.3 Preparation of standard solution

11.3.1. Dissolve exactly 50 mg of lysozyme chlorhydrate in water, and fill up to 100 ml in a graduated flask.

11.3.2. Dilute 5 ml of solution in 11.3.1 with water up to 50 ml.

11.3.3. Dilute 2 ml of this solution with a M/15 phosphate buffer up to 100 ml to obtain a 1 mg/l of lysozyme (standard solution).

11.4 A solution to analyse

Dilute the sample of wine with m/15 phosphate buffer to obtain the same concentration of standard solution (1 mg/l) in relation with the concentration of lysozyme.

11.5 Procedure

Prepare the following solutions in 180 x 80 mm test tubes

Standard solution to analyse	Buffer M/15	Lysozyme concentration
2.0 ml	3.0 ml	0.4 mg/l
2.8 ml	2.2 ml	0.56 mg/l
4.0 ml	1.0 ml	0.8 mg/l

It is recommended to repeat each dilution 3 times for the standard solution and for the solution to be tested.

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Prepare two test tubes with 5 ml of buffer as a suspension control sample for *Micrococcus luteus*. Use the first control sample in the beginning and the second one at the end of the trial.

After exactly 30 seconds, add 5 ml of *Micrococcus luteus* suspension. This must be mixed manually to avoid over spilling. Mix with a Vortex and keep the tubes in 37°C (± 5°C) water for exactly 12 minutes.

The final quantity of lysozyme in the tubes will be 0.2 – 0.28 – 0.4 mg/l.

After incubation, remove the tubes in the same order they were placed in, with an interval of 30 seconds.

Mix and take a reading of the absorbency with the 540 nm spectrophotometer for white wine and 740nm for the red wine against the control buffer.

Under normal circumstances, the test trial is acceptable when the difference between the absorbency rates for the control samples is under 5%.

11.6 Calculation

Prepare a standard curve indicating the average values of absorbency obtained for each standard solution on the y-axis. On the x-axis put the concentrations of lysozyme on a logarithm scale.

Carry over the results obtained for the dilutions to be analysed.

Draw two straight lines: one between the points obtained from the standard solution and the other between the points of the solution to be analysed. The two lines must be parallel, if not the dose is incorrect.

Then draw a line parallel to the x-axis so that the two right lines are cut about halfway the extreme limit for dosing.

In the two intersection points, which correspond to two concentrations on the x-axis (C_{st} concentration of the standard curve and C_x concentration of the curve for the solution to be analysed). The activity is calculated as follows:

$$\text{Concentration of lysozyme } (\mu\text{g/ml}) = = \frac{C_{st} \times D}{C_x}$$

Where

C_{st} = concentration of the standard solution

C_x = concentration of the solution to be analysed

D = dilution factor

12. DETERMINATION OF LYSOZYME IN WINE

(Determination by HPLC)

The lysozyme residue can be determined by HPLC according to the method described in the Compendium on International Analysis Methods of Wines and Musts.

13. PRESERVATION

Lysozyme must be stored at room temperature in a closed sealed container, away from humidity.

14. BIBLIOGRAPHY

FIP (1997), Pharmaceutical Enzymes, A. Lowers and S. Scharpe ed. 1997, vol.84 pages 375-379.

L-MALIC ACID, DL-MALIC ACID**2-hydroxybutanedioic acid****N° SIN: 296****C.A.S. number 617-48-1****Chemical formula C₄H₆O₅****Molecular mass: 134.09****(OENO 30/2004)****1. OBJECT, ORIGIN AND FIELD OF APPLICATION**

An acid of natural origin contained in most fruit (L-malic acid) or synthetically made: DL-malic.

It is used for the acidification of musts and wines in the conditions set by the regulation.

2. LABELLING

The label must mention particularly clearly that it is L-malic or D,L-malic acid, the storage conditions and date of expiry.

Malic acid content should be at least 99%.

3. CHARACTERISTICS

White or off-white crystalline powder or granules with a clearly acid flavour.

Melting point of D,L-malic: 127°C-132°C

Melting point of L-malic: 100°C.

4. SOLUBILITY

Water at 20°C: 55.8 g/100

Alcohol at 95% vol.: 45.5 g/100.

Ether: 0.84 g/ 100

5. OPTICAL ROTATION

For the L-Malic acid in aqueous solution at 8.5 g for 100 ml.

$\alpha_{20^{\circ}\text{C}}^D$ is - 2.3°

6. IDENTITY CHARACTERS**6.1 Characterisation of malic acid**

Malic acid can be determined by an enzymatic process according to the methods in the Compendium of international methods of analysis of wines and musts (specifically L-malic and D-malic acids. Malic acid can also be determined by HPLC according to the method in the Compendium of international methods of analysis of wines and musts.

7. TEST TRIALS**7.1 Preparation of the test trial solution**

For purity trials, prepare a solution containing 10% m/v of malic acid.

7.2 Sulphuric cinders

From a 2 g sample of malic acid, determine the sulphuric cinders as indicated in chapter II of the International Oenological Codex.

Content must be less than or equal to 1 g/kg.

7.3 Chlorides

To 0.5 ml of the test trial solution (7.1), add 14.5 ml of water, 5 ml of diluted nitric acid (R) and 0.5 ml of silver nitrate solution at 5% (R). The solution should satisfy for the test trial, the determination limit of chlorides described in chapter II of the International Oenological Codex.

Content must be less than 1 g/kg expressed in hydrochloric acid.

7.4 Iron

To 10 ml of the test trial solution (7.1), add 1 ml of concentrated hydrochloric acid (R) and 2 ml of potassium thiocyanate solution at 5% (R). The red colouration obtained should not be darker than that of the control prepared with 1 ml of an iron salt solution (III) at 0.010 g of iron per litre (R), 9 ml of water and the same quantities of the same reagents.

Content must be less than 10 mg/kg.

Iron can also be determined by atomic absorption spectrometry according to the method described in chapter II of the International Oenological Codex.

7.5 Lead

Using the test trial solution (7.1), apply the method described in chapter II of the International Oenological Codex.

Lead content must be less than 5 mg/kg.

7.6 Mercury

Using the test trial solution (7.1), determine the mercury according to the method described in chapter II of the International Oenological Codex.

Mercury content must be less than 1 mg/kg.

7.7 Cadmium

Using the test trial solution (7.1), determine the cadmium according to the method described in chapter II of the International Oenological Codex.

Cadmium content must be less than 1 mg/kg.

7.8 Arsenic

Using the test trial solution (7.1), determine the arsenic according to the method described in chapter II of the International Oenological Codex.

Arsenic content must be less than 3 mg/kg.

7.9 Sulphates

To 1 ml of the test trial solution (7.1), add 18 ml of water, 1 ml of diluted hydrochloric acid at 10% (R) and 2 ml of the barium chloride solution at 10% (R). The solution should satisfy for the test trial, the determination limit of sulphates described in chapter II of the International Oenological Codex. Sulphates content must be less than 1 g/kg, expressed in sulphuric acid.

7.10 Cyanides

In a 40 ml volumetric flask containing 25 ml of distilled water and 2.5 ml of buffer solution at pH 7.5 (R), introduce 0.4 ml of the test trial solution (7.1), add 0.3 ml of chloramine T solution at 0.1% (R). Wait 90 seconds and add 6 ml of pyridine-pyrazolone reagent (R). Complete to 40 ml with distilled water and mix. The colouration obtained must not be darker than that obtained by treating the same way 4 ml of a freshly prepared potassium cyanide solution titrating 1 mg of hydrocyanic acid per litre (R).

Free cyanide content expressed in hydrocyanic acid must be less than 1 mg/kg.

7.11 Sugars

Add 2 ml of the test trial solution (7.1) to 10 ml of cupro-alkaline reagent (R). No red precipitate should form.

7.12 Fumaric and maleic acids

Limit in fumaric acid: 1% in weight.

Limit in maleic acid: 0.05% in weight. These acids are determined by HPLC according to the method described in the Method of Analysis of Wines and Musts in the same way as malic and tartaric acids.

8. STORAGE

Malic acid should be stored in hermetically sealed containers away from heat and light.

METATARTARIC ACID

Ditartaric acid

Acidum ditartaricum

SIN NO. 353

(Oeno 31/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

The name metatartaric acid applies to the product obtained by dehydrating L-tartaric acid at a temperature of 150-170 °C under atmospheric pressure or under a reduced pressure.

This product can retard tartaric precipitation in the bottle.

Its effectiveness in preventing tartaric precipitation is directly related to the rate of esterification

The quantity in which it is used in wines is restricted.

The primary constituents of the product are the ditartaric monoester and diester in variable proportions based on the combination of two molecules of tartaric acid with water loss, mixed with variable quantities of non-esterified tartaric acid, pyruvic acid and small quantities of poorly known polyester acids.

This product exists in crystalline masses or in powder form with a white or greater or lesser yellowish color. It has a faint odor of toast or caramel and is very deliquescent.

It is highly soluble in water and alcohol and rapidly hydrolyzed in aqueous solution at 100 °C, but much more slowly at cold temperatures.

2. LABELING

The label should indicate the esterification rate and safety and storage conditions, as well as the optimal expiration date.

3. DETERMINATION

3.1. Place a sample of 1-10 mg of this substance in a test tube with 2 ml of concentrated sulfuric acid (R) and 2 drops of sulforesorcinic reagent (R). When heated to 150 °C, an intense violet coloration appears.

3.2. Place 2.50 ml of 10 pp 100 (m/v) tartaric acid in 20 alcohol by volume in a 100 ml cylindrical flask. Add 10 mg of metatartaric acid (0.5 ml of 2% solution), 40 ml of water and 1 ml of 25 pp 100 calcium acetate solution (R). Stir. A weak, amorphous precipitate remaining in suspension will appear for certain samples having a high ester number

when they contain a small quantity of poorly known polyesters. No crystallized precipitate should form within 24 hours, whereas a mixture of the same reagents without metatartaric acid yields a crystallized precipitate within several minutes.

4. TESTS

4.1. Appearance

A 10 pp 100 aqueous solution of metatartaric acid should be clear and almost colorless or slightly amber in color.

4.2. Preparation of the Test Solution

Prepare a metatartaric acid solution in a concentration of 20 g/l in water.

4.3. Quantitative Analysis

Place 50 ml of very recently prepared 2 pp 100 solution (1 g of metatartaric acid) in a 250 ml conical flask. Add 3 drops of bromothymol blue solution (R) in a concentration of 4 g/l and 1 M sodium hydroxide solution until the indicator turns bluish-green. Let n be the number of ml used.

Add 20 ml of 1M sodium hydroxide. Insert the stopper and let sit for 2 hours at ambient temperature. Titrate the excess alkaline solution using 0.5M sulfuric acid. Let n' be the number of millimeters used: 1 ml of 1M sodium hydroxide corresponds to 0.075 g of tartaric acid.

Content (pp 100 of total free and esterified acid) of the tested product:

$$7.5 (n + 20 - n')$$

Ester content pp 100 of total acid functions:

$$100 (20 - n') / (n + 20 - n')$$

The wine-making product must contain at least 105 pp 100 total tartaric acid after hydrolysis and 32 pp 100 esterified acid.

4.4. Heavy Metals

Using the thioacetamide technique described in the annex, determine heavy metals content in the test solution prepared in accordance with Par. 4.2 (when expressed for lead, the heavy metals content must be lower than 10 mg/kg).

4.5. Lead

Using the technique described in the Compendium, quantify the proportion of lead in the test solution (Par. 4.2). (Lead content should be less than 5 mg/kg).

4.6. Mercury

Using the technique described in the annex, determine the proportion of mercury in the test solution prepared in accordance with Par. 4.2 (content must be lower then 1 mg/kg).

4.7. Arsenic

Using the technique described in the annex, determine arsenic content in the test solution prepared in accordance with Par. 4.2 (content must be lower then 3 mg/kg).

5. STORAGE

Metatartaric acid should be stored in hermetically sealed containers away from air and moisture.

MICROCRYSTALLINE CELLULOSE
(C₁₂ H₂₀ O₁₀)_n
INS N°: 460
(Oeno 9/2002)

1. OBJECT. ORIGIN AND FIELD OF APPLICATION

Microcrystalline cellulose is purified cellulose and is partially depolymerised. It comes from the treatment of alpha-cellulose mineral acids from plant fibres. Its molecular weight is approximately 36 000. Microcrystalline cellulose plays an important role in "supporting" very clarified fermentation as it increases the fermentability of the juices.

2. LABELLING

The concentration of the product must be mentioned on the label and if there is mixing as well as the method of preservation.

3. CHARACTERISTICS

Cellulose is found in **microcrystalline powder form**. white, odourless and tasteless. It is almost insoluble in water, acetone, ethanol, toluene, diluted acids and in 50 g/l sodium hydroxide solutions.

4. IDENTIFICATION

4.1 In a watch glass, put approximately 10 mg of microcrystalline cellulose and add 2 ml of zinc chloride iodated solution (R). The solution turns bluish purple.

4.2 Degree of polymerisation

Put 1.300 g of microcrystalline cellulose in a 125 ml conical flask. Add 25 ml of water (R) and 25 ml of 1M cupriethylenediamine hydroxide. Immediately pass a nitrogen current. Close the flask and mix until completely dissolved. Pour 7 ml of the solution into an appropriate glass capillary viscosimetric tube.

Time how long it takes between two lines on the viscosimeter and express the time measured in (t_1) . Calculate the kinematic viscosity V_1 of the solution using the following formula:

$$V_1 = t_1(k_1)$$

In which k_1 is the viscosimeter constant.

Take out an appropriate volume of 1M cupriethylenediamine hydroxide and dilute with the same volume of water. (R). Using an appropriate capillary viscosimeter. determine the time flow of this solution.

Calculate the kinematics viscosity V_2 of the solvent using the following formula:

$$V_2 = t_2(k_2)$$

In which k_2 is the viscosimeter constant.

Determine the relative viscosity η_{rel} of the microcrystalline cellulose sample. using the following formula:

$$V_1/V_2$$

Determine the intrinsic viscosity $[\eta]c$ by extrapolation. using the intrinsic viscosity table in Annex.

Calculate the degree of polymerisation P. using the formula:

$$P = 95[\eta]c/m[(100-b)/100]$$

In which m is the mass. in grams of the trial and b is the value obtained in the test trial " loss through drying " in %.

The degree of polymerisation is not over 350.

4.3 pH

Shake for 20 minutes about 5 g of cellulose in 40 ml of water free of carbon dioxide. Centrifuge. The pH of the supernatant liquid must be between 5.0 and 7.5.

4.4 Soluble substances in ether

Prepare a column of 10.0 g of microcrystalline cellulose in a glass tube with an inside diameter of approximately 20 mm. Put 50 ml

of ether free of peroxides (R). through the column and evaporate the eluate until bone dry. The residue should not be above 5.0 mg (0.05%).

4.5 Soluble substances in water

Mix 5.0 g of microcrystalline cellulose with 80 ml of water (R) for 10 mn. Filter in a vacuum and collect the filtrate in a weighed vase. Evaporate over a bath of 100° C water until bone dry and dry at 100-105°C for 1 hour. The residue is not above 12.5 mg (0.25%).

4.6 Starch

Add 90 ml of water (R) to 10 g microcrystalline cellulose. and boil for 5 mn. Filter when hot. Let cool and add 0.1 ml iodine 0.05 M to filtrate. There is no blue colouring.

4.7 Loss through drying

Put 1 g of cellulose in a mass capsule for 3 hours in an incubator at 100-105°C. Loss through drying should not be more than 6.0%.

All limits set below refer to the dried product.

4.8 Ashes

Incinerate at $600 \pm 25^{\circ}\text{C}$ the residue obtained in point 4.7. for 4 hours. The mass of the ashes should not be more than 0.1%.

4.9 Preparation of test solution

After weighing. dissolve the ashes in 2 ml of concentrated hydrochloric acid (R) and 10 ml of water (R). Heat to activate the dissolution and fill up to 50 ml with water.

4.10 Iron

Determine iron with an atomic absorption spectrophotometer following the method described in Chapter II into the test solution (4.9).

Iron content must be less than less or equal to 10 mg/kg.

4.11 Lead

Determine the lead according to the method described in Chapter II. into the test solution (4.9).

Lead content must be less than 5 mg/kg.

4.12 Mercury

Determine the mercury according to the method described in Chapter II

Mercury content must be less than 1 mg/kg.

4.13 Cadmium

Determine the cadmium according to the method described in Chapter II. into the test solution (4.9).

Cadmium content must be less than 1 mg/kg.

4.14 Arsenic

Determine the arsenic according to the method described in Chapter II.

Arsenic content must be less than 1 mg/kg.

4.15 Calcium

Determine the calcium with an atomic absorption spectrophotometer. following the method described in Chapter II. into the test solution (4.9).

Calcium content must be less than 500 mg/kg.

5. STORING CONDITIONS

Cellulose must be stored in a well-ventilated place in sealed packages away from volatile substances which it might adsorb.

INTERNATIONAL CENOLOGICAL CODEX
Microcrystalline cellulose

TABLE OF INTRINSIC VISCOSITY

Intrinsic viscosity, $[\eta]_c$, according to value of relative viscosity.. η_{rel}

$[\eta]_c$

η_{rel}	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
1.1	0.098	0.106	0.115	0.125	0.134	0.143	0.152	0.161	0.170	0.180
1.2	0.189	0.198	0.207	0.216	0.225	0.233	0.242	0.250	0.259	0.268
1.3	0.276	0.285	0.293	0.302	0.310	0.318	0.326	0.334	0.342	0.350
1.4	0.358	0.367	0.375	0.383	0.391	0.399	0.407	0.414	0.422	0.430
1.5	0.437	0.445	0.453	0.460	0.468	0.476	0.484	0.491	0.499	0.507
1.6	0.515	0.522	0.529	0.536	0.544	0.551	0.558	0.566	0.573	0.580
1.7	0.587	0.595	0.602	0.608	0.615	0.622	0.629	0.636	0.642	0.649
1.8	0.656	0.663	0.670	0.677	0.683	0.690	0.697	0.704	0.710	0.717
1.9	0.723	0.730	0.736	0.743	0.749	0.756	0.762	0.769	0.775	0.782
2.0	0.788	0.795	0.802	0.809	0.815	0.821	0.827	0.833	0.840	0.846
2.1	0.852	0.858	0.864	0.870	0.876	0.882	0.888	0.894	0.900	0.906
2.2	0.912	0.918	0.924	0.929	0.935	0.94 1	0.948	0.953	0.959	0.965
2.3	0.971	0.976	0.983	0.988	0.994	1.000	1.006	1.011	1.017	1.022
2.4	1.028	1.033	1.039	1.044	1.050	1.056	1.061	1.067	1.072	1.078
2.5	1.083	1.089	1.094	1.100	1.105	1.111	1.116	1.121	1.126	1.131
2.6	1.137	1.142	1.147	1.153	1.158	1.163	1.169	1.174	1.179	1.184
2.7	1.190	1.195	1.200	1.205	1.210	1.215	1.220	1.225	1.230	1.235
2.8	1.240	1.245	1.250	1.255	1.260	1.265	1.270	1.275	1.280	1.285
2.9	1.290	1.295	1.300	1.305	1.310	1.314	1.319	1.324	1.329	1.333
3.0	1.338	1.343	1.348	1.352	1.357	1.362	1.367	1.371	1.376	1.381
3.1	1.386	1.390	1.395	1.400	1.405	1.409	1.414	1.418	1.423	1.427
3.2	1.432	1.436	1.441	1.446	1.450	1.455	1.459	1.464	1.468	1.473
3.3	1.477	1.482	1.486	1.491	1.496	1.500	1.504	1.508	1.513	1.517
3.4	1.521	1.525	1.529	1.533	1.537	1.542	1.546	1.550	1.554	1.558
3.5	1.562	1.566	1.570	1.575	1.579	1.583	1.587	1.591	1.595	1.600
3.6	1.604	1.608	1.612	1.617	1.621	1.625	1.629	1.633	1.637	1.642
3.7	1.646	1.650	1.654	1.658	1.662	1.666	1.671	1.675	1.679	1.683
3.8	1.687	1.691	1.695	1.700	1.704	1.708	1.712	1.715	1.719	1.723
3.9	1.727	1.731	1.735	1.739	1.742	1.746	1.750	1.754	1.758	1.762

INTERNATIONAL CENOLOGICAL CODEX
Microcrystalline cellulose

4.0	1.765	1.769	1.773	1.777	1.781	1.785	1.789	1.792	1.796	1.800
4.1	1.804	1.808	1.811	1.815	1.819	1.822	1.826	1.830	1.833	1.837
4.2	1.841	1.845	1.848	1.852	1.856	1.859	1.863	1.867	1.870	1.874
4.3	1.878	1.882	1.885	1.889	1.893	1.896	1.900	1.904	1.907	1.911
4.4	1.914	1.918	1.921	1.925	1.929	1.932	1.936	1.939	1.943	1.946
4.5	1.950	1.954	1.957	1.961	1.964	1.968	1.971	1.975	1.979	1.982
4.6	1.986	1.989	1.993	1.996	2.000	2.003	2.007	2.010	2.013	2.017
4.7	2.020	2.023	2.027	2.030	2.033	2.037	2.040	2.043	2.047	2.050
4.8	2.053	2.057	2.060	2.063	2.067	2.070	2.073	2.077	2.080	2.083
4.9	2.087	2.090	2.093	2.097	2.100	2.103	2.107	2.110	2.113	2.116
5.0	2.119	2.122	2.125	2.129	2.132	2.135	2.139	2.142	2.145	2.148
5.1	2.151	2.154	2.158	2.160	2.164	2.167	2.170	2.173	2.176	2.180
5.2	2.183	2.186	2.190	2.192	2.195	2.197	2.200	2.203	2.206	2.209
5.3	2.212	2.215	2.218	2.221	2.224	2.227	2.230	2.233	2.236	2.240
5.4	2.243	2.246	2.249	2.252	2.255	2.258	2.261	2.264	2.267	2.270
5.5	2.273	2.276	2.279	2.282	2.285	2.288	2.291	2.294	2.297	2.300
5.6	2.303	2.306	2.309	2.312	2.315	2.318	2.320	2.324	2.326	2.329
5.7	2.332	2.335	2.338	2.341	2.344	2.347	2.350	2.353	2.355	2.358
5.8	2.361	2.364	2.367	2.370	2.373	2.376	2.379	2.382	2.384	2.387
5.9	2.390	2.393	2.396	2.400	2.403	2.405	2.408	2.411	2.414	2.417
6.0	2.419	2.422	2.425	2.428	2.431	2.433	2.436	2.439	2.442	2.444
6.1	2.447	2.450	2.453	2.456	2.458	2.461	2.464	2.467	2.470	2.472
6.2	2.475	2.478	2.481	2.483	2.486	2.489	2.492	2.494	2.497	2.500
6.3	2.503	2.505	2.508	2.511	2.513	2.516	2.518	2.52 1	2.524	2.526
6.4	2.529	2.532	2.534	2.537	2.540	2.542	2.545	2.547	2.550	2.553
6.5	2.555	2.558	2.561	2.563	2.566	2.568	2.571	2.574	2.576	2.579
6.6	2.581	2.584	2.587	2.590	2.592	2.595	2.597	2.600	2.603	2.605
6.7	2.608	2.610	2.613	2.615	2.618	2.620	2.623	2.625	2.627	2.630
6.8	2.633	2.635	2.637	2.640	2.643	2.645	2.648	2.650	2.653	2.655
6.9	2.658	2.660	2.663	2.665	2.668	2.670	2.673	2.675	2.678	2.680
7.0	2.683	2.685	2.687	2.690	2.693	2.695	2.698	2.700	2.702	2.705
7.1	2.707	2.710	2.712	2.714	2.717	2.719	2.721	2.724	2.726	2.729
7.2	2.731	2.733	2.736	2.738	2.740	2.743	2.745	2.748	2.750	2.752
7.3	2.755	2.757	2.760	2.762	2.764	2.767	2.769	2.771	2.774	2.776
7.4	2.779	2.781	2.783	2.786	2.788	2.790	2.793	2.795	2.798	2.800
7.5	2.802	2.805	2.807	2.809	2.812	2.814	2.816	2.819	2.821	2.823
7.6	2.826	2.828	2.830	2.833	2.835	2.837	2.840	2.842	2.844	2.847
7.7	2.849	2.851	2.854	2.856	2.858	2.860	2.863	2.865	2.868	2.870
7.8	2.873	2.875	2.877	2.879	2.881	2.884	2.887	2.889	2.891	2.893
7.9	2.895	2.898	2.900	2.902	2.905	2.907	2.909	2.911	2.913	2.915

INTERNATIONAL CENOLOGICAL CODEX
Microcrystalline cellulose

8.0	2.918	2.920	2.922	2.924	2.926	2.928	2.931	2.933	2.935	2.937
8.1	2.939	2.942	2.944	2.946	2.948	2.950	2.952	2.955	2.957	2.959
8.2	2.961	2.963	2.966	2.968	2.970	2.972	2.974	2.976	2.979	2.981
8.3	2.983	2.985	2.987	2.990	2.992	2.994	2.996	2.998	3.000	3.002
8.4	3.004	3.006	3.008	3.010	3.012	3.015	3.017	3.019	3.021	3.023
8.5	3.025	3.027	3.029	3.031	3.033	3.035	3.037	3.040	3.042	3.044
8.6	3.046	3.048	3.050	3.052	3.054	3.056	3.058	3.060	3.062	3.064
8.7	3.067	3.069	3.071	3.073	3.075	3.077	3.079	3.081	3.083	3.085
8.8	3.087	3.089	3.092	3.094	3.096	3.098	3.100	3.102	3.104	3.106
8.9	3.108	3.110	3.112	3.114	3.116	3.118	3.120	3.122	3.124	3.126
9.0	3.128	3.130	3.132	3.134	3.136	3.138	3.140	3.142	3.144	3.146
9.1	3.148	3.150	3.152	3.154	3.156	3.158	3.160	3.162	3.164	3.166
9.2	3.168	3.170	3.172	3.174	3.176	3.178	3.180	3.182	3.184	3.186
9.3	3.188	3.190	3.192	3.194	3.196	3.198	3.200	3.202	3.204	3.206
9.4	3.208	3.210	3.212	3.214	3.215	3.217	3.219	3.221	3.223	3.225
9.5	3.227	3.229	3.231	3.233	3.235	3.237	3.239	3.241	3.242	3.244
9.6	3.246	3.248	3.250	3.252	3.254	3.256	3.258	3.260	3.262	3.264
9.7	3.266	3.268	3.269	3.271	3.273	3.275	3.277	3.279	3.281	3.283
9.8	3.285	3.287	3.289	3.291	3.293	3.295	3.297	3.298	3.300	3.302
9.9	3.304	3.305	3.307	3.309	3.311	3.313	3.316	3.318	3.320	3.321
10	3.32	3.34	3.36	3.37	3.39	3.41	3.43	3.45	3.46	3.48
11	3.50	3.52	3.53	3.55	3.56	3.58	3.60	3.61	3.63	3.64
12	3.66	3.68	3.69	3.71	3.72	3.74	3.76	3.77	3.79	3.80
13	3.80	3.83	3.85	3.86	3.88	3.89	3.90	3.92	3.93	3.95
14	3.96	3.97	3.99	4.00	4.02	4.03	4.04	4.06	4.07	4.09
15	4.10	4.11	4.13	4.14	4.15	4.17	4.18	4.19	4.20	4.22
16	4.23	4.24	4.25	4.27	4.28	4.29	4.30	4.31	4.33	4.34
17	4.35	4.36	4.37	4.38	4.39	4.41	4.42	4.43	4.44	4.45
18	4.46	4.47	4.48	4.49	4.50	4.52	4.53	4.54	4.55	4.56
19	4.57	4.58	4.59	4.60	4.61	4.62	4.63	4.64	4.65	4.66

INTERNATIONAL OENOLOGICAL CODEX
NITROGEN

NITROGEN
Nitrogenum
N = 14.007
SIN NO. 941
(Oeno 19/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Neutral gas used to render inert or to degas. It can be used pure or mixed with carbon dioxide.

2. LABELING

The label should mention the nature of this gas and reference its composition and purity, as well as its safety and storage conditions.

3. PROPERTIES

Colorless, odorless, flavorless gas. It is not flammable and does not maintain combustion.

The weight of a liter of nitrogen under normal conditions is 1.250 g. Under a pressure of 760 mm of mercury at 20 °C, a volume of water dissolves a 0.01507 volume of nitrogen, while a volume of alcohol dissolves a 0.1224 volume of nitrogen.

4. TESTS

The purity of nitrogen used for oenological purposes should be 99 parts per 100 by volume.

Before undertaking any measurement, the gas should be allowed to escape for several moments in order to clean out the lines.

Gas detection and quantitative analysis: oxygen, carbon monoxide, argon, carbon dioxide, etc. are most rapidly detected using gas phase chromatography. (See this method in the Annex.)

The following chemical methods can also be used.

4.1. Phosphorous-containing Hydrogen, Arsenical Hydrogen and Reducing Substances

Let 1 liter of nitrogen to flow into a mixture of 10 ml of ammoniacal silver nitrate (R) and 15 ml of water.

Regulate the flow of gas so that the gas flows into the solution in approximately 15 minutes.

There should be no clouding or brown coloration when compared with an identical control solution through which no gas will flow.

4.2. Oxygen

Prepare a flask to test for oxygen as follows:

Place 2 turned pieces of copper of approximately 2 cm², 16 ml of ammoniacal copper sulfate solution (R) and 2 ml of hydrazine dichlorhydrate in a 24 ml flask.

Stop the flask with a rubber stopper which can easily be pierced with a hypodermic needle. Seal the collar with a metal cap, then cover the cap with wax to ensure a perfectly airtight seal. Shake the flask, then let it sit in the dark until the color disappears completely, after approximately eight days.

Conducting the test:

Pierce the flask stopper with a 8/10 mm hypodermic needle (take care not to dip the needle into the liquid). This will allow gas to escape after bubbling. Next, insert a second hypodermic needle of the same diameter and plunge it into the liquid. After a minute of bubbling, there should be no significant coloring. In the presence of oxygen, the liquid will rapidly turn blue and the color becomes more intense over time.

The nitrogen must incorporate less than 10 ml/l oxygen.

5. PACKING AND STORAGE

Nitrogen is delivered in high-strength steel canisters which are painted black and equipped with a needle valve tap. The strength of the canisters should be checked periodically.

OENOLOGICAL TANNINS
INS N°: 181
(Oeno 12/2002)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Oenological tannins are extracted from nutgalls, or a wood rich in tannin: chestnut trees, oak, exotic wood, or grape seeds. Tannins are made up of a mixture of glucosides either from gallic acid (gallotannins), or from dilactone, ellagic acid (ellagitannins) (hydrolysable tannins) or from a mixture of proanthocyanidines (condensed tannins). Tannins are used to facilitate the clarification of wines and musts. Tannins must not change the olfactory properties and the colour of wine.

2. LABELLING

The nature of the extraction solvent (water or alcohol) , the botanical origin and an estimation of the total phenols contained must be clearly labelled.

3. CHARACTERISTICS

Oenological tannins range in colour from pale-yellow to reddish brown, with an astringent taste. Tannins are partially soluble in ethyl acetate, water-soluble, ethanol and methanol for condensed tannins and insoluble in most organic solvents, with the exception of ethanol and methanol for hydrolysable tannins.

4. IDENTIFYING CHARACTERISTICS

4.1 – The aqueous solution of tannins produces, along with iron (III) salts, a blue/black precipitation between pH 3 and 5. This precipitation disappears with the addition of small quantities of strong acids.

4.2 – The aqueous solution of condensed tannin precipitate gelatine, egg whites, blood serum, etc. with a pH level between 3 and 6. Tannins precipitate alkaloids (quinine, strychnine) with a pH level between 4 to 6.

5. CHARACTERISATION

It is possible to characterise the botanical origin with the aid of the following criteria: ultraviolet absorption spectrum, flavanol content, proanthocyanidines, digallic acid, and scopoletine. (see appendix)

6. TEST TRIALS

6.1 Foreign matter

Tannin must be almost completely water-soluble and the content of insoluble substances should be under 2%, after shaking for 15 minutes 10 g of tannin in one litre of water.

6.2 Loss during drying

Determine the weight loss in an incubator at 100 – 105°C for 2 hours, of 2g of test solution. The weight must be constant and weight loss must be under 10%.

The limits below refer to the dry product.

6.3 Ashes

Incinerate progressively without going over 550 °C, the residue left over in the determination of loss during drying. The weight of the ashes should be under 4%.

6.4 Preparation of test solution

Take the ashes from 2 g of tannin by 1 ml of diluted hydrochloric acid (R) and one drop of concentrated nitric acid (R). Heat in 100°C water a little to dissolve. Pour this into a 50 ml volumetric flask. Rinse the capsule with distilled water and fill up the line on the flask.

6.5 Arsenic

Take 0.25 g of tannin, and determine arsenic using the method described in Chapter II by atomic absorption spectrometer, after destroying organic matter by the wet method. (Arsenic content must be under 3 mg/kg).

6.6 Iron

Add 2 ml of 5% potassium thiocyanate solution (R) and 1 ml of concentrated hydrochloric acid (R) to 10 ml of test solution prepared according to article 6.4. The resulting colour should not be more intense than the control sample prepared with 2ml of iron (III) salt solution at 0.010 g of iron per litre (R), 8 ml of water and the same volumes of the same reagents. (Iron content must be less than 50 mg/kg). It is also possible to measure the iron with the atomic absorption spectrometer.

6.7 Lead

Measure the lead in the solution prepared according to article 6.4 and using the method outlined in the Compendium of International Methods of Analysis of Wine and Musts by atomic absorption spectro-photometer. Content must be less than 5 mg/kg.

6.8 Mercury

Measure the mercury using the method outlined in Chapter II by atomic absorption spectrometer. Content must be less than 1 mg/kg.

6.9 Estimation of total phenols

On an aqueous tannin solution at 1 g/l diluted to 1/100th, measure the absorbency at 280 nm on an optical path of 1 mm. Total phenol content is given in gallic acid equivalents/g and transformed in p. 100 of tannin powder. For total phenols the results must be greater than 65%.

6.10 Nature of tannins

6.10.1 - Proanthocyanidic tannins are estimated by the DMACH method: mix 5 ml of reagent (100 mg of dimethylaminocinnamaldehyde + 10 ml of 12 M HCl solution; after bring to 100 ml with methanol) to 1 ml of aqueous tannin solution (1g/l). Wait 10 minutes; take a reading of the absorbency at 640 nm on 1 mm optical path. The results are given in equivalent catechin. The result for condensed tannins must be greater than 10 mg/g.

6.10.2 – The nitrous acid method is used to estimate ellagitannins. Mix 1 ml of aqueous tannin solution (1 g/l), 1 ml of methanol and 160 µl of 6% acetic acid (m/v). Displace the oxygen by nitrogen sparging for 10 minutes, add 160 µl of 6% sodium nitrite (m/v) followed by a brief nitrogen sparging (1 mn), the tube is vacuum sealed and its reaction takes in 60 mn in water bath at 30°C. The intensity of the colour is measured by absorbency at 600 nm. The results are estimated in mg/g in equivalents of castalagine ($\epsilon_{600\text{nm}}$: 983 g⁻¹). For hydrolysable tannins and ellagic type, the result must be greater than 20 mg/g.

6.10.3 – Gallic like hydrolysable tannins correspond to other categories of products, and test negatively to 6.10.1 and 6.10.2.

6.11 Extraction process

6.11.1 – IS solubility indicator

It is expressed in % of solubility for 5 g of tannin in 100 ml of diethylether/ethanol (9/1, v/v) mixture. For tannins extracted from water, the indicator must be less than 5.

6.11.2 – Iex extractability indicator:

$I_{\text{Ex}} = (D.O._{370\text{ nm}} \times 2) - (D.O._{350\text{ nm}} + D.O._{420\text{ nm}})$.

When I_{Ex} is greater than 0.05, the products come solely from extraction by water.

7. STORAGE CONDITIONS

Oenological tannins must be kept in sealed closed packages.

APPENDIX

IDENTIFICATION OF THE BOTANICAL ORIGINS OF OENOLOGICAL
TANNINS

MATERIALS AND METHODS

Principle

The recognition of the botanical origin of oenological tannins requires the formulating of the following observations in order:

- 1°) The presence of condensed tannins taken from grapes,
 - 2°) The presence of tannins from nutgalls,
 - 3°) The presence of tannins from exotic wood,
 - 4°) Differentiating the tannin from oak and the tannin for chestnut wood.
-
- Tannins from grapes is characterized by high content of flavanols, as expressed in (+) catechin.
 - Nutgall tannins have a high content of digallic acid.
 - The ultraviolet spectrum for tannins from exotic wood has a specific peak.
 - Tannins from oak trees are richer in coumarines, in particular scopoletine, than chestnut tannins.

Equipment and analytical conditions

- Laboratory glassware.
- Magnetic mixer.
- UV/visible absorption spectrophotometer double beam.
- 1 cm optical pathway glass cuvette
- 1 cm optical pathway quartz cuvette,
- 100° C water bath (optional)
- Heated rotating evaporator
- Composed chromatographic system (as an example):
 - pressure gradient pump for binary mixtures
 - an injector equipped with a 20-μl loop
 - a spectrophotometer detector with wave length 280 nm
 - a fluorimetric detector
 - An reversed phase column (C18) diameter of particles 5μm, dimensions of the column: 20 cm X 4.6 mm to measure the gallic acid and the scopoletine.
- pH meter.

Reagents and reference solutions

- para-dimethylaminocinnamaldehyde
- concentrated hydrochloric acid solution(R)
- (+) catechin
- digallic acid
- absolute ethanol
- ethyl acetate
- concentrated sodium hydroxide solution(R)
- methanol
- ethyl ether
- acetonitrile
- acetic acid
- scopoletine
- umbelliferone
- distilled water or demineralised or ultra filtered water.

Preparation of reagents

p-dimethylaminocinnamaldehyde (p-DACA) solution
100 mg of p-DACA are put into a solution of 10 ml 12 M hydrochloric acid and 90 ml of methanol.

Elution solvents for digallic acid

solvent A: pure methanol

solvent B: perchloric acid solution in water at pH 2,5

Elution solvents for scopoletine

solvent A: distilled water containing 3% acetic acid

solvent B: acetonitrile containing 3% acetic acid

Preparation of reference solutions

(+) catechin solution

Dissolve 10 mg of (+) catechin in 1 l of distilled water

Digallic acid solution at 100 mg / litre of distilled water

Scopoletine solution at 20 µg / litre of distilled water.

Operating methods

There are 2 methods for identifying the presence of grapes tannins:

Measuring total flavanols.

5 ml of p-DACA reagent are added to 1 ml of aqueous solution at 200 mg / l of tannin.

After 10 mn measure the absorption of the mixture at 640 nm in a glass cuvette with an optical path of 10 mm.

The absorbance values are then read from the calibration curve obtained from an increasing concentration range in (+) catechin analysed under the same conditions.

Measuring proanthocyanic tannins.

Add 2 ml of distilled water and 6 ml of 12 M hydrochloric acid to 4 ml of solution of 200 mg/l of tannin in a hydrolysis tube. This tube is heated to 100 °C for 30 mn and cooled in a cold bath.

A second tube containing the same mixture stays at room temperature for the same amount of time.

Then, 1 ml of ethanol is placed in both tubes and the absorbance values are measured at 550 nm.

The difference between the 2 absorbance values is multiplied by 380 to give the Proanthocyanic tannin content.

Identification of tannins from nutgall

20 ml of aqueous tannin solution at 50 mg/l is brought to pH 7 with the aid of a concentrated sodium hydroxide solution (R).

An initial series of extractions carried out 3 times 20 ml of ethyl acetate to eliminate neutral substances.

Secondly, the aqueous state is brought to pH 2 by the addition of concentrated hydrochloric acid solution (R). and then followed by a new series of 3 extractions with ethyl acetate.

After the evaporation of the ethyl acetate, the residue is taken by 20 ml of methanol then analysed by chromatograph under the following conditions: (as an example):

injected volume: 20 µl of extract or standard
digallic acid solution
Detection at 280 nm

Composition of an elution gradient:
from 10 to 20% of solvent A in 35 mn
from 20 to 40% of solvent A in 15 mn
from 40 to 98% of solvent A in 20 mn
Mobile phase flow: 0.8 ml / mn.

Identification of tannins from exotic wood

Prepare an aqueous solution of tannin so that when placed in a 1 cm optical pathway quartz cuvette. The solution has an absorbency measured at 280 nm between 1 and 1.5.

Carry out a continuous absorbency readings between 250 and 300 nm.

Note the presence or the absence of a maximum absorption peak.

Identification of tannins from oak or chestnut

Scopoletine contained in the 20 ml aqueous solution of tannin at 5 g/l is extracted 3 times with 20 ml of ethylic ether.

After the total recuperation and evaporation of the ether phase, the extract is taken from 50 ml of water and then analysed by chromatography under the following conditions : (as an example):

Injected volume: 20 µl of extract or scopoletine reference solution

fluorimetric detection:

excitation wavelength: 340 nm,
emitting wavelength: 425 nm

Composition of an elution gradient:

94% of solvent A during 10 mn
from 94 to 85% in 20 mn
from 82 to 67% in 5 mn
from 37 to 42% in 5 mn.

Mobile phase flow: 1 ml/mn

CONCLUSION

Tannin is recognised as being from grapes when the total flavanol content, expressed as (+) catechin is over 50 mg/g or its proanthocyanic tannin content is over 0.5 mg/g.

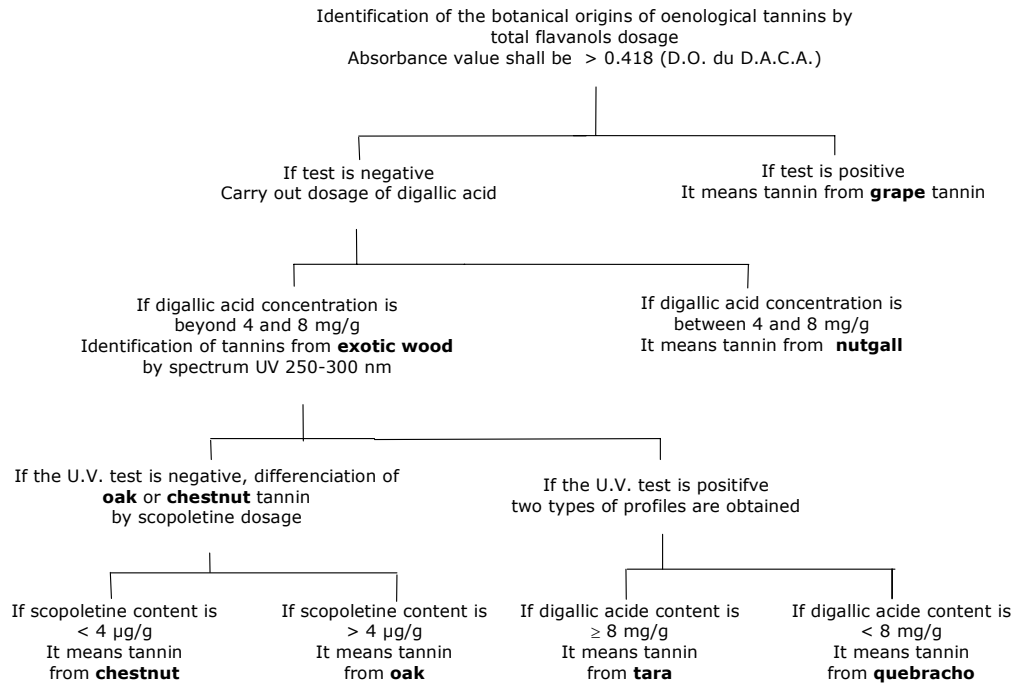
Tannin is recognized as coming from nutgall when digallic acid content is between 4 and 8 mg/g.

Tannin is recognized as coming from exotic wood when its spectrum reveals an absorption peak between 270 and 280 nm.

Tannin is recognized as coming from oak when scopoletine content is over 4 µg/g .

Tannin is recognized as coming from chestnut trees when its scopoletine content is equal to or less than 4 µg/g and if it is not identified as coming from another origin.

**BOTANICAL ORIGINS
CONCLUSION**



OXYGEN
O₂ = 32.0
N° SIN: 948
N°CAS = 7727-44-7
(OENO 32/2004)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Gas used for operations of hyperoxygenation of must or oxygenation of wine. It is also used pure or mixed with nitrogen (reconstituted air) during alcoholic fermentation (pumping).

2. LABELLING

The label must mention the nature of the gas and refer to its composition and purity. The safety conditions should also be indicated on the package.

3. CHARACTERISTICS

Colourless, odourless gas without flavour. Non flammable, it supports combustion.

The weight in grams of a litre of oxygen under normal conditions under the pressure of 760 mm of mercury and at 20°C is 1.429 g.

A volume of water dissolves 0.0325 volume of oxygen (44 mg/l). This solubility is 0.049 ml at 0°C (70 mg/l) and a volume of alcohol dissolves 0.1428 volume of oxygen.

It is therefore possible to dissolve 44 ml of oxygen at 20°C in a litre of wine with an alcoholic strength of 12% vol.

In association with nitrogen (air) the maximum solubility of oxygen is 10.27 ml/l in water at 20°C that is about 13.9 ml in a litre of wine whose alcoholic strength is 12% vol.

4. TEST TRIALS

The global purity of the oxygen used in oenology should be above or equal 99% in volume.

Before any measurement, it is advisable to allow the gas to escape for a few minutes in order to purge the piping.

4.1 Chromatographic dosage

Search and determination of gases: nitrogen, carbon monoxide (less than 10 µl/l), argon, carbon dioxide (less than 300 µl/l), etc. are quickly obtained by chromatography in gaseous phase.

4.2 Oxygen dosage

Place a sufficient quantity of ammonium hydroxide and ammonium chloride solution prepared by mixing equal volumes of water and ammonium hydroxide and by saturating with ammonium chloride at room temperature in an apparatus made up of:

- 100 ml burette calibrated with a bi-directional stopcock,
- pipette for gas absorption and
- level vase with an appropriate capacity and all the connections for linking the whole set.

Fill the pipette for gas absorption with copper turnings, wire or metallic lattice or any other appropriate system.

Eliminate all the gas bubbles from the liquid in the testing apparatus. Use the test trial solution two or three times without performing any measurements.

Fill the calibrated burette, all the connections, the two stopcock openings, and the liquid uptake tube.

Entrain 100.0 ml of oxygen in the burette while lowering the level vase.

Open the stopcock facing the absorption pipette and force the oxygen to penetrate in the absorption pipette by lifting the level vase. Shake the pipette in order to favour the close contact of the liquid, gas and copper. Continue shaking until no other decrease in volume occurs.

Entrain the residual gas again in the calibrated burette and measure its volume:

A volume of gas more than 1.0 ml should not remain.

In solution, oxygen can be determined by polarography.

5. PACKAGING

Oxygen is supplied in highly resistant steel cylinders painted white, with needle valves. The resistance of these cylinders must be periodically checked.

PERLITE

CAS no. 93763-70-3

Expanded perlite

(Oeno 10/2003)

1. OBJECT, ORIGIN, AND FIELD OF APPLICATION

Perlite is a vitreous rock of volcanic origin, belonging to the rhyolite group. Like glass, perlite is made of aluminium silicate and has a chemically bound water content of 1% to 2%.

To be used for oenological purposes, perlite must be dried at 150°C, grinded and then subjected to "expansion" by pre-heating between 200°C to 400°C followed exposing perlite in a flame at 800°C to 1100°C, which provokes swelling and causes a 60-fold increase in size.

Perlite is in white powder form and the final grain size is obtained after being grinded following expansion.

It is a filtration additive for wine.

2. LABELLING

The purity and the storage conditions must be written on the label.

3. LIMITS AND TEST TRIALS

3.1 Odour and taste

Perlite must not give any foreign odour or taste to the wine. Place 2.5 g of perlite in 1 litre of wine. Shake. Allow to stand 24 hours. Taste and compare to wine without an addition of perlite.

3.2 Loss through desiccation

Place approximately 5 g of perlite in a capsule. Put in an incubator to 103 ± 2 °C. After two hours weight loss must not be over 1%.

3.3 Loss through calcination

Heat the dry residue obtained in point 3.2 in an oven at 550 °C. Weight loss must not be over 3%.

3.4 pH measurement

In a 250 ml recipient, place approximately 10 g of perlite. Pour in slowly, while shaking by hand 100 ml of water to wet the product and obtain a homogenous suspension. Shake by hand from time to time or by using a magnetic stirrer. After 10 minutes, allow the suspension to stand and measure the pH level. Expanded perlite has a pH between 7.5 and 10.

3.5 Soluble products in diluted acids

Bring 10 g of dried perlite with 20 ml of concentrated hydrochloric acid (R) and 100 ml of water to a boil. Gather the perlite on an ashless filter and wash the residue with 100 ml of distilled water. After desiccation at 100°C to 105°C and incineration, and being separated from the insoluble residue filter, it should weigh at least 9.8 g that is 98% of the dry product.

3.6 Preparation of test trial solution

Place 200 ml of citric acid at 5 g per litre brought to pH 3 (R) and 10 g of perlite in a 500 ml flask that can be hermetically sealed. Place on a stirrer and shake 1 hour at a temperature of 20° plus or minus 2°C. Allow to stand, then filter by eliminating the first 50 ml of filtrate. Collect at least 100 ml of clear liquid.

3.7 Iron

Carry out the determination of iron according to the procedure described in Chapter II of the International Oenological Codex using the test trial solutions prepared according to point 3.6. Iron content must be less than 300 mg/kg.

3.8 Lead

Carry out the determination of lead according to the procedure described in Chapter II of the International Oenological Codex using the test trial solution prepared according to point 3.6. Lead content must be less than 5 mg/kg.

3.9 Mercury

Carry out the determination of mercury according to the procedure described in Chapter II of the International Oenological Codex using the test trial solution prepared according to point 3.6.

Mercury content must be less than 1 mg/kg.

3.10 Arsenic

Carry out the determination of arsenic according to the procedure described in Chapter II of the International Oenological Codex using 4 ml of the test trial solution prepared according to point 3.6.

Arsenic content must be less than 5 mg/kg.

3.11 Cadmium

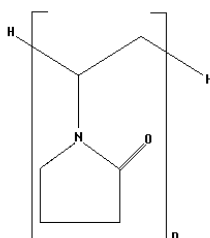
Carry out the determination of cadmium according to the procedure described in Chapter II of the International Oenological Codex using the test trial solution prepared according to point 3.6.

Cadmium content must be less than 1 mg/kg.

4. STORAGE

Perlite must be kept in a well-ventilated dry place in watertight containers under temperate conditions.

POLYVINYLPOLYPYRROLIDONE
POVIDONE
(PVPP)
(C₆H₉NO)_n = (111,1)_n
INS N°: 1202
(Oeno 11/2002)



1. OBJET, ORIGIN AND FIELD OF APPLICATION

Insoluble polyvinylpolypyrrolidone is a polymer poly[1-(2-oxo-1-pyrrolidinylethylene)] reticulated to render it insoluble. It is made by polymerisation of N-vinyl-2-pyrrolidone in the presence of different catalysers (for example sodium hydroxide) or in the presence of N'N'-divinylimidazolidone.

PVPP fixes the polyphenols in wines; this adsorption depends on the rate of polymerisation. Its application rate is limited.

2. SYNONYMS

poly(1-ethenylpyrrolidin-2-one)
Crospovidone (nomenclature of pharmacope)
Reticulated polyvidone
Reticulated homopolymer of 1-ethenyl-2-pyrrolidone
Reticulated insoluble polymer of N-vinyl-2-pyrrolidone
P.V.P. insoluble
Polyvinylpolypyrrolidone (PVPP).

3. LABELLING

The label must indicate that PVPP is for oenological usage, minimum guaranteed efficiency vis-à-vis safety test and storage conditions.

4. CHARACTERISTICS

Light powder, white and creamy white.
Insoluble in water and in organic solvents.
Insoluble in strong acid minerals and in alkaly.

5. TEST TRIALS

5.1 Loss through drying

Place 2 g of PVPP in a 70 mm diameter silica capsule; dry in an incubator at 100-105° C for 6 hours. Let cool in the desiccators. Weigh. Weight loss must be less than 5%.

It is also possible to carry this out more quickly by titration with the Karl-Fischer procedure (see annex).

Note: All limits set above refer to the dried product.

5.2 Ashes

Incinerate the residue left over in test trial 5.1 progressively without going over 600° C. (Ash mass should be less than 0.5%).

5.3 Preparation for test trial solution

After weighing the ashes, dissolve 1 ml of concentrated hydrochloric acid (R) and 10 ml of distilled water. Heat to activate the solution. Bring up to 20 ml with distilled water. 1 ml of this solution contains 0,10 g of PVPP mineral matter.

5.4 Heavy metals

10 ml of solution prepared according to point 5.3 is put in a test tube with 2 ml of a pH 3.5 (R) buffer solution and 1.2 ml of reactive thioacetamide (R). There should be no precipitation. If a brown colour appears, it should be inferior to the test sample as indicated in Chapter II (Heavy metal content, expressed in lead, must be less than 10 mg/kg).

5.5 Lead

Using the solution prepared idem, determine the lead, following the procedure in Chapter II or by atomic absorption spectrophotometer procedure. Lead content must be below 5 mg/kg.

5.6 Mercury

Determine the mercury, following the procedure in Chapter II. Mercury content must be below 1 mg/kg.

5.7 Zinc

Determine the zinc, following the procedure described in Chapter II. Zinc content must be below 5 mg/kg.

5.8 Arsenic

Determine the arsenic, following the procedure in Chapter II. Arsenic content must be below 3 mg/kg.

5.9 Cadmium

Determine the cadmium using the method described in Chapter II of the International oenological Codex by atomic absorption spectrophotometer procedure.

Cadmium content must be below 1 mg/kg.

5.10 Sulphates

Determine the sulphates, following the procedure in Chapter II. Sulphate content must be below 1 mg/kg.

5.11 Determining total nitrogen

Introduce approximately 0.20 g of PVPP weighed precisely in a 300 ml flask with 15 ml concentrated sulphuric acid (R) and 2 g of mineralisation catalyst (R) and continue the operation as indicated in Chapter II. (Total nitrogen content must be between 11 and 12.8%).

5.12 Solubility in a water medium

Introduce 10 g of PVPP in a 200 ml flask containing 100 ml of distilled water. Mix and leave for 24 hours. Filter through a gauze screen with a porosity of 2.5 µm and then through a gauze screen with a porosity of 0.8 µm. The residue left from the evaporation of dried filtrate over 100°C hot water, must be less than 50 mg (solubility in water must be less than 0.5%).

5.13 Solubility in acid and alcohol.

Introduce 1 g of PVPP in a flask containing 500 ml of the following mixture

Acetic acid	3 g
Ethanol	10 ml
Water	100 ml

Let sit 24 hours. Filter through a gauze screen with a porosity of 2.5 μm and then through a gauze screen with a porosity of 0.8 μm . Concentrate the filtrate over 100°C hot water. Stop evaporation over a 100° C hot water in a 70 mm diameter previously weighed silica capsule. The residue left by dry evaporation must be less than 10 mg, taking into account the residue left by evaporation of 500 ml of a mixture of acetic- acid ethanol (solubility in acetic acid and alcohol medium must be less than 1%).

6. PVPP EFFICIENCY WITH REGARDS TO ADSORPTION OF POLYPHENOLIC COMPOUNDS

6.1 Salicylic acid essay

6.1.1 Reagents:

- 0.1 M sodium hydroxide solution
- 0.1M salicylic acid solution (13.81 g salicylic acid are dissolved in 500 ml of methanol and diluted with 1l of water).

6.1.2 Operating mode:

- Weigh 2-3 grams of PVPP in a 250 ml conical flask and write down the MASS M at ± 0.001 g.
- Calculate the dry extract of the sample (solid weight percentage) as P in % to the nearest decimal.
- Add the 0.1M salicylic acid solution according to the following formula:

$$43. M \cdot P = \text{ml to be added}$$

- Close the flask and shake for 5 minutes.

- Pour the 25°C mixture on a filter over a Büchner funnel connected to a 250 ml tube; empty it until there is at least 50 ml of filtrate (the filtrate must be clear).
- Use a pipette take 50 ml of the filtrate and put it in a 250 ml conical flask.
- Determine the neutralisation point of phenolphthalein and write down the volume V_s with a 0.1M sodium hydroxide solution.
- Titrate 50 ml of the salicycal acid solution (sample test) in the same manner and write down the volume V_b .

6.1.3 Calculation:

$$\% \text{ activity} = \frac{V_b - V_s}{V_b} \cdot 100$$

The percentage of activity must be equal or greater to 30%.

6.2. Determining the adsorption capacity of oenocyanine (30% minimum)

6.2.1. Principle

A small amount of PVPP is put in contact with a oenocyanine solution for 5 minutes. Adsorption at 280 nm of treated oenocyanine solution is compared to a standard solution and a blank solution made up of only solvent. The decrease of adsorption to 280 nm is used as a relative measurement of PVPP capacity to adsorb oenocyanine.

6.2.2. Reagents

- oenocyanine (hydrate of)
- Ethanol (absolute)
- Distilled water.

6.2.3. Material

- Spectrophotometer, UV visible.
- Quartz cuvettes, 1 cm of optical path.
- Beakers, 150 ml.
- Graduated flask, 1 litre.
- Teflon stirring rods and magnetic mixer.
- Syringes.
- Filters for syringes, (0,45 µm porosity).

6.2.4. Methods

- Solution E. Dissolve 80 mg of oenocyanine hydrate in 50 ml of ethanol. Quantitatively transfer to a one litre graduated flask (with distilled water) and dilute to volume indicated with the distilled water. Label this solution E, and keep in an amber coloured tube. This is the standard solution.

- Solution R. Prepare the reference solution by diluting 50 ml of ethanol in 1 litre of distilled water. This is the reference solution.

- Weigh 3 volumes of, 50 mg \pm 0,1 mg of samples in 150 ml beakers. Add the Teflon mixing rods and put under the magnetic mixer.

NOTE: The contact time between the sample and the solution is *critical*. In the following steps, the addition of the solution to the samples will be in increments in order to foresee exactly 5 minutes between the introduction of the solution and the filtration of each sample.

- Using a pipette, add 100 ml of sample solution E, to 2 of the solutions and add 100 ml of solution R to the third sample. Put the timer on, once the 100 ml has been added.

- Shake for 5 minutes \pm 5 seconds.

- With the aid of syringe and a filter with pores measuring 0.45 μ m in diameter, withdraw a part of the solution immediately and filter in a clean flask. The filtered solutions can be stored in a cool and dark place for maximum 1 hour before measuring UV absorbency.

- Set up the UV spectrophotometer in compliance with manufacturers' instructions in order to measure absorbency at 280 nm. Put the machine at zero on 280 nm and use the R solution as a blank.

- Measure the degree of absorbency of each filtered extract at 280 nm compared to solution R by using quartz cuvettes with 1 cm optical path.

6.2.5. Calculations

$$\text{Absorbency capacity} = \frac{A_o - (A_T - A_B) \times 100}{A_B}$$

Given that:

A_o = Solution E absorbency

A_T = Sample solution absorbency

A_B = Blank solution absorbency (PVPP without oenocyanine)

Calculate the average for the two sample solutions.

7. DETERMINING OF THE N-VINYL-2-PYRROLIDONE MONOMER IN PVPP WITH THE AID OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV DETECTION.

7.1. Principle

The N-vinyl-2-pyrrolidone monomer is the extract PVP polymer with methanol. The methanol solution is analysed by HPLC by using C8 type deactivated reversed phase column. This quantification is carried out by UV detection at 235 nm. Soluble PVP is eliminated when entering the column by a back flush technique.

This method can be applied to samples of which the monomer concentration is between 0.4 and 100 mg/l. The content of N-vinyl-2-pyrrolidone in PVPP should not exceed 10 mg per kg.

7.2. Reagents

- Methanol, HPLC grade.
- water, micro filtered rest > 18 MΩ.
- N-vinyl-2-pyrrolidone

7.3. Equipment

7.3.1 Glassware

- Assembling HPLC to filter solvents; entirely in glass.
- Filters for mobile phases, nylon 0.45 µm.
- Graduated pipettes (10, 20 and 100 ml).
- Volumetric flasks (100 and 1000 ml).
- 7.5 ml polyethylene pipettes

- Spatulas used for the handling of powder grams.
- Small flasks with polyethylene stoppers.
- Filters with porosity 0.45 μm in glass microfibers.

7.3.2. Instruments

- Scale, which can measure to the nearest 0.1 mg.
- Magnetic mixer
- HPLC system with type C8 column and UV-Visible detector.

7.4. Procedure

7.4.1. Preparation of the mobile phase

- Using a pipette, introduce 200 ml of HPLC grade methanol in a 1000 ml flask. Dilute as needed, with HPLC grade water and mix.
- Filter/degasify the mobile phase and then transfer to the solvent reservoir to pump HPLC.

7.4.2. Preparation of reference solution

- VP 1000 mg/l reference solution
Weigh about 100 mg of N-vinyl-2-pyrrolidone to the nearest 0.1 mg in a 100 ml volumetric flask. Dilute the volume as needed with the mobile phase
- VP 100 mg/l reference solution
Dilute 10 ml of the solution at 1000 mg/l to the needed volume, with the mobile phase, in a 100 ml volumetric flask.
- VP 10 mg/l reference solution
Dilute 10 ml of the solution at 100 mg/l to the needed volume, with the mobile phase, in a 100 ml volumetric flask.
- VP 1 mg/l reference solution
Dilute 10 ml of the solution at 10 mg/l to the needed volume, with the mobile phase, in a 100 ml volumetric flask.

7.4.3. Preparation of the sample

- In a small flask, weigh about 2.0 g of PVPP \pm 0.1 mg.
- Using a pipette, introduce 20 ml of HPLC grade methanol in the flask containing the sample.
- Close the flask vacuum tight and put it under an automatic mixer. Extract for 1 hour at a speed of 130 rotations per minute.
- After one hour, remove the flask from the mixer. Filter the supernatant with a filter with a porosity of 0.45 μm in glass micro fibres.

7.4.4. Analysis by HPLC

- Install the HPLC equipment in compliance with the manufacturers' instructions and balance the column and the detector with the mobile phase for at least one hour before analysing the reference test specimen and the samples.

HPLC conditions (as an example)

Vol. injection	20 micro litres
Solvent flow	1 ml/minute
Detection	235 nm
Duration	10 minutes for reference solutions without back flushing (60 minutes for samples with back flushing of columns) of which 10 minutes for back flushing and 50 minutes for the reconditioning of the column.

- Inject a reference specimen of 10 mg/l de N-vinyl-2-pyrrolidone (absolute concentration) three times every 6 to 10 samples to control the performance of the system.

7.4.5. Calculations

$$\text{mg/l of VP} = \frac{20 \times (\text{peak surface area of the sample}) \times (\text{response factor})}{\text{sample in grams}}$$

$$\text{with responsivity} = \frac{(\text{concentration of reference solution in mg/l})}{(\text{peak surface area of the reference solution})}$$

Comment

- Detection limit and minimum quantifiable quantity
Detection limit (signal/noise = 3 for PVPP sample with a content of 0.27 mg/l in N-vinyl-2-pyrrolidone) is ~ 0.10 mg/l with a minimum quantifiable (signal/noise = 10) of 0.33 mg/l.

- Recovery

During a laboratory test, the N-vinyl-2-pyrrolidone, overloaded with PVPP with 1.10 and 100 mg/l of VP, was respectively recovered at 108%, 99.0% and 102%.

- Retention time

The average length of peak retention of N-vinyl-2-pyrrolidone (at a rate of 10 mg/l) is 6.34 ± 0.08 minutes, for a column system + 13 cm long precolumn.

- Interferences

The appropriate duration for back flushing will be set for each system, otherwise a rigorous blocking of the column will take place.

8. DETERMINING THE FREE N,N'- DIVINYLMIDAZOLIDONE IN THE PVPP BY GAS CHROMATOGRAPHY.

This must be determined when the PVPP preparation technique N,N'-divinylimidazolidone.

the free N,N'-divinylimidazolidone in PVPP must not exceed 2 mg per kg.

8.1. Principle

Measuring by gas chromatography on a capillary column of free N,N'-divinylimidazolidone in a solvent (acetone) from non-soluble PVPP. Detection limit is 1 mg/kg.

8.2. Internal test specimen solution:

Dissolve 100 mg \pm 0.1 mg, of heptanoic acid in 500 ml of acetone.

8.3. Preparation of the sample

Weigh 2 to 2.5 g \pm 0.2 mg of polymer and pour into a 50 ml conical flask. Using a pipette, add 5 ml of internal standard solution, then 20 ml of acetone. Shake the mixture for 4 hours. Leave for 15 hours to stabilize and analyse the supernatant by gas chromatography.

8.4. Calibration solution

Weigh 25 mg \pm 0.2 mg of N,N'-divinylimidazolidone (The analytical standard can be obtained from specialized laboratories, actually : BASF, D-67056 Ludwigshafen) and pour into a volumetric flask; add acetone up to 100 ml. Using a pipette, transfer 2.0 ml of this solution in a 50 ml volumetric flask and add acetone up to 50ml. Transfer 2 ml of this solution to a 25 ml volumetric flask, add 5 ml of internal standard solution (see above) and adjust the volume with acetone.

8.5. Gas chromatography conditions (as an example):

Column (fused silica) capillary (cross linked carbowax - 20 M),
length 30 m, innerdiameter 0.25 mm, film thickness 0.5 µm.

Programmed column temperature 140°C to 240°C, 4°C/ minute.

Injector split injector, 220°C.

Flow rate 30 ml/min.

Detector Thermionic detector
(optimised in compliance with
manufacturer's instructions),
250°C.

Carrier gas Helium, 1 bar (suppression).

Volume injected 1 µl of sample floating to the
up solution or reference test
sample solution.

8.6. Procedure

Validation of response factor for specific conditions of analysis is
possible thanks to repeated injections of calibration solutions.

Analyse the sample. The N,N'-divinylimidazolidone content in
non-soluble PVPP must not exceed 0.1%.

8.7. Calculation of response factor:

$$f = \frac{W_d \times A_{se}}{W_{se} \times A_d}$$

W_d - quantity of N,N'-divinylimidazolidone used (mg)

W_{se} - quantity of internal standard used (mg)

A_{se} - peak area of standard solution

A_d - peak area of N,N'-divinylimidazolidone .

8.8. Calculation of N,N'-divinylimidazolidone content:

$$C_D = \frac{1000f.A_d.W_{se}}{A_{se}.W_s} \text{ (mg/kg)}$$

C_D = concentration of N,N'-divinylimidazolidone (mg/kg)

f = response factor

A_d = peak area of N,N'-divinylimidazolidone

W_{se} = quantity of internal standard added to the sample (mg)

A_{se} = peak area of internal standard solution

W_s = quantity of sample used (g)

9. STORING CONDITIONS

PVPP must be kept in a ventilated place in vacuum packed containers away from volatile elements that is might adsorb.

ANNEX

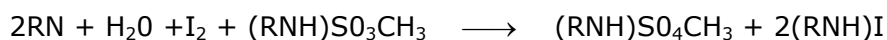
Karl-Fischer procedure

1.FIELD OF APPLICATION

This method is used to determine the water content in a transverse link PVP. Vinylpyrrolidone residue does not interfere with the usual rate present (0.1%). This method is able to detect water with concentrations above 0.05% (m/m).

2.PRINCIPLE

The sample is dissolved in anhydrous methanol and titrated using a Karl-Fischer reagent (KF) without pyridine. Water reacts to the titrating solution in the following way:



The final point (excess I_2) is determined by controlling the change in current between two micro-electrodes and the polarized platinum. The typical KF titration is completely automated and directly produces the calculated water levels.

3.REAGENTS

1. Karl Fischer reagent without pyridine (example by AQUASTAR AXI698A or the equivalent)
2. anhydrous methanol
3. Silica gel with humidity indicator for desiccation of the tube in the cell.
4. The analytic al standard can be obtained from specialized laboratories (actually : BASF, D-67056 Ludwigshafen)

4.APPARATUS

Karl Fischer Titrimeter

5. METHOD

1. Fill the titration recipient with 50 ml of anhydrous methanol or an amount sufficient to cover the electrodes. Fill the desiccation tubes above the cell of the fresh silica gel.
2. Calibrate the titration solution by using distilled water as a specimen.

Record the weight of the sample and the tare, as indicated in the instrument instruction booklet.

The apparatus will automatically calculate the average titer and will store the figure for three testings. (the assay solution H₂O/ml in grams). If an analytical balance is available for reporting the sample weight, follow instructions in the manual.

3. Add 0.075 g to 0.150 g of sample (to the nearest 0.1 mg) in a reaction recipient and mix for 2 minutes. Report the weight of the sample and the tare. The apparatus will measure the assay and automatically determine the % water content.
4. Carry out analysis in duplicate

6. CALCULATION

1. Titrate the assay solution KF, T

$$\frac{\text{water specimen in mg}}{\text{assay solution used in ml}}$$

2. % of water in the sample

$$\frac{0.1 \text{ TV}}{S}$$

Where V = ml of assay solution used
S = weight of samples in grams

7. INTERFERENCES

High concentrations of vinylpyrrolidone (>0.5%) residues react with iodine and produce very imprecise results.

(A 1% vinylpyrrolidone residual rate corresponds to a H₂O rate taken from 0.16% (m/m).

An excess base in the sample risks changing the solution pH and can produce low level results. Samples with pH levels >8 should be buffered with 5 g benzoic acid for 50 ml.

POTASSIUM ALGINATE
Kalii Alginas
(Oeno 33/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This is a potassium salt of alginic acid extracted from various types of pheophyceae algae, in particular laminaria, by means of alkaline digestion and purification.

It is a clarifying agent used during the second fermentation in the bottle for sparkling wines.

2. LABELING

The label should indicate the product's purity and safety and storage conditions.

3. PROPERTIES

Potassium alginate is a white or yellowish powder which is nearly odorless and tasteless and which is composed of fiber fragments, when seen under a microscope.

With water, it produces a viscous solution. The pH of this solution is typically between 6 and 8. It is soluble in strong alcohol and in most organic solvents.

A gelatinous calcium alginate precipitate forms if a 0.50 ml of 20 pp 100 calcium chloride solution (R) is added to 5 ml of an aqueous 1 pp 100 potassium alginate (m/v) solution.

A gelatinous alginic acid precipitate form if 1 ml of sulfuric acid diluted to 10 pp 100 (R) is added to 10 ml of an aqueous 1 pp 100 potassium alginate (m/v) solution.

4. TESTS

4.1. Starch

Add 5 ml of iodinated water (R) to 5 ml of aqueous 1 pp 100 potassium alginate (m/v) solution. No blue coloration should develop.

4.2. Gelatin

Add 1 ml of 2 pp 100 hot tannin (R) to 10 ml of aqueous 1 pp 100 potassium alginate (m/v) solution. No precipitate should form.

4.3. Desiccation loss

Desiccation loss determined up to constant weight of a precisely-weighed sample of approximately 1 g. The weight loss of the potassium alginate at 100-105 °C should not be greater than 15 pp 100.

All limits indicated below are for dry product.

4.4. Sulfur Ash

The sulfur ash content using the method indicated in the Annex is determined by analyzing the residue from the previous test (4.3). The concentration of sulfur ash in the potassium alginate sulfuric ashes should not exceed 40 pp 100.

4.5. Preparing the Solution for Tests

In a silica dish, calcine a sample whose weight corresponds to 2.5 g of dry product, without exceeding 550 °C. Take up the residue with 10 ml of water and 2 ml of concentrated nitric acid (R). Decant in a 50 ml volumetric flask. Add 2 ml of concentrated ammonium hydroxide (R). Top off to 50 ml with distilled water. Filter.

4.6. Sulfates

To 2 ml of the solution prepared for tests under paragraph 4.5, add 2 ml of dilute hydrochloric acid (R) and top off to 20 ml. Add 2 ml of 10 pp 100 barium chloride solution (R). The mixture should be clear ; or else, the opalescence observed after 15 minutes should be less intense than that observed in a control prepared as indicated in the Annex. (Sulfate content expressed in terms of sulfuric acid should be less than 1 g/kg).

4.7. Chlorides

Add 5 ml of dilute 10 pp 100 nitric acid (R), 14 ml of distilled water and 0.5 ml of 5 pp 100 silver nitrate (R) to 1 ml of the test solution (Par. 4.5). Any opalescence that appears should be less intense than that of a control prepared as indicated in the Annex. (Chloride content, expressed in terms of hydrochloric acid, should be less than 1 g/kg).

4.8. Iron

Add 8 ml of water, 1 ml of concentrated hydrochloric acid (R), 1 drop of 1 pp 100 potassium permanganate solution (R) and 2 ml of 5 pp 100 potassium thiocyanate solution (R) to 2 ml of the test solution prepared under paragraph 4.5.

Any red coloration that appears should be less intense than that of a control prepared with 3 ml of iron (III) solution in a concentration of

0.010 g iron per liter (R), 7 ml of water and the same quantities of concentrated hydrochloric acid (R) and 5 pp 100 potassium thiocyanate solution (R). (Iron content should be less than 300 mg/kg).

Iron content can also be determined by atomic absorption spectrometry, by implementing the technique described in the compendium.

4.9. Cadmium

Using the technique described in the annex, determine cadmium content in the test solution (Par. 4.5). Content should be less than 1 mg/kg.

4.10. Lead

Using the technique described in the Compendium, determine lead content in the test solution (Par. 4.5). Content should be less than 5 mg/kg.

4.11. Mercury

Using the technique described in the annex, determine mercury content in the test solution (Par. 4.5). Content should be less than 1 mg/kg.

4.12. Arsenic

Using the technique described in the annex, determine arsenic content in the test solution (Par. 4.5). Content should be less than 3 mg/kg.

4.13. Sodium

Using flame photometry, determine sodium content in the test solution (Par. 4.5). Sodium content should be less than 1 pp 100.

5. STORAGE

Potassium alginate should be stored in hermetically sealed packages.

POTASSIUM ANHYDROUS SULFITE

Potassium pyrosulfite

Potassium disulfite

Potassium metabisulfite

Kalii metabisulfis

$K_2S_2O_5 = 222.3$

SIN No. 224

(Oeno 34/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Potassium anhydrous sulfite, commonly called potassium metabisulfite, is used because of the sulfur dioxide it makes available. Potassium metabisulfite, which is sold in powdered form, contains 52-55% by weight SO_2 .

There are regulatory limits restricting the sulfur dioxide content of wines.

2. LABELING

The label should indicate the product's purity as well as its safety and storage conditions.

3. CENTESIMAL COMPOSITION

Sulfur dioxide	57.63
Potassium	35.17

4. SOLUBILITY

Water at 20 °C	454.5 g/l
Alcohol, 95% by vol.	insoluble

5. IDENTIFYING PROPERTIES

5.1. 5 ml of aqueous 10 pp 100 (m/v) solution treated with 5 ml of 1/10 diluted sulfuric acid (R) releases sulfur dioxide and reduces iodine and potassium permanganate.

5.2. The 10 pp 100 (m/v) aqueous solution is acidic as indicated by methyl red (R) of (pH approximately 5).

5.3. The 1 pp 100 (m/v) aqueous solution produces potassium-based reactions.

6. TESTS

6.1. Preparing the Test Solution in a Concentration of 10 pp 100

Prepare a solution in a concentration of 10 pp 1000 (m/v).

6.2. Preparing a Test Solution in a Concentration of 1 pp 100

Prepare a 1 pp 100 (m/v) solution by diluting the previous solution (6.1) to 1/10.

6.3. Lead

Using the technique described in the Compendium, determine the lead content in the 10 pp 100 test solution (6.1). (Lead content should be less than 5 mg/kg.)

6.4. Mercury

Using the technique described in the annex, determine the mercury content in the 10 pp 100 test solution (6.1). (Content should be less than 1 mg/kg.)

6.5. Arsenic

Using the technique described in the annex, determine the arsenic content in the 10 pp 100 test solution (6.1). (Content should be less than 3 mg/kg.)

6.6. Selenium

Weigh 2.60 g potassium anhydrous sulfite, a quantity which contains 1.5 g sulfur dioxide. Dissolve it under heat in 7 ml of distilled water and 2 ml of concentrated hydrochloric acid (R). Let cool, then add 3 ml of formaldehyde solution (R). Let sit for 10 minutes. Place the tube in a 100 °C water bath and add 50 mg of pulverized potassium anhydrous sulfite which is free of selenium (R). Leave the tube in the 100 °C water bath for 15 minutes. If a pink coloration develops, it should be less intense than that of a control prepared in the same way using 2.60 g of selenium-free potassium anhydrous sulfite (R) to which was added 0.45 ml of a selenium dioxide solution in a concentration of 100 mg of selenium per liter (R). (Selenium content, with respect to the sulfur dioxide, should be less than 10 mg/kg).

6.7. Sodium

Prepare 10 ml of a 1 pp 100 (m/v) solution as indicated in paragraph 6.2 with 2 ml of acetic acid (R). Evaporate the solution in a 100 °C water bath until it is reduced to 1/2.

Pour into a 100 ml volumetric flask. Fill with water to the gauge line. Quantitatively analyze the sodium using flame photometry. (Sodium content should be less than 2 pp 100).

6.8. Chlorides

Place 0.5 ml (concentration: 10 pp 100) of solution as prepared under paragraph 6.1 in a dish with 10 ml of water and 3 ml of 10 pp 100 sulfuric acid solution (R). Evaporate in a 100 °C water bath to reduce the volume to 5 ml. Decant in a test tube. Bring the volume up to 15 ml, then add 5 ml of 10 pp 100 nitric acid (R) and 0.5 ml of 5 pp 100 silver nitrate solution (R). The liquid should remain clear ; or else, any clouding which occurs should be less intense than that in a control prepared as indicated in the Annex. (Chloride content, expressed in terms of hydrochloric acid, should be less than 1 g/kg).

6.9. Iron

Using the technique described in the Compendium, determine the iron content in the 10 pp 100 (m/v) test solution (6.1) using atomic absorption spectrophotometry. (Iron content should be less than 50 mg/kg SO₂.)

7. QUANTITATIVE ANALYSIS

Sulfur dioxide - Place 50 ml of a disodium ethylene diamine tetraacetate solution (120 mg per liter) in a 200 ml conical flask. Add 10 ml of the freshly prepared 1 pp 100 potassium anhydrous sulfite solution and titrate with 0.05M iodine. Let n be the volume in ml ; 1 ml of 0.05M iodine corresponds to 3.2 mg of sulfur dioxide.

Sulfur dioxide content per 100 g: $3.2n$

Potassium anhydrous sulfite should contain at least **51.8 pp 100** sulfur dioxide.

8. STORAGE

This product reacts with air and should be kept in hermetically sealed containers.

POTASSIUM CASEINATE
(Oeno 35/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Potassium caseinate is obtained from fresh or pasteurized skimmed milk by acid coagulation of the casein (see monograph), neutralization using potassium hydroxide and drying with a spray dryer. It is used for the fining of wines.

2. LABELING

The label should indicate the product's purity and safety and storage conditions.

3. PROPERTIES

Potassium caseinate is a white powder with a slightly yellowish tint, whose characteristic odor is typical of that of milk proteins. It exhibits no unusual odor or taste. It yields a colloidal solution in water.

4. TESTS

4.1. pH

In a water solution with 5 g of caseinate per 100 ml of water, the pH should be 7.0 ± 0.5 .

4.2. Desiccation loss

As determined up to constant weight in a sample of approximately 2 g, weight loss at 100-105 °C should not be greater than 6 pp 100.

All of the following limiting values are for dry product.

4.3. Ash

Without exceeding 550 °C, burn the residue from the desiccation loss test. The weight of the ash should not be greater than 6 pp 100.

4.4. Preparing the Test Solution

After weighing, dissolve the ash in 2 ml of concentrated hydrochloric acid (R) and 10 ml of water. Heat to trigger dissolution and fill to 50 ml with water.

4.5. Potassium

Determine the potassium content using flame photometry on the test solution prepared under Paragraph 4.4. (Potassium content should be less than 2 pp 100).

4.6. Iron

Determine the iron content using atomic absorption spectrophotometry on the test solution prepared under paragraph 4.4. (Iron content should be less than 200 mg/kg).

4.7. Lead

Using the technique described in the Compendium, determine the lead content in the test solution (4.4). (Lead content should be less than 5 mg/kg.)

4.8. Mercury

Using the technique described in the annex, determine the mercury content in the test solution (4.4). (Mercury content should be less than 1 mg/kg.)

4.9. Arsenic

Using the technique described in the annex, determine lead the arsenic content in the (4.4). (Arsenic content should be less than 3 mg/kg.)

4.10. Total Nitrogen

Place about 0.20 g of precisely-weighed potassium caseinate in a mineralization cucurbit with 15 ml of concentrated sulfuric acid (R), 2 g of mineralization catalyst (R) and proceed as indicated in the method described in the Annex. Total nitrogen content should not be less than 13 pp 100.

4.11. Fats

The fat content measured as per the method described in the Annex should not exceed 2 pp 100 by weight.

5. STORAGE

Potassium caseinate should be stored in airtight containers, for example, in paper bags lined with polyethylene, at a temperature of between 5 and 20 °C at a relative humidity of less than 65%. The shelf life of potassium caseinate is 24 months.

POTASSIUM D,L-TARTRATE
Potassium D,L-2,3-dihydroxybutanedioate
Potassium racemate
COOK-CHOH-CHOH-COOK = 226.3
(Oeno 42/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Potassium D,L-tartrate is a salt used to deacidify musts and wines and to remove excess calcium.

Its use is subject to certain regulations.

2. LABELING

The label should indicate product purity as well as its safety and storage conditions. It should also clearly state that this is a racemic mixture of the two isomers D and L of tartaric acid, thereby avoiding the supposition that it is the natural L-tartaric acid found in grapes.

3. PROPERTIES

This product is the dipotassic salt of D,L-tartaric acid or racemic tartaric acid $K_2C_4H_4O_6$.

It is found in the form of white crystals or granulated white powder and is highly soluble in water.

4. TESTS

4.1. Desiccation Loss (volatile substances)

After 4 hours of desiccation in a 105 °C oven, weight loss should not exceed 1 pp 100.

4.2. Preparing the Solution for Tests

Place 10 g of potassium racemate in a 100 ml volumetric flask and fill to the gauge line with water.

Perform the same tests on this solution as indicated in the monograph on neutral potassium tartaric, including sodium, and observe the same limits.

4.3. Distinguishing Potassium D,L-Tartrate from Neutral Potassium Tartrate

Proceed as indicated in the monograph on neutral potassium tartrate. No white, crystalline precipitate should form instantaneously.

4.4. Lead

Using the technique described in the Annex, determine the lead content. Content to be less than 5 mg/kg.

4.5. Mercury

Using the technique described in the Annex, determine the mercury content. Content to be less than 1 mg/kg.)

4.6. Arsenic

Using the technique described in the Annex, determine the arsenic content. Content to be less than 3 mg/kg.

4.7. Oxalate

Using the technique described in the Annex, determine the mercury content in the test solution (4.2) (The content, expressed as oxalic acid, should be less than 100 mg/kg.)

5. STORAGE

Potassium tartrate should be stored in hermetically sealed containers.

POTASSIUM HEXACYANOFERRATE (II)

Potassium ferrocyanide

Cianuretum ferroso - Kalium

$K_4[Fe(CN)_6] \cdot 3H_2O = 422.40$

SIN NO. 536

(Oeno 36/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Potassium hexacyanoferrate (II) is found in the form of yellow, monoclinic crystals having no odor and with a bitter, salty flavor. Density is 1.935 at 20 °C.

This salt is slightly efflorescent and begins to lose its water of crystallization at approximately 60 °C. In an oven at 100 °C, it dehydrates completely, becoming white and hygroscopic.

Freshly prepared aqueous solutions are yellow and decay slowly in light with the release of alkalinity. They take on a greenish color by forming a small quantity of Prussian blue.

Potassium hexacyanoferrate (II) is used to remove iron (III) and iron (II) ions in wines, which could cause iron breakdown. It is also used to avoid copper breakdown. It is used, more generally, to reduce the heavy metal content.

Its use must be strictly controlled by mandatory monitoring.

2. LABELING

The label should indicate the product's purity as well as its safety and storage conditions.

3. IDENTIFYING PROPERTIES

The aqueous solution at 1 pp 100 (m/v) yields hexacyanoferrate (II) ion and potassium reactions, particularly with the iron (III) cation, resulting in a dark blue iron (III) hexacyanoferrate (II) (Prussian blue) precipitate which is insoluble in dilute mineral acids. With the copper cation, it forms a purple copper (II) hexacyanoferrate (II) precipitate that is insoluble in dilute mineral acids.

4. SOLUBILITY

Water at 20 °C	265 g/l
Water at 100 °C	740 g/l

5. TESTS

5.1. Desiccation Loss

Place 1 g of powdered potassium hexacyanoferrate (II) in a calibrated dish and dry in an oven at 100 °C until it has a constant weight. Weight loss should be between 12 and 13 pp 100.

5.2. Insoluble Products

Dissolve 10 g of potassium hexacyanoferrate (II) in 100 ml of water. The solution should be clear.

5.3. Preparing the Test Solution

Calcine 1 g of potassium hexacyanoferrate (II) in a silica dish, but without exceeding 550 °C. Take up the residue with 10 ml of water and 2 ml of concentrated nitric acid (R). Decant in a 50 ml volumetric flask. Add 5 ml concentrated ammonium hydroxide (R). Fill to 50 ml with distilled water. Filter.

5.4. Chlorides

To 2.5 ml of this test solution (5.3), add 5 ml nitric acid diluted to 10 pp 100 (R), 12.5 ml of distilled water and 0.5 ml of 5 pp 100 silver nitrate (R). If any opalescence develops, it should be less intense than that observed in a control prepared as indicated in the Annex. (Chloride content, expressed in terms of hydrochloric acid content, should be less than 1 g/kg).

5.5. Sulfates

Add 2 ml hydrochloric acid diluted to 10 pp 100 (R) to a 5 ml test solution (5.3), increase to 20 ml with distilled water and add 2 ml of a barium chloride solution (R). The mixture should be clear; or else, any opalescence observed after 15 minutes should be less intense than that of the control prepared as indicated in the annex. (Sulfates content, as expressed for sulfuric acid, should be lower than 1 g/kg.)

5.6. Sulfides

In the 100 ml flask of a distilling apparatus equipped with a small rectifying column or other anti-priming device (designed to prevent the direct flow of liquid fractions in the flask into the distillate), dissolve 1 g of potassium hexacyanoferrate (II) in 10 ml of hydrochloric acid diluted to 10 pp 100 (R) and 10 ml of distilled water. Distill and collect 5 ml of distillate in 5 ml of 1M sodium hydroxide.

Take 0.5 ml of this distillate and add 18.0 ml of distilled water and 1 ml of a lead nitrate solution in a concentration of 1 g per liter (R). The

resulting brown coloration should be less intense than that of a control prepared by adding 0.5 ml of hydrogen sulfide solution in a concentration of 1 g of sulfur per liter (R), 18 ml of distilled water and 1 ml of lead nitrate in a concentration of 1 g per liter (R). (Sulfide content, expressed in terms of sulfur, should be less than 100 mg/kg).

5.7. Cyanides

In a 40 ml volumetric flask containing 25 ml of distilled water and 2.5 ml of pH 7.5 buffer solution (R), place 40 mg of potassium hexacyanoferrate (II). After dissolving, add immediately 0.3 ml of 0.1 pp 100 T chloramine solution (R). Wait 90 seconds, then add 6 ml of pyridine-pyrazolone reagent (R).

Fill to 40 ml with distilled water and mix. The resulting coloration should not be more intense than that obtained by treating in the same way 4 ml of freshly prepared potassium cyanide solution assayed at 1 mg of hydrogen cyanide per liter (R). (Free cyanide content, expressed in terms of hydrogen cyanide, should be less than 100 mg/kg).

5.8. Lead

Using the technique described in the Compendium, determine the lead content in the solution (5.3). (Lead content should be less than 5 mg/kg.)

5.9. Mercury

Using the technique described in the annex, determine the mercury content in the test solution (5.3). (Mercury content should be less than 1 mg/kg.)

5.10. Arsenic

Using the technique described in the annex, determine the arsenic content in the test solution (5.3). (Arsenic content should be less than 3 mg/kg.)

5.11. Ammonia

Place 2 g of potassium hexacyanoferrate (II), 25 ml of distilled water and 5 ml of 30 pp 100 sodium hydroxide (R) in the flask of a distilling apparatus. Distill and collect 20 ml of distillate in 40 ml of 4 pp 100 boric acid (R) in the presence of methyl red. 1.2 ml of 0.1M hydrochloric acid should be sufficient to turn the indicator. (Total ammonia content should be less than 100 mg/kg).

6. STORAGE

Potassium hexacyanoferrate (II) should be stored in airtight bags away from moisture.

INTERNATIONAL CENOLOGICAL CODEX
POTASSIUM BICARBONATE

POTASSIUM HYDROGEN CARBONATE
Potassium bicarbonate
 $\text{KHCP}_3 = 100.1$
(Oeno 37/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This product is used to deacidify musts and wines. The transport of potassium ions causes salification of free tartaric acid and the formation of potassium hydrogen tartrate.

The use of this product is subject to regulation.

2. LABELING

The label should indicate the product's purity and storage and storage conditions.

3. CENTESIMAL COMPOSITION

Carbon dioxide	43.97
Potassium	39.06

4. PROPERTIES

Potassium hydrogen carbonate is found in the form of a white, odorless powder which is slightly hygroscopic. It leads to carbonate-based reactions.

5. SOLUBILITY

Water at 20 °C 600 g/l

Insoluble in alcohol, 95% by vol.

Soluble with effervescence in dilute acid solutions (acetic, hydrochloric, etc.).

6. TESTS

6.1. Desiccation Loss

After 4 hours of desiccation in an oven at 105 °C, weight loss should be no more than 2 pp 100.

6.2. Preparing the Solution for Tests

Place 10 g of potassium hydrogen carbonate in a 100 ml volumetric flask and fill with water.

6.3. Substances Insoluble in Water

Filter the solution prepared for testing under Paragraph 6.2. The residue, when dried at 105 °C then calcined at 550 °C, should not be greater than 0.1 g (or 1 pp 100).

6.4. Iron

Using the atomic absorption spectrometry technique detailed in the Compendium, analyze the iron content in the test solution (6.2).

6.5. Lead

Using the technique set forth in the Annex, analyze lead content in the test solution (6.2). (Lead content should be less than 5 mg/kg).

6.6. Mercury

Using the technique described in the Annex, determine the mercury content in the test solution (6.2). (Content should be less than 1 mg/kg.)

6.7. Arsenic

Using the technique described in the Annex, determine the arsenic content in the test solution (6.2). (Content should be less than 3 mg/kg.)

6.8. Sodium

Analyze the sodium content in the test solution (6.2) using flame photometry. (Sodium content should be less than 1 pp 100).

6.9. Potassium Hydrogen Carbonate Content

Dissolve approximately 2 g of a test sample, weighed precisely, in 50 ml of 1M hydrochloric acid solution. Titrate the excess hydrochloric acid using a 1M sodium hydroxide solution in the presence of methyl red.

The product intended for wine-making should contain a minimum of 98 pp 100 potassium hydrogen carbonate.

7. STORAGE

Potassium hydrogen carbonate should be stored in airtight containers away from moisture.

POTASSIUM HYDROGEN SULFITE

Potassium bisulfite

Potassium acid sulfite

$\text{KHSO}_3 = 120.2$

SIN No. 228

(Oeno 38/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Potassium hydrogen sulfite is used in oenology because of the proportion of sulfur dioxide it contains.

2. LABELING

The label should indicate the weight per liter or per kilogram of sulfur dioxide and the storage and safety conditions.

There are regulatory limits restricting the sulfur dioxide content in wines.

3. CENTESIMAL COMPOSITION

SO ₂	53.30
K	32.53

4. PROPERTIES

Potassium hydrogen sulfite is found in the form of a colorless or slightly yellow solution obtained by passing a current sulfur dioxide through an aqueous potassium hydroxide solution.

Potassium hydrogen sulfite solutions used in wine-making usually contain between 281 and 375 g/l potassium hydrogen sulfite, these values corresponding to 150 to 200 g/l sulfur dioxide.

5. IDENTIFYING CHARACTERISTICS

Potassium hydrogen sulfite solutions yield reactions of potassium and sulfur dioxide and are slightly acidic (pH of approximately 5).

6. TESTS

The tests are identical to those detailed in the monograph on potassium anhydrous sulfite, as are the limiting content levels for lead, mercury, iron, arsenic, selenium and chlorides.

7. QUANTITATIVE ANALYSIS

Place 50 ml of cold water in a 200 ml conical flask, then add 5 ml of potassium hydrogen sulfite solution. Dilute so that the solution has a concentration of approximately 1 pp 100 SO₂ and titrate with 0.1M iodine in the presence of starch. Let n be the volume of iodine used.

The sulfur dioxide (SO₂) content of the solution, expressed in pp 100 (m/v), is $0.64 \times n$ (concentration cannot be less than 150 g/l).

8. STORAGE

Potassium hydrogen sulfite solutions containing more than 15 pp 100 (m/v) of sulfur dioxide must not be stored at low temperatures, in order to avoid the risk of crystallization.

POTASSIUM HYDROGEN TARTRATE
Potassium L-2,3-dihydroxy hydrogen butanedioate
Monopotassic tartrate
Potassium bitartrate
COOH-CHOH-CHOH-COOK = 188.17
SIN No. 336 i
(Oeno 39/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This addition of potassium hydrogen tartrate, commonly called potassium bitartrate, promotes the crystallization of tartaric acid salts when cold-treating wines.

2. LABELING

The label should indicate the product's purity, size grading, and safety and storage conditions.

3. PROPERTIES

This is an anhydrous monopotassic salt of L(+) tartaric acid $C_4H_5O_6K$. It is found in the form of white crystals or white granulated powder having a slightly acidic taste.

4. SOLUBILITY

Water at 20 °C 5.2 g/l
Water at 100 °C 61 g/l
Insoluble in alcohol

5. TESTS

5.1. Desiccation Loss (Volatile Substances)

After 4 hours of drying in an oven at 105 °C, weight loss should be no more than 1 pp 100.

5.2. Preparing the Solution for Tests

Place 10 g potassium hydrogen tartrate, 50 ml water and 1 ml concentrated hydrochloric acid in a 100 ml volumetric flask. Stir and fill to the top with water.

Perform the same tests on this solution as those indicated in the monograph on L(+) tartaric acid (with the exception of chlorides), and observe the same limits.

5.3. Sodium

Using the flame photometry technique described in the Compendium, analyze sodium content in the test solution (5.2). (Sodium content should be less than 1 pp 100,)

5.4. Iron

Add 1 ml concentrated hydrochloric acid (R) and 2 ml potassium thiocyanate solution having a concentration of 5 pp 100 (R) to 10 ml test solution (5.2). The red color produced should not be more intense than that of a control prepared using 1 ml of an iron (III) salt solution in a concentration of 0.010 g iron per liter (R), 9 ml water, and the same quantities of the same reagents (content should be less than 10 mg/kg).

Iron can also be analyzed quantitatively by atomic absorption spectrometry, in accordance with the technique described in the Compendium.

5.5. Lead

Using the technique described in the Compendium, determine lead content in the test solution (5.2). (Lead content should be less than 5 mg/kg.)

5.6. Mercury

Using the technique described in the annex determine the mercury content in the test solution (5.2). (Mercury content should be less than 1 mg/kg.)

5.7. Arsenic

Using the technique described in the annex, determine the arsenic content in the test solution (5.2). (Arsenic content should be less than 3 mg/kg.)

5.8. Oxalate

Using the technique described in the annex, determine oxalate content in the test solution (5.2). (Oxalate content, expressed in the form of axalic acid, should be less than 100 mg/kg.)

6. STORAGE

Potassium hydrogen tartrate should be stored in hermetically sealed containers.

POTASSIUM SORBATE
Potassium-2,4-hexadienoate
Kalii sorbas
CH₃-CH=CH-CH=CH-COOK
C₆H₇O₂K = 150.2
SIN No. 202
(Oeno 42/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This product is used as a preservative. Potassium sorbate releases 74% sorbic acid, whose anti-fungal properties inhibit the spread of yeast. Its use is limited to 200 mg/l, expressed in the form of sorbic acid.

Sorbic acid is not a bactericide. It is metabolized by certain bacteria and has a characteristic "geranium" taste.

For this reason, its presence in wine does not make it possible to remove SO₂.

2. LABELING

The label should indicate the purity of the product, its sorbic acid content and its safety and storage conditions.

3. CENTESEMAL COMPOSITION

Sorbic acid	74.64
Potassium	26.03

4. SOLUBILITY

Water at 20 °C	highly soluble
Alcohol, 95% by vol.	moderately soluble (≅14 g/l)
Ethyl ether	insoluble

5. IDENTIFYING PROPERTIES

5.1. White, water soluble powder or granules; the solution thereof is neutral when phenolphthalein (R) is added, and alkaline when adding methyl red (R).

5.2. Stir 20 mg potassium sorbate with 1 ml brominated water (R) and 1 drop of acetic acid (R). The color should disappear.

5.3. A solution containing 5 mg potassium sorbate per liter of water has an absorption band of 256 nm.

5.4. A aqueous solution (concentration: 10 pp 100) precipitates using acids and exhibits the characteristics of potassium.

6. TESTS

6.1. Solubility

Verify complete solubility in water and in alcohol.

6.2. Desiccation Loss

1 g potassium sorbate in an oven set at 105 °C should not lose more than 1/100 of its weight in 3 hours.

6.3. Preparing the Solution for Tests

Dissolve 1 g of potassium sorbate in 40 ml of water in a 50 ml volumetric flask. Add 0.5 ml concentrated nitric acid (R). Fill to the gauge line with water and filter.

6.4. Chlorides

Add 0.5 ml of nitric acid diluted to 10 pp 100 (R), 17 ml of water and 0.5 ml of 5 pp 100 silver nitrate (R) to 2.5 ml of the test solution as prepared under Paragraph 4. The resulting opalescence should be less than that of a control prepared as indicated in the Annex. (Chloride content, expressed in terms of hydrochloric acid, should be less than 1 g/kg).

6.5. Sulfates

Add 1 ml of diluted hydrochloric acid diluted to 10 pp 100 (R), 14 ml of water and 2 ml of barium chloride solution (R) to 5 ml of the test solution as prepared under paragraph 6.4. The mixture should be clear; or else, the opalescence observed after 15 minutes should be less than that of a control prepared as indicated in the Annex. (Sulfate content, expressed in terms of sulfuric acid, should be less than 1 g/kg).

6.6. Heavy Metals

Dissolve 1 g of potassium sorbate in 15 ml of water. Add 2 ml of pH 3.5 buffer solution (R) and 1.2 ml of thioacetamide reagent (R). The mixture should remain colorless, or less intensely colored than a solution containing 1 g of the same potassium sorbate in 15 ml of water. If there is an increase in color, it should be equal to that of the control containing 20 µg of lead. For this comparison, use the same system described for sorbic acid. (Heavy metal content, expressed in terms of lead, should be less than 10 mg/kg).

6.7. Lead

Using the technique described in the Compendium, determine lead content in the test solution (6.3). (Lead content should be less than 5 mg/kg).

6.8. Mercury

Implementing the technique detailed in the Annex, determine mercury content in the test solution (6.3). Content should be less than 1 mg/kg.

6.9. Arsenic

Implementing the technique detailed in the Annex, determine the arsenic content in the test solution (6.3). Content should be less than 3 mg/kg.

6.10. Aldehyde Determination

Add 05 ml nitric acid diluted to 10 pp 100 (R) and 14 ml water to 2.5 ml of the test solution (6.3). Add 0.5 ml fuchsin solution bleached using sulfuric acid (R) to 1 ml of this solution and, after 15 minutes, compare to a control tube obtained using 0.5 ml of the same reagent and 1 ml formaldehyde in solution in a concentration of 20 µg per milliliter. The color should be less intense than that of the control. (Aldehyde content, expressed in the form of formaldehyde, should be less than 1 g/kg.).

6.11. Quantitative Analysis

This analysis should be performed using product to be analyzed that has been previously dried in a desiccator with sulfuric acid for 24 hours.

Add a weight, **p** (in g) of dried product of about 0.2 g to the wash bottle of a steam distillation device, along with 1 g of tartaric acid and 10 ml of water. Distill at least 250 ml (until the steam does not entrain any more acid). Titrate the distilled acidity with 0.1M sodium hydroxide solution ; Let **n** be the number of ml used. 1 ml 0.1M sodium hydroxide corresponds to 0.01502 g potassium sorbate.

Potassium sorbate content in percent of the product tested:

$$1.502n / p$$

Titration of the potassium sorbate analyzed should give at least 98 pp 100 for the dried product.

7. STORAGE

Potassium sorbate should be stored in an airtight container away from light to retard oxidation.

POTASSIUM-L(+)- TARTRATE
Potassium-L-2,3-dihydroxybutanedioate
Dipotassium tartrate
Neutral potassium tartrate
COOK-CHOH-COOK, (H₂O)_{1/2} = 235.3
SIN No. 336 ii
(Oeno 41/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

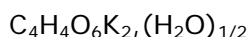
Dipotassium L-tartrate is used to deacidify musts and wines. Its use is subject to the regulatory restrictions in force in certain countries.

2. LABELING

The label should indicate product purity (greater than or equal to 98% in the product by dry weight), its safety and storage conditions, and the fact that deacidification of wine is subject to certain requirements.

3. PROPERTIES

This is the dipotassium salt of L-tartaric acid (positive rotatory power, sometimes written as L(+) tartaric), which crystallizes with a half-molecule of water:



It is made in the form of white crystals or granulated white powder.
It is highly soluble in water.

4. TESTS

4.1. Desiccation Loss (Volatile Substances)

After 4 hours of desiccation in a 105 °C oven, weight loss should not exceed 4 pp 100.

4.2. Preparing the Solution for Tests

Place 10 g of neutral potassium tartrate in a 100 ml volumetric flask and fill to the gauge line with water.

Perform the same tests on this solution as indicated in the monograph on L(+) tartaric acid and observe the same limits.

4.3. Sodium

Implementing the flame photometry technique detailed in the Compendium, determine sodium content in the test solution (4.2). (Sodium content should be less than 1 pp 100.)

4.4. Iron

Add 1 ml concentrated hydrochloric acid (R) and 2 ml potassium thiocyanate solution (concentration : 5 pp 100) (R) to 10 ml of the test solution (4.2). The red color should not be more intense than that of the control prepared using 1 ml of an iron (III) salt solution (concentration : 0.010 g iron per liter) (R), 9 ml water, and the same quantities of the same reagents. (Content should be less than 10 mg/kg.)

The iron content may also be analyzed using the atomic absorption spectrometry technique described in the Compendium.

4.5. Lead

Applying the method set forth in the Compendium, analyze the lead content in the test solution (4.2). Lead content should be less than 5 mg/kg.)

4.6. Mercury

Using the technique described in the Annex, determine the mercury content in the test solution (4.2). (Content to be less than 1 mg/kg.)

4.7. Arsenic

Using the technique described in the Annex, determine the arsenic content in the test solution (4.2). (Content to be less than 3 mg/kg.)

4.8. Distinguishing Between Potassium Tartrate and Potassium Racemate

Place 10 ml of water in a test tube with 1 ml of the test solution prepared under paragraph 4.2, 1 ml crystallizable acetic acid (R) and 2 ml of 25% calcium acetate solution (R). No white, crystalline precipitate should form instantaneously.

4.9. Oxalate

Using the technique described in the Annex, determine the oxalate content in the test solution (4.2). (The oxalate content, expressed in terms of oxalic acid, should be less than 100 mg/kg after drying.)

5. STORAGE

Potassium tartrate should be stored in hermetically sealed containers.

PROTEIN PLANT ORIGIN FROM WHEAT and PEAS
(OENO 28/2004)

1 OBJECT, ORIGIN AND FIELD OF APPLICATION

Currently, the only plant proteins, described in this monograph, is extracted from wheat (*Triticum Sp.s*) and peas (*Pisum sativum*). It is mainly made up of proteins but can also naturally contain carbohydrates (fibres, starch, sugars), fats and minerals. It is intended for human consumption..

The plant protein matter is used for the fining of musts and wines.

It comes in the form of a whitish, beige or yellowish powder. It is totally or partially soluble in water depending on the pH. It can also be in liquid form with content more than or equal to 50 g/l. The solutions are stabilised with sulphur dioxide.

2 LABELLING

The following indications must appear on the label of the package: plant origin of the protein, minimal protein content, safety and storage conditions and expiry date. Without prejudice to the provisions in force in the countries where these products are marketed to be used, GMO origin of the raw material is indicated on the package label.

3 TEST TRIALS

3.1 Loss from desiccation

In a silica capsule with a 70 mm diameter with a lid, place 2 g of proteins. Dry in incubator at 105°C for 6 hours. Allow to cool in open capsule and desiccator. Weigh.

Weight loss must not be more than 12% of the powder preparation.

All limits set below concern dry weight.

3.2 Determination of total nitrogen

On a 0.2 g test sample proceed as indicated in chapter II of the Oenological Codex.

The total nitrogen must be more than 10% of the powder weight (corresponding to about 65% in protein).

3.3 Ashes

Incinerate the residue left from the determination of the loss from desiccation (3.1) by progressively heating at 600°C in a muffle oven until a white residue is obtained and after having sprinkled it with 0.2 to 0.3 g of ashes paraffin in order to avoid mass overflow.

Total ashes must be less than 8%.

3.4 Preparation of the test trial solution

After weighing, dissolve the ashes in 2 ml of concentrated hydrochloric acid (R) and 10 ml of water. Heat in order to activate the dissolving and add distilled water until a volume equal to 25 times the weight of dry protein is obtained. 1 ml of this solution contains mineral substance of 0.04 g of dry protein.

3.5 Iron

1 ml of concentrated hydrochloric acid (R), a drop of potassium permanganate at 1% (R) and 2 ml of potassium thiocyanate at 5% (R) were added to 10 ml of the test solution prepared according to 3.4.

If a red colouration appears, it must be lighter than the control prepared with 6 ml of iron solution (III) at 0.010 g per litre (R), 4 ml of water and the same quantities of concentrated hydrochloric acid (R) and potassium thiocyanate at 5% (R).

Iron content must be less than 150 mg/kg.

It is also possible to proceed with the determination of iron by spectrophotometric atomic absorption according to the method described in chapter II of the International Oenological Codex.

3.6 Chromium

In a 50 ml conical flask, place 10 ml of solution prepared according to 3.4, 1 ml of 15% (R) ammonium persulphate solution at 0.5 ml of a 1% (R) silver nitrate solution at. Heat and add drop by drop until a persistent pink colouration appears of the 3% (R) potassium permanganate solution at. Put a few drops in excess and maintain a gentle boil for 10 minutes. If during boiling, the solution becomes discoloured, add potassium permanganate. After 10 minutes, introduce drop by drop diluted hydrochloric acid at 1/10 (R) until the solution is once again colourless.

After cooling, transfer to a 20 ml graduated flask and add 2 ml of 0.05% diphenylcarbazide in solution at in freshly prepared alcohol (R). Bring to 20 ml.

If a red purplish colouration appears, it must be lighter than that obtained by treating 4 ml of 0.001 g of chromium per litre (R) potassium dichromate solution at by 2 ml sulphuric acid at 5% (R), 5 ml of distilled

water, by adding after mixing 2 ml of diphenylcarbazide solution at 0.05% in alcohol (R) and by bringing to 20 ml.

Chromium content must be less than 10 mg/kg.

It is also possible to proceed with the determination of chromium by atomic absorption according to the method described in chapter II of the International Oenological Codex.

3.7 Copper

2.5 ml of the test trial solution prepared according to 3.4, are placed in a test tube with 7.5 ml of water, 0.5 ml of hydrochloric citric solution (R), 1 ml of ammonium hydroxide 5 M (R), 0.5 ml of sodium diethyldithiocarbamate reagent (R). If a yellow colouration appears, it must not be darker than that obtained by adding the same quantities of the same reagents to 4.7 ml of a copper solution at 1 mg per litre (R) brought to 10 ml.

Copper content must be less than 35 mg/kg.

It is also possible to proceed with the determination of copper by atomic absorption according to the method described in chapter II of the International Oenological Codex.

3.8 Zinc

To 1.25 ml of the test solution prepared according 3.4, add 3.75 ml of distilled water, 5 ml of acetate buffer solution (R), 1 ml of sodium thiosulphate solution at 25% (m/v) (R), 5 ml of dithizone solution at 25 mg per litre in chloroform or dichloromethane (R). Shake for 2 minutes. Separate the organic phase; its colouration must be lighter than that obtained by treating 2 ml of zinc solution at 1 mg per litre (R) with the same quantities of the same reagents.

Zinc content must be less than 50 mg/kg.

It is also possible to proceed with the determination of zinc by atomic absorption according to the method described in chapter II of the International Oenological Codex

3.9 Lead

Using the test trial solution (3.4), perform the determination using the method described in chapter II of the International Oenological Codex.

Lead content should be less than 5 mg/kg.

3.10 Mercury

Perform the determination of mercury using the method described in chapter II of the International oenological Codex.

Mercury content should be less than 1 mg/kg

3.11 Arsenic

Perform the determination of arsenic using the method described in chapter II of the International oenological Codex.

Arsenic content should be less than 3 mg/kg.

3.12 Cadmium

Perform the determination of cadmium using the method described in chapter II of the International Oenological Codex.

Cadmium content should be less than 1 mg/kg.

4 MICROBIOLOGICAL CONTROL

4.1 Total viable micro-organisms

Proceed as described in Chapter II of the International Oenological Codex.

Content less than $5 \cdot 10^4$ CFU/g.

4.2 *Escherichia coli*

Proceed with counting as described in Chapter II of the International Oenological Codex.

Absence checked on a 1 g sample.

4.3 Salmonella

Proceed with counting as described in Chapter II of the International Oenological Codex.

Absence checked on a 25 g sample.

4.4 Coliforms

Proceed with counting as described in Chapter II of the International Oenological Codex.

Content less than 10^2 CFU/g.

4.5 Yeasts

Proceed with counting as described in Chapter II of the International Oenological Codex.

Content less than 10^3 CFU/g.

4.6 Moulds

Proceed with counting as described in Chapter II of the International Oenological Codex.

Content less than 10^3 CFU/g.

5 SEARCH FOR MYCOTOXINS AND PESTICIDE RESIDUES

5.1 Aflatoxins B₁

Proceed with analysis according to ISO method 16050
Content less than 4 µg/kg.

5.2 Aflatoxin B₁, B₂, G₁, G₂

Proceed with analysis according to ISO method 16050
Content less than 4 µg/kg in total.

5.3 Organophosphorous pesticide residues *

Content less than 10 mg/kg.

5.4 Organochlorine pesticide residues*

Content less than 0.1 µg/kg.

5.5 Ochratoxine A

Using an aqueous solution of 5 g/l of plant protein, perform the determination using the method described in the Compendium of methods of analysis of musts and wines.

Content less than 5 µg/kg.

6 STORAGE

The plant proteins should be stored in closed containers or in watertight bags impervious to humidity under temperate conditions.

*Method to be determined at a later date.

RECTIFIED ALCOHOL OF AGRICULTURAL ORIGIN
(Oeno 11/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Rectified, or "neutral," alcohol obtained by distilling and rectifying alcohol from wine, wine sediments or alcoholic fermentation products derived from from grape or raisin marcs, and all other plant-based substances of agricultural origin.

Rectified alcohol of agricultural origin forms an ingredient of some spirits and special wines.

2. COMPOSITION

At a temperature of 20 °C, 100 parts of this alcohol contain at least 96 parts ethanol.

Note: **The tests and controls described below in italics are not mandatory and are performed only upon request.**

3. PROPERTIES

Colorless, clear, volatile liquid with a penetrating odor and fiery flavor. It is flammable and burns without smoke and with a blue flame.

It should be distilled completely at between 78 and 79 °C.

3.1. Solubility

Neutral alcohol is miscible in water in all proportions with a notable release of heat and contraction of volume. It is also mixable with in acetone, chloroform, ethyl ether, glycerol, and an equal volume of castor oil.

3.2. Characterization Procedure

- Slowly heat a mixture of 1 ml neutral alcohol, twenty drops of concentrated sulfuric acid (R) and 10 g of sodium acetate (R) in a test tube. A strong, characteristic odor of ethyl acetate will be released.

- Mix several drops of alcohol and 1 ml of concentrated sulfuric acid (R), then add several drops of 10 pp 100 potassium dichromate solution. The liquid will become green and emit the odor of ethanal.

- Dilute 0.5 ml of alcohol with 4.5 ml of water. Add 1 ml of 1M sodium hydroxide solution, then slowly add 2 ml of iodized potassium iodide (R). An odor of iodoform will be produced, following by the formation of a yellow precipitate.

3.3. Analysis of Agricultural Origin

This analysis is carried out by measuring the ethanol 14C/12C ratio (scintillation) in accordance with the method described in the Spirits Compendium.

4. TESTS

4.1. Appearance

Take two identical test tubes made of alkali-lime glass about 250 mm high, fill one with alcohol, the other with water, which will serve as a control. Examine the liquids along the cylinder's axis. The alcohol should not exhibit any noticeable coloration.

In one test tube about 250 mm high and 25 mm in diameter, pour 40 ml of alcohol, then dilute it with 80 ml of water. The mixture should not cloud nor present any odor or foreign taste.

4.2. Foreign Odoriferous Substances

Let 10 ml of alcohol evaporate spontaneously on a strip of white filter paper. No foreign odor should be perceived during or after evaporation.

4.3. Dry Extract or Non-Volatile Residue

In a 25 ml calibrated dish, heat to 100 °C in a water bath, then slowly evaporate 100 ml of alcohol. Weigh. The dry extract should be less than 1.5 g/hl 100% ethanol by volume.

4.4. Heavy Metals

Take up, using 10 ml dilute chlorhydric acid (R), any residue left from the evaporation of 100 ml alcohol during the dry extract analysis. After heating for several minutes in a 100 °C water bath to stimulate dissolution of this residue, decant the acid solution in a 25 ml volumetric flask after the calibrated dish has been washed three times with 5 ml of water and the volume raised to 25 ml. Take a 5 ml sample of this solution in a test tube. Add 2 ml of a pH 3.5 (R) buffer solution, 7.5 ml of water and 1.2 ml of thioacetamide reagent (R). The solution should not produce any white or black precipitates nor any brown or other coloration. At the very least, any coloration produced should be no more intense than that obtained using the general method. (Heavy metal content expressed in lead, after 50% concentration of the alcohol, should be 0.5 mg/l).

4.5. Lead

Using the method set forth in the Compendium, perform the lead analysis lead in the solution obtained in the previous paragraph. (Lead content should be less than 0.5 mg/l).

4.6. Mercury

Using the method described in the annex, carry out the mercury analysis in the solution obtained in Paragraph 4.4. (Mercury content should be less than 0.2 mg/l).

4.7. Arsenic

Using the method described in the annex, carry out the arsenic analysis in the solution obtained in Paragraph 4.4. (Arsenic content should be less than 0.5 mg/kg after 50% alcohol concentration).

4.8. Ketones, propan-2-ol and 2-methylpropan-1-ol

Add 3 ml of water and 10 ml of mercury sulfate (II) solution (R) to 1 ml of alcohol, then heat in a 100 °C water bath. No precipitate should form in the first three minutes.

4.9. Permanganate Decolorization Time (Barbet Test)

Pour 50 ml of the alcohol sample into a flask. Add 2 ml of freshly prepared potassium permanganate solution to 0.20 g/l (R). Place the container in a 15 °C water bath and start a stopwatch. Avoid directly exposing the sample to natural or artificial light during the test.

Simultaneously, place 50 ml of the comparison solution in the 15 °C water bath. This solution is obtained by mixing 3 ml of 5 pp 100 cobalt chloride solution (R), 4.2 ml of 4 pp 100 uranyl nitrate solution (R) and filling to 50 ml with distilled water. Compare the test color to the standard. Stop the timer when the colors are identical. Note the amount of time elapsed. The decolorization time of the permanganate should be at least 20 minutes.

4.10. Sulfured Derivatives

Add approximately 1 ml of mercury, then 20 ml of alcohol to a test tube. Agitate for 1-2 minutes. The surface of the mercury should remain brilliant with no black clouding.

4.11. Methanol

4.11.1 Colorimetric Analysis

Standard solution: weigh 5 g of methanol in a 50 ml volumetric flask, then top off to the line with ethanol (free of methanol).

In a 1-liter volumetric flask, place 1 g of the preceding solution (i.e., 1.25 ml) containing 125 mg of methanol, 250 ml of pure alcohol (methanol free). Top off with water to 1000 ml.

Test technique: place 1250/A ml of alcohol in a volumetric flask (A is the alcoholmetric titer of the alcohol to be tested.) and fill to the

gauge line with water. Place 1 ml of alcohol, diluted to 25 pp 100 in a test tube. Add four drops of 50 pp 100 (m/m) phosphoric acid (R), four drops of 5 pp 100 (m/m) potassium permanganate solution (R), then stir and let sit 10 minutes. Decolorize the permanganate with several (typically 8) drops of 2 pp 100 (m/v) of potassium anhydrous sulfite (metabisulfite) (R), avoiding any excess. Add 5 ml of chromotropic sulfuric acid solution (R). Place in a 70 °C water bath for 20 minutes. No violet color should appear, or in the event it does appear, it should not be more intense than that of a control prepared using the same technique and the same reagents, with 1 ml of the aforementioned standard solution (maximum methanol content is 50 g/hl at 100% vol.).

4.11.2 *Gas phase chromatography Analysis*

Equipment (example):

Gas phase chromatograph with a flame ionization detector

Semi-polar capillary columns, for example Carbowax 20 M ®.

Test technique:

Prepare a water-alcohol solution using 1 g per liter of the internal standard (4-methylpentane-2-ol) in 50 pp 100 alcohol by volume.

Prepare the solution to be analyzed by adding 5 ml of this solution to 50 ml of alcohol reduced to 50 pp 100 by volume.

Prepare a reference solution of methanol at 100 mg per liter of alcohol at 50 pp 100 by volume. Add 5 ml of the internal standard solution to 50 ml of this solution.

Inject 2 microliters of the solution to be analyzed added to the internal standard solution, into the chromatograph.

The oven temperature should be 90 °C and the supporting gas flow rate should be 25 ml per minute. These settings are given as an example.

S: surface of the methanol peak of the reference solution

S_x: surface of the methanol peak of the solution to be analyzed

i: surface of the internal standard solution peak in the solution to be analyzed

I: surface of the internal standard solution peak in the reference solution

The methanol content, expressed in milligrams per liter of alcohol at 50 pp 100 by volume, is given by the formula:

$$C = 100(I/i)(S_x/S)$$

The content in grams per hectoliter of pure alcohol is 0.20C (maximum content in methanol 50 g/hl of ethanol at 100% by volume).

4.12. Ammonium Hydroxide and Nitrogenous Bases

Pour 50 ml of the alcohol to be examined into a 200 ml flask. Add 40 ml of water and two drops of phosphoric acid ($\rho_{20} = 1.58$). Distill and collect the 80 ml that are returned. Add 2 ml of 10 pp 100 sodium hydroxide ® to the cooled residue. Distill again and collect approximately 7 ml of distillate in a test tube to which had previously been added 2 ml of water and one drop of methyl red solution ®. The distillate should be drawn to the bottom of the tube using a slender tube. Titrate using a solution of 0.01 M hydrochloric acid until the indicator turns to red. Let n be the number of milliliters of 0.01 M hydrochloric acid solution used.

1 ml of 0.01 M hydrochloric acid solution corresponds to 0.00014 g of nitrogen (ammoniacal or volatile nitrogen bases).

The quantity of ammoniacal nitrogen or nitrogenous bases expressed in milligrams of nitrogen per liter of ethanol is:

$$280n/A$$

Where A is the alcohometric titer by volume of the alcohol studied.

Neutral alcohol should not contain more than 1 mg of nitrogen (ammoniacal or of volatile nitrogenous bases) per liter of ethanol.

(Maximum ammonium hydroxide and nitrogenous base content is expressed in terms of nitrogen is 0.1 g/hl of ethanol at 100% by volume).

4.13. Acidity

Place 100 ml strengthened of 50 pp 100 by volume alcohol in a 250 ml conical flask. Add one drop of phenol red solution (R) and add 0.01 M sodium hydroxide, one drop at a time, until red, where n is the number of milliliters used.

1 ml of 0.01 M sodium hydroxide corresponds to 0.0006 g of acetic acid.

Acidity expressed in milligrams of acetic acid per liter of ethanol is equal to $12n$.

This acidity should be less than 15 mg/l of ethanol (or 1.5 g/hl) at the time the alcohol is delivered.

(Maximum acidity expressed in terms of acetic acid is 1.5 g/hl of ethanol at 100% by volume).

Note: Indicator movement should be stable and clear cut during quantitative analysis of the acidity. If it is not, and especially if the acidity exceeds 15 mg/l, a new test should be conducted after the sample is degassed using the following technique.

100 ml of alcohol at 50 pp 100 by volume is placed in a 250 ml flask whose stopper has two tubes through it.

One tube permits the flask to be kept under a vacuum using a glass filter pump. Pressure is kept between 55 and 65 cm of mercury.

During the procedure, the other tube allows air bubbling from which carbon dioxide is removed by using a sode wash bottle. To accomplish this, the tube has a capillary portion which is submerged in the alcohol. The rate of air flow through the wash bottle is approximately 1 ml per second.

The procedure should last between 3 and five minutes. Titration is accomplished in the same flask.

4.14. Esters

Add 10 ml of 0.1 M sodium hydroxide solution measured with precision to the solution prepared to analyze acidity as detailed under 4.13 (or 100 ml of alcohol at 50% by volume). Cork the flask and stir while maintaining a temperature equal to or slightly higher than 20 °C. After 24 hours of contact, titrate the excess sodium hydroxide using a 0.1 M solution of hydrochloric acid, where n is the number of milliliters used.

To determine the quantity of 0.1 M hydrochloric acid solution which will neutralize 10 ml of 0.1 M sodium hydroxide solution in the presence of the same quantity of alcohol and of the same indicator movement obtained by decreased pH intervals, perform the following test: place 100 ml of degasified 50 pp 100 alcohol in a 250 ml conical flask. Add one drop of phenol red solution (R) and n milliliters of 0.1 M sodium hydroxide. Which cause the indicator to turn to red. Add 10 ml of the 0.1 M sodium hydroxide solution, and, immediately thereafter, add 0.1 M hydrochloric acid solution to obtain the same movement of the indicator, that is, n^n of the volume used.

1 ml of 0.1 M sodium hydroxide solution corresponds to 0.0088 g of ethyl acetate. The ester concentration, expressed in milligrams of ethyl acetate contained in 1 liter of ethanol is:

$$176(n^n - n')$$

This content level should not exceed 13 mg for 1 liter of ethanol (or 1.3 g/hl) at the time the alcohol is delivered.

(Maximum ester content expressed in terms of ethyl acetate is 1.3 g/hl of ethanol at 100% of volume).

4.15. Aldehydes

Standard solution: Place 268.3 mg of pure acetal (boiling point : 102°C) in a 100 ml volumetric flask. Top off to the line with 50 pp 100 alcohol by volume, free of aldehydes.

Dilute this solution to 1/10 in 50 pp 100 alcohol by volume, is free of aldehydes. The solution obtained contains 100 mg of ethanal per liter of 50 pp 100 alcohol by volume, or 20 g in 100 liters of ethanol.

Test procedure: Place 10 ml of alcohol reduced to 50 pp 100 by volume in a test tube. In a second test tube, place 5 ml of the solution containing 100 mg of ethanal per liter of alcohol at 50 pp 100 and 5 ml of alcohol at 50 pp 100 by volume which is free of aldehydes. Add to the two tubes 4 ml aniline red chlorhydrate solution decolorized by sulfuric acid (R), stir, and compare the colorations obtained after 20 minutes.

The alcohol to be tested should have a color approximately equal to that of the standard solution.

(Maximum aldehyde content expressed in ethanal is 0.5 g/hl at 100% of volume).

Note concerning 50 pp 100 alcohol by volume without aldehydes: Place 100 ml of alcohol diluted to 50 pp 100 by volume in a 250 ml flask with 2 g of metaphenylene diamine (R) and two pieces of pumice stone. Connect the flask to a reflux condenser and maintain a gentle boil for one hour. After cooling, connect the flask to the distilling apparatus and slowly distill without overheating the walls. Collect 75 ml of distillate in a 100 ml volumetric flask. Fill to the line with distilled water.

4.16. Superior Alcohols

Propan-1-ol, 2-methylpropan-1-ol, 2- and 3-methylbutan-1-ol. Quantitative analysis by gas phase chromatography (see methanol). Maximum content for the sum of each of the alcohols: 0.5 g/hl of ethanol at 100% of volume.

4.17. Furfural

Place 10 ml of alcohol reduced to 50% by volume in a test tube with an emery stopper. Add 0.5 ml of aniline (R) and 2 ml of crystallizable acetic acid (R). Stir. No salmon pink coloration should be perceptible after 20 minutes.

5. STORAGE

The alcohol should be stored in inert containers which will not give off metals, ions or plastic constituents.

INTERNATIONAL ŒNOLOGICAL CODEX
RECTIFIED ALCOHOL OF AGRICULTURAL ORIGIN

The containers, as well as storage methods, must be in compliance with safety standards.

RECTIFIED ALCOHOL OF VITI-VINICULTURAL ORIGIN
(Oeno 12/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

Alcohol obtained exclusively by distillation and rectification from wine, grape marcs, wine sediments, or fermented raisins.

Rectified alcohol of viti-vinicultural origin constitutes a constituent of some spirits and special wines.

2. COMPOSITION

At a temperature of 20 °C, 100 parts of this alcohol contain at least 96 parts ethanol.

Note: **The tests and controls described below in italics are not mandatory and are performed only upon request.**

3. PROPERTIES

Colorless, clear, volatile liquid with a penetrating odor and fiery taste. It is flammable and burns without smoke and with a blue flame.

It should be distilled completely at between 78 and 79 °C.

3.1. Solubility

Neutral alcohol is miscible in water in all proportions with a notable release of heat and contraction of volume. It is also mixable with acetone, chloroform, ethyl ether, glycerol, and an equal volume of castor oil.

3.2. Characterization Procedure

- Slowly heat a mixture of 1 ml neutral alcohol, twenty drops of concentrated sulfuric acid (R) and 10 g of sodium acetate (R) in a test tube. A strong, characteristic odor of ethyl acetate will be released.

- Mix several drops of alcohol and 1 ml of concentrated sulfuric acid (R), then add several drops of 10 pp 100 potassium dichromate solution. The liquid will become green and emit the odor of ethanal.

- Dilute 0.5 ml of alcohol with 4.5 ml of water. Add 1 ml of 1M sodium hydroxide solution, then slowly add 2 ml of iodized potassium iodide (R). An odor of iodoform will be produced, following by the formation of a yellow precipitate.

3.3. Determination of Viti-vinicultural Origin

This analysis is carried out by measuring the ethanol 14C/12C ratio (scintillation) in accordance with the method described in the Spirits Compendium.

3.4. If necessary, the viti-vinicultural source of the alcohol can be determined using isotopic methods detailed in the Compendium of Wine and Must Analysis Methods.

4. TESTS

Test are identical to those for rectified alcohol of agricultural origin , but with the following content limits:

4.1. Methanol

Maximum content 50 g/hl of ethanol at 100% by volume.

4.2. Acidity

Maximum acetic acid content 1.5 g/hl of ethanol at 100% by volume.

4.3. Esters

Maximum content of ethyl acetate 1.3 g/hl of ethanol at 100% by volume (or 5 g/hl).

4.4. Aldehydes

Maximum ethanal content 0.5 g/hl of ethanol at 100% by volume.

4.5. Superior Alcohols

Maximum content 0.5 g/hl of ethanol at 100% by volume.

4.6. Preparing the solution for tests

Using 10 ml of dilute hydrochloric acid (R), take up the residue left by evaporating 100 ml of alcohol during the dry extract analysis. After heating for several minutes in a 100 °C water bath to stimulate dissolution of this residue, decant the acid solution in a 25 ml volumetric flask, and wash the dish three times with 5 ml of water and filled to 25 ml.

4.7. Heavy metals

Place 5 ml of the prepared solution in a test tube in accordance with paragraph 4.6. Add 2 ml of pH 3.5 (R) buffer solution, 7.5 ml of water and 1.2 ml of thioacetamide reagent (R). The solution should not yield any white or black precipitate nor any brown or coloring. At the very least, any coloring produced should be no more intense than that obtained using the general method (heavy metals content expressed in terms of lead, after 50% concentration of the alcohol, should be 0.5 mg/l).

4.8. Lead

Using the method set forth in the Compendium, conduct the quantitative lead analysis on the solution prepared for testing (under paragraph 4.6) (lead content should be less than 0.5 mg/l).

4.9. Mercury

Carry out the quantitative mercury analysis on the solution prepared for testing (under Paragraph 4.6), implementing the technique described in the annex (mercury content should be less than 0.2 mg/l).

4.10. Arsenic

Conduct the quantitative arsenic analysis on the solution prepared for testing (Paragraph 4.6), using the method described in the annex (Arsenic content should be less than 0.5 mg/kg).

5. STORAGE

Alcohol should be stored in inert containers which will not give off metals, ions or plastics constituents.

The containers as well as the storage methods must comply with safety standards.

REVERSE OSMOSIS MEMBRANES

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

A reverse osmosis membrane is a membrane belonging to the group of semi-permeable thin-layer composites (known as TFC, or Thin Film Composites).

Reverse osmosis is a must-enrichment treatment. It entails the use of a membrane to remove pure water, thus increasing the concentration of sugars and other constituents in solution in grape musts.

2. PRINCIPLE UNDERLYING THE PROCEDURE

This is a physical process for removing a portion of the water in a must using a semi-permeable membrane acted upon by a pressure gradient at ambient temperature and without changing or degrading its condition.

The equipment used consists essentially of a so-called « booster » pump which feeds a high-pressure pump (under 100 bars, for example) which allows osmotic pressure to be overcome, a membrane block and control apparatuses such as a flow meter, pressure indicator, pressure regulator, etc.

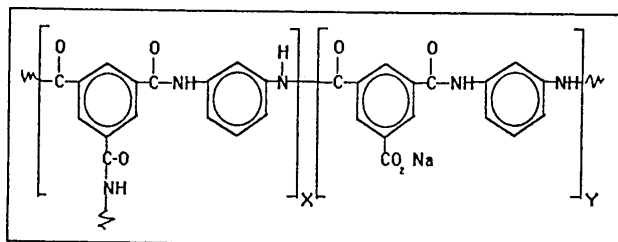
3. COMPOSITION

All equipment used in this Prescriptions must be in compliance with the regulations concerning fittings which come into contact with foodstuffs (pipes, pumps, control apparatuses, joints, etc.) and in particular, the reverse osmosis membrane.

The substances which make up the membrane must be in compliance with the regulations in force.

These membranes are prepared by *in situ* polymerization of a polymer on the surface of a porous substrate. The substrate is typically an polysulfone ultrafilter. The thin layer serves as a discriminating membrane, while the porous substrate provides physical support.

As an example, the structural formula of the polyamide base is as follows:



4. IMPLEMENTATION

During the manufacturing process, the membrane passes through a number of extraction baths containing hot water in order to eliminate traces of solvent and residual monomers.

In particular, under normal or unforeseen circumstances it cannot give off any constituents which could pose a threat to human health (with respect to the component most easily measured, i.e., sodium chloride, in particular, it should exhibit a substance-retention rate greater than 99%). It must not cause an undesirable change of the composition of the grape must (or of a solution containing 170 g/l of sugar and 5 g/l of tartaric acid neutralized to a pH of 3.5 by potassium hydroxide), nor can it alter the organoleptic properties of the must.

5. MEMBRANE REGENERATION

As regenerating agent, the operator can use inorganic products permitted under the regulations, provided that the operation ends by washing with water so as to completely remove the regenerating agent before adding the must.

6. LIMITS

- All equipment/materials in contact with food products must be in compliance with the standards in force.
- There should be no perceptible change of the organoleptic properties of the processed must.

Any release of product or derivative constituting a constituent of the membrane must be less than 50 µg/l in its entirety, which is the

recommended value, and it must comply with the regulatory limits governing the specific migration of the various materials constituents.

7. SPECIAL RESTRICTIONS

Membranes may be supplied only by approved suppliers or distributors.

Use of the membrane must be monitored and restricted by :

- installing a time meter and a volumeter which are sealed at the permeate outlet,
- the physical impossibility inhering in the process of increasing the concentration of the must beyond the established threshold.

SORBIC ACID

Trans,trans-hexa-2-4-dienoic acid

CH₃-CH=CH-CH=CH-COOH

C₆H₈O₂ = 112.1

SIN NO. 200

(Oeno 45/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This product falls under the category of antifungal preservatives (see potassium sorbate). Because it is not soluble in wine, it cannot be used as is, but rather in its potassium salt form. It may be soluble in some spirits.

Its use is subject to regulatory restrictions on content.

2. LABELING

The label should indicate the product's purity and its safety and storage conditions.

3. SOLUBILITY

Water at 20 °C	1.6 g/l
Water at 100 °C	38 g/l
Alcohol	55 g/l
Ethyl ether	104 g/kg

This acid can be entrained in steam. At 100 °C, the steam has a sorbic acid concentration equal to 59% of the concentration of the dilute, boiling solution.

The ethyl ether/water partition coefficient is 32.

4. IDENTIFYING PROPERTIES

4.1. Melting point: 134 ± 2 °C. Boiling point: 228 °C.

4.2. Stir 20 mg of sorbic acid with 1 ml of brominated water (R). The color should disappear.

4.3. A solution containing 4 mg of sorbic acid per liter of water containing 0.5 g of monosodium carbonate per liter has an absorption band of 256 nm.

5. TESTS

5.1. Moisture

Not more than 0.5% of sorbic acid must be made up of water (Karl Fisher method).

5.2. Sulfuric Ash

The proportion of sulfuric ash is determined as indicated in the Annex. It should be less than 0.2 per 100.

5.3. Preparing the Solution for Tests

Shake 0.5 g of sorbic acid with 70 ml of boiling water. Let the solution cool. Filter and collect the filtrate in a 100 ml volumetric flask. Wash the first container and the precipitate and the filter several times with several ml of water until 100 ml of filtrate is obtained.

5.4. Sulfates

To 20 ml of solution prepared for tests under paragraph 5.3, add 1 ml hydrochloric acid diluted to 10 pp 100 (R) and 2 ml of barium chloride solution (R). The mixture should be clear, or the opalescence observed after 15 minutes should be less than that of the control solution prepared as indicated in the Annex. (Sulfate content expressed in terms of sulfuric acid should be less than 1 g/kg).

5.5. Chlorides

To 10 ml of solution prepared for tests under paragraph 5.3, add 5 ml of water, 5 ml of nitric acid diluted to 10 pp 100 (R) and 0.5 ml of 5 pp 100 silver nitrate solution (R). The mixture should be clear or the opalescence observed after 15 minutes should be less than that of the control solution prepared as indicated in the Annex. (Chloride content expressed in terms of hydrochloric acid should be less than 1 g/kg).

5.6. Heavy Metals

Take 10 ml of the solution prepared under paragraph 5.3. Add 2 ml of pH 3.5 (R) buffer solution, and 1.2 ml of thioacetamide reagent (R). Use the method described in the Annex. (Heavy metal content expressed in terms of lead should be less than 10 mg/kg).

5.7. Lead

Using the technique described in the Compendium, determine lead content in the test solution (5.3). (Lead content to be less than 5 mg/kg.)

5.8. Mercury

Using the technique described in the annex, determine mercury content in the test solution (5.3). (Content to be less than 1 mg/kg.)

5.9. Arsenic

Using the technique described in the annex, determine arsenic content in the test solution (5.3). (Content to be less than 3 mg/kg.)

5.10. Aldehydes

Prepare a saturate aqueous sorbic acid solution by agitating 1 g of sorbic acid with 35 ml of very hot water. Let cool in a corked flask. Filter and collect the filtrate in a 50 ml volumetric flask. Wash the flask, the precipitate and the filter several times with several ml of water until 50 ml of filtrate are obtained. Treat the solution with 0.5 ml of fuchsin solution bleached out with sulfuric acid (R). After 15 minutes, compare it to a control tube produced with 0.5 ml of the same reagent and 1 ml of formaldehyde in solution with 20 µg per ml. The resulting coloration should be less intense than that of the control. (Aldehyde content, expressed in terms of formaldehyde, should be less than 1 g/kg).

5.11. Quantitative Analyses

These analyses must be performed using sorbic acid which has previously been dried in a desiccation chamber with sulfuric acid for 24 hours.

1° Weigh a quantity **p** of sorbic acid of about 0.20 g and dissolve it in 10 ml of pure alcohol. Then dilute in 100 ml of water. Titrate the acidity using a 0.1M solution of sodium hydroxide in the presence of phenolphthalein solution (R). Let *n* be the amount in ml used:

1 ml of 0.1M sodium hydroxide solution corresponds to 0.0112 g of sorbic acid. Content in pp 100 of sorbic acid in the product tested:

$$1.12 \, n/p$$

2° The same procedure should be performed after entrainment in steam. Place 10 ml of the alcoholic solution containing a quantity of **p** grams of sorbic acid (about 0.2 g) in the bubble chamber of a steam distillation machine. Add a crystal (about 0.5 g) of tartaric acid and distill at least 250 ml (until the steam no longer distills acid). Titrate the distilled acidity using a 0.1M sodium hydroxide solution.

Using these two analyses, the product tested should contain at least 98 pp 100 sorbic acid.

6. STORAGE

Sorbic acid should be stored in hermetically sealed, airtight containers.

THIAMINE HYDROCHLORIDE
3-[(4-amino-2-methyl-5-pyrimidinyl)methyl-5-
(2-hydroxyethyl-4-methylthiazolium hydrochloride
Thiaini hydrochloridum
 $C_{12}H_{18}Cl_2N_4OS = 337.3$
(Oeno 50/2000)

1. OBJECTIVE, ORIGIN AND SCOPE OF APPLICATION

This product is used to promote alcohol fermentation. Its use is subject to statutory restrictions placed on limiting thiamine content.

2. LABELING

The label should indicate the purity percentage of the product, the use-by date, and the safety and storage requirements.

3. PROPERTIES

White or substantially white crystalline powder or colorless crystals with a slight characteristic odor. Easily soluble in water, soluble in glycerol, minimally soluble in alcohol, virtually insoluble in chloroform and ethyl ether.

4. SOLUBILITY

Water at 20 °C	1000 g/l
Alcohol, 95% by vol.	12.5 g/l
Glycerol	63.3 g/l
Ethyl ether,	insoluble

5. PROOF

Proof test **5.1** may be omitted when tests **5.2** and **5.3** are performed. Proof test **5.2** may be omitted when tests **5.1** and **5.3** are performed (methods described in the Annex).

5.1. Examine the thiamine hydrochloride by absorption spectrophotometry in infrared light.

The maximum absorption values of the spectrum obtained from the substance tested correspond, in position and relative intensity, to those obtained from thiamine hydrochloride SCR. If the spectra exhibit differences, dissolve the substance to be tested and the chemical reference substance, respectively, in water. Evaporate the solutions until dry and produce new spectra from the resulting residues.

5.2. Dissolve about 20 mg thiamine hydrochloride in 10 ml of water. Add 1 ml dilute acetic acid (R) and 1.6 ml of 1M sodium hydroxide (R). Heat in a 100 °C water bath for 30 minutes and let cool. Add 5 ml of dilute sodium hydroxide solution (R), 10 ml potassium hexacyanoferrate (III) solution (R) and 10 ml of butanol. Shake vigorously for 2 minutes. An intense light blue fluorescence will spread in the alcoholic layer, especially under ultraviolet light at 365 nm. Repeat the test using 0.9 ml 1M sodium hydroxide and 0.2 g sodium sulfite replacing the 1.6 ml 1M sodium hydroxide. Virtually no fluorescence will be observed.

5.3. Thiamine hydrochloride produces chloride reactions (method described in the Annex).

5.4. Thiamine hydrochloride contains at least 98.5 pp 100, and at most the equivalent of 101.5 pp 100, 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium hydrochloride, as calculated with respect to the anhydrous substance.

6. TESTS

6.1. Desiccation Loss

Place 2 g of thiamine in an oven at 105 °C for 3 hours. Weight loss should not be greater than 5 pp 100.

6.2. Sulfur Ash

Using the method described in the annex, analyse 2 g thiamine hydrochloride. The sulfur ash content should not be greater than 0.1 pp 100.

6.3. Preparing the Solution for Tests

Dissolve 5 g thiamine hydrochloride in water and fill to 100 ml.

6.4. Determining pH

The pH of the solution prepared for tests under Paragraph 5.3 and diluted by one-half should have a pH of between 2.7 and 3.3.

6.5. Nitrates

Add 1 ml water and 1 ml concentrated sulfuric acid (R) to 1 ml of the solution prepared for tests under Paragraph 5.3. Cool. Deposit 2 ml of the extemporaneously prepared 5 pp 100 iron (II) sulfate solution on the surface of the liquid. No brown ring should form at the interface of the 2 layers.

6.6. Heavy Metals

Test for heavy metals in 10 ml of the solution prepared for tests under Paragraph 5.3 using the method described in the Annex. (Heavy metal content expressed in terms of lead should be less than 10 mg/kg).

6.7. Lead

Using the method indicated in the annex, determine lead content in the test solution (5.3). (Lead content should be less than 5 mg/kg).

6.8. Mercury

Using the method indicated in the Annex, determine the mercury content in the test solution (5.3). (Mercury content should be less than 1 mg/kg).

6.9. Arsenic

Using the method indicated in the Annex, determine the arsenic content in the test splution (5.3). (Arsenic content should be less than 3 mg/kg).

7. QUANTITATIVE ANALYSIS

Dissolve 0.150 g of thiamine hydrochloride in 5 ml anhydrous formic acid. Add 65 ml anhydrous acetic acid, then, while stirring, 10 ml mercuric acetate solution. Quantitatively analyze organic base halogenated salts in a non-aqueous medium by titrating with 0.1M perchloric acid. Determine the point of equivalence by potentiometric analysis. 1 ml of 0.1M perchloric acid corresponds to 16.86 mg of $C_{12}H_{18}Cl_2N_4OS$.

8. STORAGE

Thiamine hydrochloride should be stored in properly sealed, non-metal containers kept away from light.

UREASE
E.C. 3.5.1.5.
CAS N°: 9002-13-5
(Oeno 5/2005)

GENERAL SPECIFICATIONS

The specifications must be in compliance with general specifications for enzymatic preparations as provided for in the International Oenological Codex.

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

The purpose of an enzyme is to break down urea into ammonia and carbon dioxide. Urease is produced from *Lactobacillus fermentum*. It belongs to the urease group collectively called "urease acids". They are activated at low pH levels.

L. fermentum is grown in a synthetic environment. After fermentation, the culture is filtered, washed in water and the cells are killed in 50% vol alcohol. The suspension is freeze dried or dried by pulverisation.

The preparation consists of a powder made up of whole dead cells containing enzymes.

Urease contains no substances, nor micro-organisms nor collateral enzymatic activities which are:

- harmful to health,
- harmful to the products treated,
- lead to the formation of undesirable products,
- produces or facilitates fraud

2. LABELING

The concentration of the product must be indicated on the label in addition to security and storage conditions and the to the expiration date.

3. ENZYMATIC ACTIVITY

The claimed enzymatic specific activity is posted at 3.5 U/mg. Note that one unit is defined as the quantity of enzymes which release one micromole molecule of ammonia hydroxide from 5 g/l dose of urea, per minute at pH level 4 in a citrate buffer 0.1 M medium, at 37 °C. This activity is the only isolation.

4. CHARACTERISTICS

Urease can be found in the crystal powder form, white, odourless, with a mild taste

5. SUPPORTS, DILUENTS, PRESERVATION AGENT

The only substance added for conditioning is dextrin.

6. TRIALS

6.1 Sulphuric ashes

Determine sulphuric ashes according to the method in Chapter II in the International Oenological Codex. The rate of sulphuric ashes in urease must not be over 8%.

6.2 Solution for trials:

Dissolve 5 g of urease in 100 ml of water.

6.3 Heavy metals

A 10 ml of solution for trials (6.2), add 2 ml of buffer solution pH 3.5 (R), 1.2 ml of thioacetamide (R) reagent. There should be no precipitation. If brown colouring occurs, it should be less than demonstrated in the trial prepared as indicated in Chapter II of the International Oenological Codex.

The contents of heavy metals expressed in lead, must be less than 30 mg/kg.

6.4 Arsenic

Measure arsenic according to the method which appears in Chapter II of the International Oenological Codex from the trial solution (6.2).

The contents of arsenic must be less than 2 mg/kg.

6.5 Lead

Measure lead according to the method which appears in Chapter II of the International Oenological Codex from the trial solution (6.2).

The contents of lead must be less than 5 mg/kg.

6.6 Mercury

Measure mercury according to the method which appears in Chapter II of the International Oenological Codex from the trial solution (6.2).

The contents of mercury must be less than 0.5 mg/kg.

6.7 Cadmium

Measure cadmium according to the method which appears in Chapter II of the International Oenological Codex from the trial solution (6.2).

The contents of cadmium must be less than 0.5 mg/kg.

7. BIOLOGICAL CONTAMINANTS

Carry out a counting according the method described in Chapter II of the International Oenological Codex

7.1 Total bacteria	under 5×10^4 CFU/g
7.2 Coliformesteneur	under 30 CFU/g of preparation
7.3 <i>Escherichia coli</i>	absence checked on 25 g sample
7.4 <i>St. aureus</i>	absence checked on 1 g sample
7.5 Salmonella	absence checked on 25 g sample.

No mutagenic or bacterial activity should be detectable

It is also admitted that no *Lactobacillus* strain should produce antibiotics.

8. APPLICATION TO WINE

Urease must be carefully incorporated and mixed in wine to be aged more than 1 year if it contains more than 3 mg/l of urea. The dose to be used will be 25 mg/l to 75 mg/l, according to tests carried out beforehand. This procedure is carried out in less than 4 weeks at a temperature above 15°C and when there is less than 1 mg/l fluoride ions.

- After a noticeable decrease in urea, for example less than 1 mg/l, all enzymatic activity is eliminated by filtering the wine. (diameter of pores under 1 µm).

9. STORAGE CONDITIONS

Urease can be stored for several months at a low temperature (+ 5 °C). There is a 50% loss in activity annually.

PIECES OF OAK WOOD
(Oeno 3/2005)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Pieces of oak wood used for winemaking and for passing on certain constituents to the wine in conditions set by regulations.

The pieces of oak wood must come exclusively from the *Quercus* genus.

They can possibly be left in their natural state or they can be heated to a low, medium or high temperature but they must not be charred including on the surface, nor be carbonaceous, nor friable when touched.

No compound should be added to them for the purpose of increasing their natural aromatising capacity or their extractible phenolic compounds.

Likewise, they must not undergo any chemical, enzymatic or physical treatment other than heating.

2. LABELLING

The label must mention the varietal origin of the oak and the intensity of any heating, the storage conditions and safety precautions.

3. DIMENSIONS

The dimensions of these particles must be such that at least 95% in weight be retained by the screen of 2 mm mesh (9 mesh).

4. PURITY

The pieces of oak wood must not release any substances in concentrations which may be harmful to health,

5. STORAGE CONDITIONS

The pieces of oak wood must be stored in sufficiently dry and odourless conditions free from substances liable to contaminate them.

WOOD FOR WINE CONTAINERS
(OENO 4/2005)

1. SUBJECT, ORIGIN AND SCOPE

The wood of containers used during the making, storage or transport of wines.

The pieces of wood must exclusively originate from species recognized as being suitable to store wine (oak, chestnut)

They can possibly be left in their natural state or they can be heated to a low, medium or high temperature, but they must not be charred, including on the surface, nor be carbonaceous, nor friable when touched.

No compound should be added to them for the purpose of increasing their natural aromatizing capacity or their extractable phenolic compounds.

They must not undergo any chemical, enzymatic or physical treatment other than heating when used for new containers.

If they have undergone chemical or physical treatment, in particular to clean containers having already been used, it is recommended to ensure the perfect harmlessness of any such treatment for materials in contact with foodstuffs, and in particular to ensure that sufficient rinsing has eliminated any trace of certain products that are not authorized in wine.

2. CONTAINER MARKING AND/OR ACCOMPANYING DOCUMENT

Container markings or the accompanying document must indicate the origin of the botanical species of wood, the intensity of any heating and the safety instructions.

3. PURITY

Wooden containers must not release substances in concentrations which may be harmful to health.

4. STORAGE

Wooden containers must be washed before first use and then stored under suitable conditions to prevent any development of undesirable micro-organisms when the containers are empty.

YEAST MANNOPROTEINS

(Oeno 26/2004)

1. OBJECT, ORIGIN AND FIELD OF APPLICATION

Mannoproteins are extracted from *Saccharomyces cerevisiae* yeast cell walls by physico-chemical or enzymatic methods.

Mannoproteins are different structures depending on their molecular weight, their degree and type of glycosylation, and their load size. Depending on their extraction mode, they have different tartaric and/or protein stabilisation of wine.

2. LABELLING

The label must indicate the field of application (tartaric and/or protein stabilisation of wine), security and storage conditions in addition to the date of expiry.

For solution preparations, the concentration of mannoproteins, the content of sulphur dioxide must likewise be indicated.

3. CHARACTERISATION

3.1 - Mannoproteins are found in powder form, usually microgranulated, white or beige in colour, odorless, or in a colloidal solution, yellow in colour, translucent.

3.2 - Mannoproteins are water soluble and insoluble in ethanol. In solution form, they precipitate when 1 volume of ethanol is added.

3.3 - Optical rotation

The specific optical rotation is measured at 589 nm (sodium D line) and is related to a

10 g/l mannoprotein solution with a length 1dm.

Certain mannoproteins with a $[\alpha]_D^{20^\circ C}$ rotary power between 80 ° and 150° can be distinguished from the arabic gum with a rotary power below 50°.

Other preparations can only be distinguished by the percent composition in sugar (see point 4.12)

TRIALS

4.1 Loss through desiccation

3.1.1 Powder mannoprotein:

Put 5 g of mannoproteins in a 70 mm diameter silica capsule. Place in 100-105 °C drying chamber for 5 hours. Weight loss must not be more than 15%.

3.1.2 Mannoproteins in solution:

Put 10 g of mannoproteins in a 70 mm diameter silica capsule. Put over 100 °C water bath for 4 hours in a 100-105 °C drying chamber for 3 heures.

The quantity of dry residue must be at least 10%.

The limits set below are related to dry products.

4.2 Ashes

Incinerate dry residue at 550-600 °C. Ash content must not be more than 8%.

4.3 Preparation of solution for trials

Prepare a 10 g/l mannoprotein solution in water.

In the case of mannoprotein solution, weigh amount corresponding to 5 g of dry residue, evaporate until almost dry and dissolve again to 10 g/l in water.

4.4 Heavy metals

Determine iron on the solution prepared for trials (4.3) according to the method described in Chapter II of the International Oenological Codex

The content expressed in lead must be less than 30 mg/kg.

4.5 Lead

Determine lead on the solution prepared for trials (4.3) according to the method described in Chapter II of the International Oenological Codex

The content expressed in lead must be less than 5 mg/kg.

4.6 Mercury

Determine mercury on the solution prepared for trials (4.3) according to the method described in Chapter II of the International Oenological Codex without evaporating the solution.

The content of mercury must be less than 0.15 mg/kg.

4.7 Arsenic

Determine arsenic according to the method described in Chapter II of the International Oenological Codex on a solution for trials (4.3)
The content of arsenic must be less than 1 mg/kg.

4.8 Cadmium

Determine cadmium according to the method described in Chapter II of the International Oenological Codex on a solution for trials (4.3)
The content of cadmium must be less than 0.5 mg/kg.

4.9 Total nitrogen

Introduce 5 g of mannoproteins in a 300 ml mineralisation flask with 15 ml of concentrated sulphuric acid (R) and 2 g of mineralisation catalyser (R). Continue the determination as indicated in Chapter II of the International Oenological Codex.

In the case of mannoprotein solution, weigh an amount corresponding to 5 g of dry residue, evaporate until almost dry then proceed as in the above.

The content of nitrogen must be between 5 and 75 g/kg

4.10 Microbiological analysis

4.10.1 Total aerobic mesophile flora

Proceed with counting according to method described in Chapter II of the International Oenological Codex.

Not more than 10 000 total aerobic mesophile germs in 1 g.

4.10.2 Coliforms

Proceed with counting according to method described in Chapter II of the International Oenological Codex.

Not more than 10 CFU/g of preparation.

4.10.3 *Staphylococcus aureus*

Proceed with counting according to method described in Chapter II of the International Oenological Codex.

Absence checked for *Staphylococcus aureus* on a 1 g sample.

4.10.4 *Salmonella*

Proceed with counting according to method described in Chapter II of the International Oenological Codex.

Absence checked for salmonella on a 25 g sample

4.10.5 *Escherichia coli*

Proceed with counting according to method described in Chapter II of the International Oenological Codex.

Absence checked for *Escherichia coli* on a 25 g sample.

4.10.6 Lactic bacteria

Proceed with counting according to method described in Chapter II of the International Oenological Codex.

Not more than 10^4 CFU/g of perparation.

4.10.7 Mould

Proceed with counting according to method described in Chapter II of the International Oenological Codex.

Not more than 50 CFU/g of preparation.

4.10.8 Yeasts

Proceed with counting according to method described in Chapter II of the International Oenological Codex.

Not more than 10^2 CFU/g of preparation.

4.11 Polysaccharides

4.11.1 Principle:

Measure colour intensity using hot phenol solution in a sulfuric medium.

4.11.2 Products:

4.11.2.1 15 mg/l mannoprotein solution

Dissolve 150 mg of mannoproteins in 100 ml of distilled water, then dilute this solution 1/100 with distilled water.

4.11.2.2 50 g/l phenol solution

Dissolve 5 g of pure phenol in 100 ml of distilled water.

4.11.3 Protocol:

200 µl of phenol (4.11.2.2) then 1 ml of pure sulphuric acid (R) are added to 200 µl of solution to be determined (4.11.2.1). After immediately mixing, the tubes are heated at 100°C in a bath water for 5 minutes and then cooled to 0 °C.

After reaching room temperature, absorbance is measured at 490 nm. 100 mg/l mannose solution is the reference solution.

(Content of polysaccharides expressed in equivalent of mannose above 600 g/kg)

4.12 Centesimal composition of glucidic monomers

4.12.1 Principle:

Enzymatic determination of glucose and mannose after acid hydrolysis.

The determination of mannose is carried out following the determination of fructose and the addition of phosphomannose isomerase (PMI).

4.12.2 Products:

4.12.2.1 Mannoprotein solution 5 g/l

Dissolve 500 mg of mannoproteins in 100 ml of distilled water.

4.12.2.2 Sulphuric acid solution 5 M

Place 28 ml of sulphuric acid in 100 ml of distilled water.

4.12.2.3 Potassium hydroxide solution 10 M

Dissolve 46 g of potassium hydroxide in 100 ml of distilled water.

4.12.2.4 Phosphomannose isomerase 616 U/ml.

4.12.3 Protocol:

Place 100 µl of solution to be determined (4.12.2.1) in airtight sealed tubes and add 1 ml of sulphuric acid (4.12.2.2). After mixing, the tubes are heated at 100 °C in a water bath for 30 minutes and then cooled to 0 °C. After room temperature is reached, 1 ml of potassium hydroxide is added to neutralise the medium.

The determination of glucose and mannose can be carried out according to the method described in the compendium. Mannoproteins must contain at least 70% of mannose compared to total polysaccharides determined in 4.11.

4.13 Mannoprotein efficiency test with regards to tartaric precipitation

4.13.1 Principle:

Determination of dose of mannoproteins to delay crystallisation of potassium hydrogenotartrate in a hydroalcoholic solution.

4.13.2 Produits:

Crystallised tartaric Ac: PM = 150.05

Ethanol at 95% volume

Potassium chloride: PM= 74,5

Potassium hydrogenotartrate: PM= 188

4.13.3 Protocol:

4.13.3.1 Mannoprotein solution 10 g/l

Dissolve 1 g of mannoproteins in 100 ml of distilled water.

4.13.3.2 Hydro-alcoholic matrix

In a 1 liter volumetric flask half filled with distilled water dissolve:

- Tartaric acid: 2.1 g
- Potassium chloride: 1.1 g
- Ethanol at 95 % volume: 110 ml

Homogenise and fill up with distilled water.

4.13.4 Test:

Place increasing quantities of mannoprotein solution (4.13.3.1) in a 100ml volumetric flask 0 – 1 – 2 – 3 – 4 ml and the volume is brought up to 100 ml with hydro-alcoholic matrix (4.13.3.2). These quantities correspond to final quantities of 0 – 100 – 200 – 300 – 400 mg/l of mannoproteins.

Add potassium hydrogenotartrate in each 100 mg flask.

Heat at 40 °C in a water bath for 1 hour until the complete solubilization of potassium hydrogenotartrate.

Stack the flasks in a refrigerator at 4 °C.

Observation after 48 hours:

The reference flask containing 0 ml of mannoprotein solution (4.13.3.1)

Presents potassium hydrogenotartrate crystals.

The absence of crystals in flasks containing mannoproteins aids in appraising the effectiveness. In all cases, crystals must be absent in a solution containing 400 mg/l of mannoproteins.

4.14 Mannoprotein efficiency test regarding protein casses

4.14.3 Principle

Determination of mannoprotein dose needed to improve protein stabilisation of wine.

4.14.4 Product:

Bovine serum albumen (Fraction V) (BSA)

4.14.5 Protocol:

4.14.5.1 10 g/l bovine serum albumen solution

Dissolve 2 g of bovine serum albumen in 200 ml of distilled water.

4.14.3.2 20 g/l mannoprotein solution

Dissolve 2 g of mannoproteins in 100 ml of distilled water.

4.14.4 Test

Place 1 ml of BSA solution (4.14.3.1) in two 100 ml volumetric flasks and bring up to 100 ml in each flask with dry white wine which presents no cloudiness with heating (or stabilised if necessary with an adequate dose of bentonite treatment), and homogenize.

Adjust 0 and 1 ml of mannoprotein solution (4.14.3.2) and homogenize. These quantities correspond to 0 and 200 mg/l final doses of mannoproteins.

Filter reference and treated solutions through a membrane with a 0.45 µm pore diameter. Pour the filtered solutions in two 50 ml flasks.

Place the 2 50 ml flasks and heat at 80 °C in a water bath for 30 minutes. Let cool to room temperature for 45 minutes, measure turbidity of reference and treated solution.

The decrease in turbidity between the reference sample and the treated sample must be at least 50%.

4.15 Dosage in wine

Principle

The dosage of mannoproteins in wine can be carried out after precipitating ethanol (5 volumes), acid hydrolysis from the precipitate and determination of released mannose according to the method listed in the annex.

5. STORAGE CONDITIONS

Solid mannoproteins have a 2 year shelf life if they are stored away from humidity in a sealed pack in a temperate room.

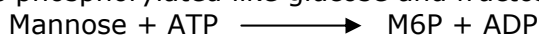
Mannoproteins present in colloidal solutions ready to be used must be stored in a hermetically sealed container.

Annex

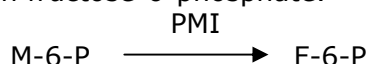
Determination of mannose using enzymatic method

Principle

Mannose is phosphorylated like glucose and fructose:



Following the determination of glucose and fructose, mannose-6-phosphate is transformed due to the action of phosphomannose isomerase (PMI) in fructose-6-phosphate.



Fructose-6-phosphate formed again is transformed as before in glucose-6-phosphate which is dosed.

Protocol

Place 5 ml of wine in a centrifuge tube and add 25 ml of 95% ethanol, after mixing the tubes are put in a 4°C refrigerator for 12 hours. The precipitate formed is recuperated by a centrifuge, washed 2 times by 10 ml of 95% ethanol. The hydrolysis of the precipitate is carried out as in 4.12.

This determination does not enable the differentiation of mannoproteins added and natural mannoproteins.

Additional reagent regarding the method of the Compendium of International Methods of Analysis of Wines and Musts

Solution 6: phosphomannose isomerase (616 U/ml).
the suspension is used without diluting.

Determination

After measuring A_3 following the methods of the Compendium of Methods of Analysis of Wines and Musts, add

	Reference	Determination
Solution 6	0.02 ml	0.02 ml

Mix; carry out the determination after 30 min; monitor the end of the reaction after 2 min. (A_4)

Determine the absorbances differences:

$A_4 - A_3$ corresponding to mannose for the reference and the determination

Subtract the absorbance difference for the reference (ΔA_T) and for the determination (ΔA_D) and establish: $\Delta A_M = \Delta A_D - \Delta A_T$ for mannose.

Results

For mannose: $Cg/l = 0.423 \times \Delta A_M$ is obtained.

Remark: If the measurements were carried out with wave lengths 334 or 365 nm, we obtain:

For a 334 nm measurement:

For mannose: $Cg/l = 0.430 \times \Delta A_M$

365 nm measurement

For mannose: $Cg/l = 0.783 \times \Delta A_M$

CHARBON ANIMAL PURIFIÉ

Charbon animal lavé

Noir en pâte

Carbo ossium depuratus

Le charbon animal purifié est obtenu par traitement du charbon animal par de l'acide chlorhydrique dilué et lavage prolongé à l'eau. Après essorage, on obtient une pâte épaisse qui constitue le *noir en pâte*, qui contient 75 à 85 p. 100 d'eau.

Par dessiccation du noir en pâte à l'étuve à 150 °C environ, on obtient une poudre noire que l'on passe au tamis de soie et conserve en flacon; c'est le *charbon animal lavé en poudre*, moins actif que le précédent pour la même quantité de charbon sec.

Essais :

1. Humidité :

Placer 5 g de charbon en pâte ou 5 g de charbon lavé en poudre dans une capsule de silice de 70 mm de diamètre. Placer dans une étuve à 100 °C aérée. La perte de poids ne doit pas être supérieure à 85 p. 100 pour le noir en pâte après 8 heures de dessiccation et à 15 p. 100 pour le noir en poudre après 3 heures de dessiccation.

Toutes les limites fixées pour le charbon animal lavé en pâte ou en poudre sont rapportées au poids de charbon sec.

2. Cendres :

Incinerer ce résidu à 500-600 °C. Le résidu doit peser au plus 0,25 g pour 1 g de charbon sec. Ces cendres, insolubles dans l'eau, doivent être presque entièrement insolubles dans l'acide chlorhydrique dilué (R).

3. Calcination incomplète :

0,5 g de charbon lavé en poudre ou le résidu de la dessiccation à l'étuve à 150 °C de 2,5 g de charbon en pâte chauffé fortement dans un tube à essai, ne doivent pas dégager d'odeur empyreumatique.

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Charbon animal purifié

4. Chlorures :

Agiter la quantité de noir en pâte ou de charbon lavé en poudre correspondant à 0,067 g de charbon sec avec 20 ml d'eau distillée. Filtrer. A 5 ml de filtrat, ajouter 5 ml d'acide nitrique dilué (R). Compléter à 20 ml et ajouter 0,5 ml de solution de nitrate d'argent à 5 p. 100 (R).

Comparer l'opalescence ou le trouble éventuel à celui d'un témoin préparé comme il est indiqué à la page 134. (Teneur en chlorures inférieure à 3 p. 1 000.)

5. Cyanures :

Placer dans une fiole conique de 100 ml, la quantité de charbon lavé en poudre, ou la quantité de noir en pâte contenant 1 g de charbon sec, avec 10 ml d'acide sulfurique dilué (R). Adapter à la fiole conique un tube à dégagement plongeant dans 2 ml environ de solution saturée de borax (R) placés dans un tube à essai. Distiller et recueillir 2 à 3 ml de distillat. Ajouter V gouttes d'une solution de métabisulfite de potassium à 2 p. 100 (R), laisser en contact pendant 5 minutes. Ajouter 1 ml de sulfate ferreux à 5 p. 100 (R), laisser en contact 15 minutes. Ajouter alors II gouttes de phénolphthaléine (R) et alcaliniser légèrement avec une solution saturée de borax (R); laisser 5 minutes en contact. Ajouter II gouttes d'une solution de sulfate ferrique et d'ammonium à 10 p. 100 (R) et 1 ml d'acide chlorhydrique pur (R); il ne doit se produire ni coloration, ni précipité bleu.

6. Hydrocarbures aromatiques supérieurs :

Épuiser 1 g de charbon sec par 10 g de cyclohexane pur pendant 2 heures. L'extract ne doit présenter aucune coloration; en lumière ultraviolette il ne doit présenter aucune absorbance entre 230 et 400 nm examiné sous 1 cm d'épaisseur et il ne doit pas être plus fluorescent qu'une solution de 0,1 mg de sulfate de quinine dans 1 l d'acide sulfurique 0,1 N; par évaporation, il ne doit pas laisser de résidu.

7. Sulfures :

Placer dans un ballon de 50 ml une quantité de charbon contenant 1 g de charbon sec avec 10 ml d'acide chlorhydrique dilué (R) et 10 ml d'eau. Distiller en recueillant 5 ml de distillat dans un tube à essai contenant 5 ml de solution normale d'hydroxyde de sodium.

1 ml de solution d'essai est additionné de 0,5 ml d'une solution de nitrate de plomb à 1 g par litre (R). On ne doit pas observer de coloration brune ou de précipité noir. (Teneur en sulfures exprimés en soufre inférieure à 20 parties par million.)

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Charbon animal purifié

8. Arsenic :

Placer dans un ballon à fond rond de 500 ml une quantité de noir en pâte ou de charbon lavé en poudre contenant 0,200 g de charbon sec, avec 20 ml d'acide nitrique pur (RAs) et 8 ml d'acide sulfurique pur (RAs). Porter à ébullition et chauffer jusqu'à disparition de toute trace de charbon. Chasser les vapeurs nitreuses en reprenant le résidu refroidi, à deux reprises, par 10 ml d'eau distillée. Rechercher l'arsenic par la méthode décrite page 126. (Teneur en arsenic inférieure à 5 parties par million.)

9. Préparation de la solution pour les essais :

Dans un flacon, placer une quantité (p g) de charbon lavé contenant 2,50 g de charbon sec avec 25 ml de solution d'acide citrique à 20 g par litre (R). Ajouter (27,50-p) ml d'eau, agiter énergiquement pendant 5 minutes et laisser reposer 12 heures. Filtrer.

10. Fer :

A 5 ml de la solution obtenue selon l'alinéa 9, ajouter 5 ml d'eau, 1 ml d'acide chlorhydrique pur (R), 2 ml d'une solution de thiocyanate de potassium à 5 p. 100 (R). La coloration obtenue doit être inférieure à celle d'un témoin préparé avec 10 ml d'une solution de sel ferrique à 0,010 g de fer par litre (R), 1 ml d'acide chlorhydrique pur (R), 2 ml de solution de thiocyanate de potassium à 5 p. 100 (R). (Teneur en fer inférieure à 400 parties par million.)

11. Métaux lourds :

A 20 ml de la solution préparée au 9^e alinéa, ajouter 2 ml de solution de fluorure de sodium pur à 4 p. 100 (R), 0,5 ml d'acide acétique pur (R). Certains charbons cédant du calcium à la solution citrique, donnent un précipité blanc avec la solution de fluorure de sodium. Dans ce cas, filtrer après 15 minutes de repos. Au filtrat, ajouter 2 ml de solution d'acide sulfhydrique (R). La coloration brune éventuellement observée doit être inférieure à celle du témoin préparé comme il est indiqué à la page 130, mais en portant le volume à 24 ml. (Teneur en métaux lourds, exprimés en plomb, inférieure à 20 parties par million.)

12. Calcium :

Dans un verre cylindrique de 100 ml, placer 10 ml de la solution préparée selon l'alinéa 9, la chauffer à 70-80 °C, ajouter goutte à goutte 5 ml de solution d'oxalate d'ammonium (R). Laisser refroidir 1 heure et filtrer. Après lavage, le précipité d'oxalate de calcium est dissous dans 5 ml d'acide sulfurique dilué (R) et 20 à 30 ml d'eau bouillante. L'acide oxalique est titré par la solution de permanganate de potassium 0,1 N.

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Charbon animal purifié

Soit n le volume utilisé :

— 1 ml de solution 0,1 N de permanganate correspond à 2 mg de calcium.

— 100 g de charbon sec contiennent donc $0,4 n$ g de calcium.

Cette quantité doit être inférieure ou égale à 1 g pour 100 g de charbon sec.

13. Détermination du pouvoir décolorant :

Dans un tube à centrifuger, placer 10 ml de vin rouge de moins de 2 ans dont la coloration est identique en intensité à celle d'une solution saturée de sulfate de cobalt (32,6 g de CoSO_4 anhydre pour 100 ml d'eau (*)), 25 mg de charbon lavé en poudre sec ou la quantité de noir en pâte correspondant à 25 mg de charbon sec et une goutte d'acide acétique pur (R).

Agiter pendant 30 minutes. Centrifuger et prélever le liquide clair surnageant. Comparer sa coloration à celle d'une gamme établie par addition de 0, 10, 20, 30, 40, 50, 60, 70, 80 p. 100 d'eau au vin rouge.

Le pouvoir décolorant du charbon est chiffré par référence à l'échantillon de la gamme ci-dessus, présentant la même intensité de coloration que le vin traité par le charbon.

Le pouvoir décolorant d'un charbon lavé en poudre ou d'un noir en pâte doit être au moins de 40 dans ces conditions.

(*) Soit 58 g $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ pour 100 ml de solution ($d_{20}^{20} = 1,29$).

CHARBON ACTIVÉ
Carbo activatus

Le charbon activé œnologique est un charbon végétal doué d'un pouvoir adsorbant élevé, obtenu par un traitement approprié sans intervention de matières d'imprégnation à base de métaux.

Caractères :

Poudre noire, inodore et sans saveur, combustible au rouge sans flamme.

Essais :

On utilise pour le charbon activé les mêmes méthodes d'essais que pour le charbon animal purifié, avec les limites suivantes :

1. Humidité :

Inférieure à 15 p. 100.

2. Cendres :

Inférieures à 10 pour 100 grammes de charbon sec.

3. Chlorures :

Inférieurs à 3 p. 1 000.

4. Cyanures :

Absence.

5. Hydrocarbures aromatiques supérieurs :

Absence.

6. Sulfures :

Teneur en soufre inférieure à 20 parties par million.

CODEX ŒNOLOGIQUE INTERNATIONAL
Charbon activé

7. Arsenic :

Inférieur à 5 parties par million.

8. Fer :

Inférieur à 200 parties par million.

9. Métaux lourds :

Exprimés en plomb, inférieurs à 20 parties par million. Certains charbons activés au chlorure de zinc mal lavés donnent un précipité blanc au cours de cet essai après addition de la solution d'acide sulfhydrique; ces charbons doivent être rejetés.

10. Calcium :

Inférieur à 1 g pour 100 g.

11. Détermination du pouvoir adsorbant vis-à-vis de l'iode :

Dans un tube à centrifuger, placer 10 ml de solution titrée d'iode 0,05 N, 25 mg de charbon activé sec et une goutte d'acide acétique pur (R). Agiter pendant 30 minutes. Centrifuger et prélever 5 ml de liquide clair surnageant. Titrer l'iode restant par le thiosulfate 0,05 N. Soit n ml versés : calculer la quantité d'acide adsorbée pour 100 parties d'iode utilisées : $20(5 - n)$.

Le pouvoir adsorbant vis-à-vis de l'iode d'un charbon végétal activé doit être au moins de 30 p. 100 dans ces conditions.

CODEX ŒNOLOGIQUE INTERNATIONAL

Ecorces de levures

SPECIFICATIONS GENERALES DES PREPARATIONS DES ECORCES DE LEVURE DESTINEES AU TRAI- TEMENT DES MOOTS ET DES VINS EN VUE DE STIMULER LEUR FERMENTATION

1°- NOM : Enveloppes cellulaires de levure ou "écorces de levure".

2°- DESCRIPTION :

21 - Les écorces de levure à usage œnologique sont obtenues à partir de levures *Saccharomyces cerevisiae* cultivées sur mélasse de betterave. Ces levures ont subi une autolyse par leurs propres enzymes protéolytiques. Les enveloppes cellulaires, insolubles, sont recueillies par centrifugation, lavées avec une solution hydroalcoolique et séchées par une technique qui respecte leur surface et en conséquence leur capacité adsorbante.

22 - Les écorces de levure se présentent sous la forme d'une poudre fine, non hygroscopique, de couleur crème, peu odorante. Elles sont à base de composants ne laissant aucun résidu nuisible à la santé dans le moût de raisin et le vin.

23 - Les écorces sont conditionnées sous vide, en emballage étanche, pour éviter les phénomènes d'oxydation.

3°- IDENTIFICATION :

Distinction des écorces de levure d'avec les levures sèches actives :

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Ecorces de levures

Ajoutées à la dose de 0,2 g/l à un milieu sucré nutritivement complet, les écorces de levure, contrairement aux levures sèches actives, ne doivent pas provoquer la fermentation du sucre.

4°- COMPOSITION DES ECORCES DE LEVURE (VALEURS MOYENNES)

Extrait Sec	96± 2 g pour 100 g tel quel
Matières protéiques	15± 3 g pour 100 g tel quel
Matières Grasses	20± 2 g pour 100 g tel quel
Matières Minérales	4± 1 g pour 100 g tel quel
Matières glucidiques	57± 2 g pour 100 g tel quel

41. -Matières protéiques : elles proviennent du complexe glucolipidoprotéique qui compose la paroi cellulaire des levures.

42. - Matières grasses : Les lipides présents dans les enveloppes cellulaires se répartissent pour :

50 % sous forme libres, et

50 % sous forme intimement liées aux autres composants.

Cette phase lipidique renferme une partie sous forme d'ergostérol.

43. - Matières minérales : Les matières minérales des enveloppes cellulaires sont particulièrement riches en phosphore (principalement, sous forme de phosphates).

Phosphore	1,2 g pour 100 g tel quel
Calcium	0,3 g pour 100 g tel quel
Magnésium	0,10 g pour 100 g tel quel
Sodium	0,50 g pour 100 g tel quel
Potassium	0,10 g pour 100 g tel quel
Chlorures	0,10 g pour 100 g tel quel

44. -Matières glucidiques : Cette fraction ne renferme pas de cellulose mais différents sucres qui se répartissent de la façon suivante :

Glucides solubles dans l'acide trichloracétique	10 % +/-5
Mannane	15 % +/- 5
Glycogène acido et alcalino-soluble	20 % +/-5
Glucane	55 % +/-5

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Ecorces de levures

5° - ADDITIFS ET INGREDIENTS: n é a n t

6° - CONTAMINANTS

- | 61. chimiques | Concentration maximale |
|-----------------------------------|------------------------|
| arsenic | 3 mg/ kg |
| cadmium | 0,5 mg/kg |
| mercure | 0,5 mg/kg |
| plomb | 10 mg/kg |
| métaux lourds (exprimés en plomb) | 50 mg/kg |
| mycotoxines | 5 microgrammes/ kg. |
62. Microbiens:
- Salmonelles: absence contrôlée sur un échantillon de 20 g.
- Pseudomonas aeruginosa: absence contrôlée sur un échantillon de 1 g
- Escherichia coli: absence contrôlée sur un échantillon de 1 g
- Staphylocoques: absence contrôlée sur un échantillon de 1 g
- Coliformes: moins de 10 par gramme
- Germes banaux: moins de 10.000 par gramme.

71 - HYGIENE

71. Les écorces de levure sont produites conformément aux bonnes pratiques de fabrication des aliments.
72. Elles sont exemptes d'impuretés nocives pour la santé, conformément aux paragraphes 2.2. et 6.1
73. Elles ne doivent pas présenter d'odeur de rance et ne doivent pas céder de goût anormal au vin (goût de levure).

8° - ACTIVITE

81. L'action stimulante des écorces de levure est basée sur leur capacité d'adsorber certaines substances toxiques pour la levure, formées par elle-même au cours de sa croissance. Parmi celles-ci l'acide décanoïque est le plus inhibiteur. Il peut être utilisé comme indice de la capacité adsorbance des écorces et en conséquence de leur propriété activatrice à l'égard de la fermentation alcoolique.
82. L'activité technologique (AT) ramenée au gramme (g) de produit peut être ainsi déterminée:
- 1 g d'écorce de levure ajouté à une solution contenant:
- alcool 10 % vol, ac. décanoïque 2 mg/l, doit adsorber, après 24 h de contact à 18-22° C, 50 % de cet acide.
- 8.2.1. - Le contrôle de l'activité technologique peut être effectué par le dosage de l'acide décanoïque effectué par chromatographie en phase gazeuse selon (à titre d'exemple) les modalités suivantes:

CODEX OENOLOGIQUE INTERNATIONAL

Ecorces de levures

- appareil Carlo Erba Série 2900
- colonne F.F.A.P.
- support colonne de verre capillaire W.C.O.T.
- température 60° → 180°C, 4°C/mn
- volume injecté 1 µl de solution hydroalcoolique (10 % vol.) à 2 mg/l d'acide décanoïque traitée par les écorces de levure
- étalon interne: acide heptanoïque
- solution de référence: solution hydro-alcoolique (10 % vol) à 2 mg/l d'acide décanoïque.

822. Autre procédé de contrôle de l'activité technologique:

Utilisées à la dose de 0,2 g/l dans un jus de raisin limpide, riche en sucre (250 g/l),ensemencé avec 10⁵ cells/ml de *S. cerevisiae*, fermentant en semi-anaérobiose, les écorces de levure doivent permettre la formation de 1 à 2 % vol.d'éthanol supplémentaire.

9°- EMPLOI DES ECORCES DE LEVURE

91 - Dans le moût, pour prévenir les arrêts de fermentation.

Ajouter 10 à 15 g/hl d'écorces de levure, à l'occasion d'un remontage, de préférence après la fermentation des premiers cinquante grammes par litre de sucre, sous le chapeau en vinification en rouge. En vinification en blanc si des phénomènes de production de mousse sont redoutés, ajouter les écorces dans le moût clarifié avant fermentation; leur efficacité est moins grande, mais encore importante.

92 - Dans le vin, pour traiter les arrêts de fermentation, deux cas se présentent :

a) vins rouges bien structurés.

Ajouter 20 g/hl d'écorces de levure. Procéder à deux remontages pour homogénéiser la suspension dans l'ensemble du volume. Inoculer une deuxième population de levure après 24 h.

b) vins blancs et vins rouges plus délicats qui doivent l'essentiel de leurs caractères aromatiques aux processus fermentaires.

Utiliser les écorces de levure, à la dose de 20 à 40 g/hl, uniquement dans le pied de cuve.

SODIUM (ALGINATE DE)
Natrii alginas

Sel de sodium de l'acide alginique, extrait de diverses algues Phéophycées, surtout les laminaires, par digestion alcaline et purification.

Caractères :

L'alginate de sodium est une poudre blanche ou jaunâtre, à peu près inodore et insipide, qui se présente, au microscope, composée de fragments de fibres.

Il donne avec l'eau une solution visqueuse. Le pH de cette solution est généralement compris entre 6 et 8. Il est insoluble dans l'alcool fort et dans la plupart des solvants organiques.

Si, à 5 ml d'une solution aqueuse d'alginate de sodium à 1 p. 100 on ajoute 0,50 ml de solution de chlorure de calcium à 20 p. 100 (R), il se forme un précipité gélatineux d'alginate de calcium.

Si, à 10 ml de solution aqueuse à 1 p. 100 d'alginate de sodium, on ajoute 1 ml d'acide sulfurique dilué à 10 p. 100 (R), il se forme un précipité gélatineux d'acide alginique.

Essais :

1. Amidon :

A 5 ml de solution aqueuse à 1 p. 100 d'alginate de sodium, ajouter 5 ml d'eau iodée (R); il ne doit pas se produire de coloration bleue.

2. Gélatine :

A 10 ml de solution aqueuse à 1 p. 100 d'alginate de sodium, ajouter 1 ml de solution chaude de tanin à 2 p. 100 (R) : il ne doit pas se produire de précipité.

3. Perte à la dessiccation :

Déterminée jusqu'à poids constant sur une prise d'essai exactement pesée voisine de 1 g, la perte de poids à 100-105 °C de l'alginate de sodium ne doit pas être supérieure à 25 p. 100.

CODEX ŒNOLOGIQUE INTERNATIONAL

Sodium (alginate de)

Toutes les limites fixées ci-dessous sont rapportées au produit sec.

4. Cendres sulfuriques :

Déterminé comme il est indiqué à la page 121 sur le résidu de l'essai précédent, le taux des cendres sulfuriques de l'alginate de sodium ne doit pas être supérieur à 40 p. 100.

5. Préparation de la solution pour les essais :

Dans une capsule de silice, calciner un poids d'échantillon correspondant à 2,5 g de produit sec, sans dépasser 550 °C. Reprendre le résidu par 10 ml d'eau et 2 ml d'acide nitrique pur (R). Transvaser dans un ballon jaugé de 50 ml; ajouter 2 ml d'ammoniaque pure (R). Porter à 50 ml avec de l'eau distillée. Filtrer.

6. Sulfates :

A 2 ml de la solution d'essai selon l'alinéa 5, ajouter 2 ml d'acide chlorhydrique dilué (R), porter à 20 ml avec de l'eau distillée et ajouter 2 ml de solution de chlorure de baryum (R). Le mélange doit être limpide, ou l'opalescence observée après 15 minutes doit être inférieure à celle présentée par le témoin préparé comme il est indiqué page 131. (Teneur en sulfates exprimés en acide sulfurique inférieure à 1 000 parties par million.)

7. Chlorures :

A 1 ml de la solution selon l'alinéa 5, ajouter 5 ml d'acide nitrique dilué (R), 14 ml d'eau distillée et 0,5 ml de nitrate d'argent à 5 p. 100 (R). Si une opalescence se produit, elle doit être moins intense que celle du témoin préparé comme il est indiqué page 134. (Teneur en chlorures exprimés en acide chlorhydrique inférieure à 1 000 parties par million.)

8. Fer :

2 ml de la solution selon l'alinéa 5 sont additionnés de 8 ml d'eau, 1 ml d'acide chlorhydrique pur (R), d'une goutte de permanganate de potassium à 1 p. 100 (R) et de 2 ml de thiocyanate de potassium à 5 p. 100 (R)

Si une coloration rouge apparaît, elle doit être inférieure à celle d'un témoin préparé avec 3 ml de solution ferrique à 0,010 g de fer par litre (R), 7 ml d'eau et les mêmes quantités d'acide chlorhydrique pur (R) et de thiocyanate de potassium à 5 p. 100 (R). (Teneur en fer inférieure à 300 parties par million.)

CODEX ŒNOLOGIQUE INTERNATIONAL
Sodium (alginate de)

9. Métaux lourds :

20 ml de solution selon l'alinéa 5 sont placés dans un tube à essais avec 2 ml de solution de fluorure de sodium à 4 p. 100 (R), 0,5 ml d'ammoniaque pure (R), 0,5 ml d'acide acétique pur (R) et 2 ml de solution d'acide sulfhydrique (R). Aucun précipité ne doit se produire. Si une coloration brune apparaît, elle doit être inférieure à celle présentée par le témoin préparé comme il est indiqué page 130, porté à 25 ml. (Teneur en métaux lourds, exprimés en plomb, inférieure à 20 parties par million.)

10. Arsenic :

Minéraliser par la méthode nitro-sulfurique 0,2 g d'alginate de sodium et continuer la recherche de l'arsenic comme il est indiqué page 126. (Teneur en arsenic inférieure à 5 parties par million.)

CODEX ŒNOLOGIQUE INTERNATIONAL
Sodium (chlorure de)

SODIUM (CHLORURE DE)

Sel

Natrii chloridum

NaCl = 58,5

Composition centésimale :

Chlore.	60,7
Sodium	39,3

Caractères :

Le chlorure de sodium se présente en cristaux cubiques, incolores, souvent accolés en trémies et retenant un peu d'eau d'interposition. Sa saveur, dite salée, est caractéristique.

Le chlorure de sodium est hygroscopique par la petite quantité de magnésium qu'il contient; chauffé, il perd d'abord l'eau d'interposition qu'il renferme, puis il fond vers 800 °C.

Solubilité :

Eau.	2,8
Eau à 100 ° C.	2,5
Alcool à 90 % vol.	peu soluble
Alcool absolu	insoluble
Glycérol.	12

Caractères d'identité :

- A) La solution aqueuse de chlorure de sodium est neutre.
- B) Elle donne les réactions des chlorures.
- C) Elle donne les réactions du sodium.

Essais :

1. Arsenic :

Rechercher l'arsenic sur 0,500 g de chlorure de sodium suivant la méthode indiquée page 126 pour les produits ne nécessitant pas de minéralisation. (Teneur en arsenic inférieure à 2 parties par million.)

CODEX CENOLOGIQUE INTERNATIONAL
Sodium (chlorure de)

2. Baryum :

Dissoudre 1 g de chlorure de sodium dans 5 ml d'eau. Ajouter 2,5 ml de solution saturée de sulfate de calcium (R). La solution doit rester limpide.

3. Métaux alcalino-terreux et terreux :

Dissoudre 5 g de chlorure de sodium dans 20 ml d'eau; ajouter 5 ml de solution de carbonate disodique à 25 p. 100 (R). Recueillir, laver, sécher et peser le précipité éventuellement formé. Son poids doit être inférieur à 1 p. 100.

4. Fer :

Dissoudre 1 g de chlorure de sodium dans 10 ml d'eau, ajouter une goutte de permanganate de potassium à 1 p. 100, 1 ml d'acide chlorhydrique concentré (R), 2 ml d'une solution de thiocyanate de potassium à 5 p. 100 (R). La coloration obtenue ne doit pas être plus intense que celle d'un témoin préparé avec 1 ml d'une solution de sel ferrique à 0,010 g de fer par litre (R), 9 ml d'eau, 1 ml d'acide chlorhydrique concentré (R), 2 ml de la solution de thiocyanate de potassium à 5 p. 100 (R). (Teneur en fer inférieure à 10 parties par million.)

5. Métaux lourds :

Dissoudre 1 g de chlorure de sodium dans 15 ml d'eau, ajouter 0,5 ml d'une solution de fluorure de sodium à 4 p. 100 (R), 0,5 ml d'acide acétique (R) et 2 ml de solution d'acide sulfhydrique (R). La solution doit satisfaire à l'essai limite des métaux lourds (voir p. 130). (Teneur en métaux lourds, exprimés en plomb, inférieure à 20 parties par million.)

6. Dosage :

Préparer une solution contenant 1 g de chlorure de sodium desséché pour 100 ml. Prélever 10 ml de cette solution et les placer dans un vase cylindrique; ajouter 20 ml de solution 0,1 N de nitrate d'argent, 1 ml d'acide nitrique (R), 5 ml de solution de sulfate ferrique et d'ammonium (R). Titrer l'excès de nitrate d'argent par une solution 0,1 N de thiocyanate de potassium, soit n le nombre de millilitres employés.

1 ml de solution 0,1 N de nitrate d'argent correspond à 0,00585 g de chlorure de sodium.

Teneur p. 100 en chlorure de sodium :

$$5,85 (20 - n).$$

Le produit œnologique doit contenir au minimum 98 p. 100 de chlorure de sodium rapporté au produit desséché.

CODEX ŒNOLOGIQUE INTERNATIONAL
Sodium (monosulfure de)

SODIUM (MONOSULFURE DE)

Natrium sulfuratum

$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O} = 240,2$

Composition analytique :

Sulfure de sodium.	32,50
Eau	67,50
Soufre.	13,35

Caractères :

Cristaux incolores ou légèrement colorés, très déliquescents, oxydables par l'air.

Solubilité :

Eau.	0,4
Alcool.	soluble

Le sel fond à 50 °C dans son eau de cristallisation.

Caractères d'identité :

Ce sel, entièrement soluble dans l'eau, présente en solution une odeur d'hydrogène sulfuré et une réaction alcaline à la phénolphthaléine.

La solution aqueuse précipite les solutions de sels de cuivre, de plomb et de fer, en donnant un précipité noir de sulfure. Avec un sel de zinc, on obtient un précipité blanc de sulfure.

La solution aqueuse donne les réactions du sodium.

Essais :

1. Préparation de la solution pour les essais :

Préparer une solution à 10 g pour 100 ml de monosulfure de sodium à analyser.

CODEX ŒNOLOGIQUE INTERNATIONAL

Sodium (monosulfure de)

2. Cette solution doit être à peu près incolore et limpide. Il ne doit rester aucune partie insoluble.

3. Polysulfures et thiosulfates :

A 20 ml de solution à 10 p. 100 de monosulfure de sodium, ajouter 4 ml d'acide chlorhydrique pur (R). L'acide sulfhydrique se dégage et la solution doit rester limpide ou tout au plus opalescente.

4. Arsenic :

La solution précédente obtenue en 3 est évaporée dans un ballon de 500 ml jusqu'à l'obtention d'un volume de 10 ml environ. Ajouter 10 ml d'acide nitrique pur (RAs) et 10 ml d'acide sulfurique pur (RAs) et terminer l'oxydation jusqu'à émission de vapeurs blanches d'anhydride sulfurique. Après avoir chassé les vapeurs nitreuses par reprise avec 10 ml d'eau et évaporation, diluer le liquide sulfurique à 100 ml avec de l'eau. Rechercher l'arsenic par la méthode décrite page 126 sur 25 ml de cette solution. (Teneur en arsenic inférieure à 2 parties par million.)

5. Dosage :

10 ml de solution à 10 p. 100 obtenue à l'alinéa 1 sont dilués au 1/10. 20 ml de cette solution à 1 p. 100 sont placés dans une fiole conique de 250 ml; ajouter 50 ml d'eau et 5 ml d'acide acétique (R) et titrer immédiatement par l'iode 0,1 N jusqu'à virage au bleu de l'empois d'amidon. Soit n le volume employé :

1 ml de solution 0,1 N d'iode correspond à 0,01201 g de monosulfure de sodium hydraté.

Teneur p. 100 en monosulfure de sodium du produit essayé :

$$6,00.n$$

Cette teneur doit être de 90 p. 100 au moins.

Conservation :

Le monosulfure de sodium doit être conservé à l'abri de l'air dans des récipients hermétiquement fermés. Ce produit ne doit pas être conservé longtemps.

Chapter II

Analytical and Control Techniques

DETERMINATION OF 5-(HYDROXYMETHYL)FURFURAL
(OENO 18/2003)

1. PRINCIPLE

The 5-(hydroxymethyl)furfural (HMF) is determined by HPLC (sharing liquid chromatography in reverse phase).

2. APPARATUS AND SOLUTIONS

2.1 Instrumental parameters (for example)

Chromatograph in liquid phase
UV/visible detector
column: octadecyl type grafted silica (C18), (length: 20 cm; internal diameter: 4.6 mm; granulometry of phase: 5 µm)
mobile phase: ultra filtered demineralised water - methanol - acetic acid (80, 10, 3: v/v/v)
flow: 0.5 ml/min
detection wave length: 280 nm
injected volume: 20 µl

2.2 Preparation of calibration solutions

Solution HMF at 20 mg/l:
In a 100 ml graduated flask, introduce 20 mg of HMF weighed within 0.1 mg and complete to the graduated line with ultra filtered demineralised water,
introduce 10 ml of this solution in a 100ml graduated flask and complete with ultra filtered demineralised water;
the solution HMF at 20 mg/l is to be prepared each day.

3. PREPARATION OF SAMPLES

The samples and the calibration solution HMF are injected after filtration on a 0.45 µm membrane.

4. PROCEDURE

The chromatographic column is stabilised with the mobile phase for about 30 min.

Calculate the concentration of HMF of the sample from the peak surfaces.

**MEASURING ARSENIC BY HYDRIDE GENERATION
AND ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1 – FIELD OF APPLICATION

This method applies to the analysis of arsenic in the concentration range of 0 to 200 µg/l with prior mineralisation for oenological products.

2 – DESCRIPTION OF THE TECHNIQUE

2.1. Principle of the method

After reducing arsenic (V) into arsenic (III), arsenic is determined by hydride generation and atomic absorption spectrometry.

2.2. Principle of the analysis (figure n°1)

The peristaltic pump draws up the borohydride solution, hydrochloric acid solution and calibration or sample.

The hydride formed in the gas-liquid separator is entrained by a neutral gas (argon).

The gaseous current passes in a dessicator made up of calcium chloride.

The arsenic hydride is analysed in an quartz absorption cell in the flame of a air-acetylene burner.

The optical path of the hollow-cathode lamp of the atomic absorption spectrometer passes in the quartz cell.

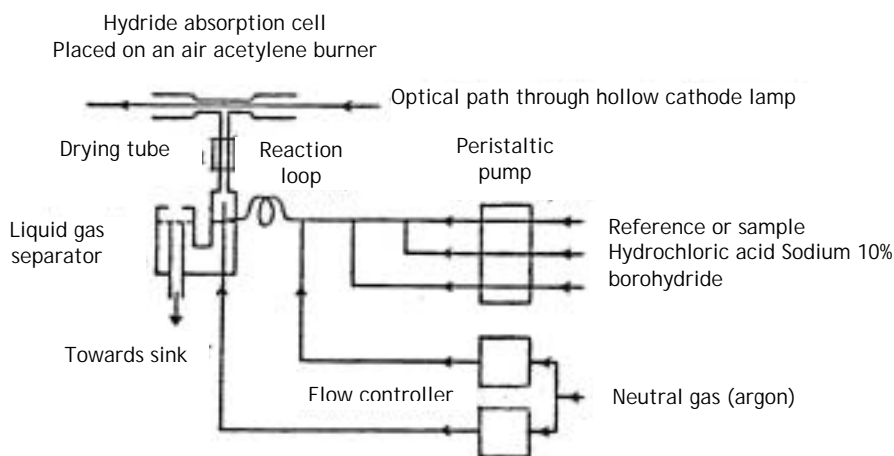


Figure n°1. Hydride generator

3 – REAGENTS AND PREPARATION OF REAGENT SOLUTIONS

- 3.1. Ultra-pure demineralised water**
- 3.2. Ultra-pure nitric acid at 65%**
- 3.3. Potassium iodide KI**
- 3.4. Potassium iodide at 10% (m/v)**
- 3.5. Concentrated hydrochloric acid**
- 3.6. Hydrochloric acid at 10% (m/v)**
- 3.7. Sodium borohydride** NaBH_4
- 3.8. Sodium hydroxide** NaOH in patches
- 3.9. Sodium borohydride solution at 0.6%** (containing 0.5% of NaOH)
- 3.10. Calcium chloride** CaCl_2 (used as a dessicator)
- 3.11. Silicone antifoam**
- 3.12. Arsenic calibration solution at 1 g/l** containing 2% of nitric acid and prepared from the following acid: $\text{H}_3\text{AsO}_4 \frac{1}{2} \text{H}_2\text{O}$
- 3.13. Arsenic solution at 10 mg/l:** place 1 ml of the calibration solution (3.12.) in a 100 ml flask; add 1% of nitric acid (3.2.); complete to volume with demineralised water (3.1.).
- 3.14. Arsenic solution at 100 µg/l:** place 1 ml of the arsenic solution at 10 mg/l (3.13.) in a 100 ml flask; add 1% of nitric acid (3.2.); complete to volume with demineralised water (3.1.).

4 – APPARATUS

- 4.1. Glassware:**
 - 4.1.1. graduated flasks 50 and 100 ml (class A)
 - 4.1.2. graduated pipettes 1, 5, 10 and 25 ml (class A)
 - 4.1.3. cylindrical vases 100 ml
- 4.2. Hot plate** with thermostat
- 4.3. Ashless filter paper**
- 4.4. Atomic absorption spectrophotometer:**
 - 4.4.1. air-acetylene burner
 - 4.4.2. hollow-cathode lamp (arsenic)
 - 4.4.3. deuterium lamp
- 4.5. Accessories:**
 - 4.5.1. vapour generator (or gas-liquid separator)
 - 4.5.2. quartz absorption cell placed on the air-acetylene burner
 - 4.5.3. bottle of neutral gas (argon)

5 – PREPARATION OF THE SET OF CALIBRATION SOLUTIONS AND SAMPLES

5.1. Set of calibration solutions 0, 5, 10, 25 µg/l

Place successively 0, 5, 10, 25 ml of the arsenic solution at 100 µg/l (3.14.) in 4, 100 ml flasks; add to each flask 10 ml potassium iodide at 10% (3.4.) and 10 ml of concentrated hydrochloric acid (3.5.); complete to volume with demineralised water (3.1.); allow to stand at room temperature for one hour.

5.2. Samples of oenological products

The sample is mineralised by wet process (cf. mineralisation methods of samples before determination by atomic absorption spectrometry) then filtered. Transfer 10 ml of filtered mineralisate to a 50 ml flask; add 5 ml of potassium iodide at 10% (3.4.) and 5 ml of concentrated hydrochloric acid (3.5.); add a drop of anti-foam (3.11.); adjust to volume with demineralised water (3.1.). Allow to stand at room temperature for one hour. Filter on an ashless filter paper.

6. PROCEDURE

6.1. Instrumental parameters of the atomic absorption spectrophotometer (given as an example)

6.1.1. oxidant air-acetylene flame

6.1.2. wave length: 193.7 nm

6.1.3. width of the monochromator's slit: 1.0 nm

6.1.4. intensity of the hollow-cathode lamp: 7 mA

6.1.5. correction of the non specific absorption with a deuterium lamp

6.2. Analytical determination

The peristaltic pump draws up the reagent solutions (3.6.) and (3.9.) and the calibrations or samples (5.1.) or (5.2.).

Present successively the calibration solutions (5.1.); wait long enough so that the hydride formed in the gas-liquid separator, passes in the absorption cell; perform an absorbance reading for 10 seconds; perform two measurements; the spectrometer's computer software sets up the calibration curve (absorbance depending on the concentration of arsenic in µg/l).

Then present the samples (5.2.). Perform two measurements.

6.3. Self-check

Every five determinations, an analytical blank solution and a calibration are analysed in order to correct a possible deviation of the spectrometer.

7. EXPRESSION OF RESULTS

The results are directly printed by the printer connected to the computer.

The concentration of arsenic in oenological products is expressed in µg/kg while taking into account the test sample.

8. CONTROL OF RESULTS

The quality control is performed by placing, after the set of calibration solutions and every five samples, a reference material whose content in arsenic is known with certainty.

A control card is set up for each reference material used. The control limits were set at: $\pm 2S_R$ intra (S_R intra : standard deviation of reproductibility).

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AROMATIC POLYCYCLIC HYDROCARBONS
DETERMINATION OF BENZO[a]PYRENE IN OENOLOGICAL
CHARBONS BY HPLC
(OENO 18/2003)

1. PRINCIPLE

Polycyclic aromatic hydrocarbons including benzo[a]pyrene are extracted by hexane; the solvent is evaporated and the residue is taken up by the methanol-tetrahydrofuran for analysis by HPLC.

2. APPARATUS AND REAGENTS

2.1 Reagents and calibrations

Acetonitrile for HPLC
Hexane for pesticide residues
Tetrahydrofuran for HPLC (THF)
Deionised and microfiltered water
Benzo[a]pyrene for HPLC.

2.2 Apparatus and chromatographic conditions

octadecyl type HPLC column
fluorimetric detector adjusted to the following detection conditions:

excitation wave length: 300 nm,
emission wave length: 416 nm.

Mobile phase:

solvent A: Deionised and microfiltered water
solvent B: acetonitrile

variations in the composition of the solvent

TIME in min	% solvent A	% solvent B
0	50	50
15	20	80
40	0	100
45	50	50

Flow 1.0 ml/mn

2.3 Preparation of reference solutions

Benzo[a]pyrene reference solution at about 100 mg/l in a methanol/THF mixture (50/50) stored for 3 years maximum in cold conditions.

Daughter solution at about 20 µg/l, prepared extemporaneous (0.5 ml of reference solution in 50 ml of methanol/THF then 1 ml of this intermediate solution in 50 ml de methanol/THF).

2.4 Preparation of samples

2 g of oenological charbon are mixed in a 50 ml volumetric flask with 30 ml of hexane.

The polycyclic aromatic hydrocarbons are extracted for 5 min using a magnetic stirrer. The organic phase recovered by filtration is gathered in a evaporating flask and evaporated. The extract is taken up by 2 ml of a methanol/THF mixture (1/1, v/v) and injected.

3. RESULTS

The benzo[a]pyrene content must not be higher than 1 µg/kg.

REMARK: It is also possible to determine benzo[a]pyrene by chromatography in gaseous phase by an apolar capillary column with detection by mass spectrometry.

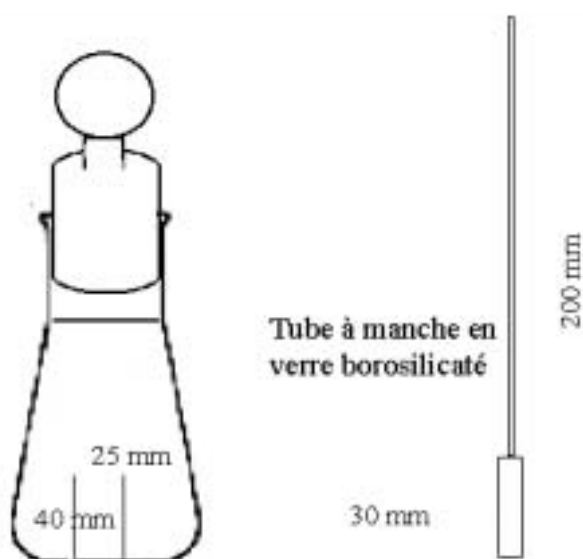
BROMINE INDEX
(OENO 18/2003)

The bromine index is the quantity of bromine expressed in grammes, that 100 g of the substance can set.

1. APPARATUS

A graduated flask of 300 to 400 ml with an interior tube welded at the bottom, an emery stopper and a tube with a handle, compliant with the following diagram

Bromination flask 300 ml in borosilicate glass.
Stopper with ground-glass joints
standardised 24/40.



2. SOLUTIONS

2.1 Potassium bromate solution 0.016 M

This solution contains for 1000 ml:

Potassium bromate KBrO_3	2.783 g
-----------------------------------	---------

Weigh exactly 2.783 g of potassium bromate and introduce into a 1000 ml graduated flask containing about 500 ml of distilled water; shake in order to dissolve and complete to 20°C with distilled water the volume of 1000 ml of solution. Mix and store in a flask with a glass stopper.

2.2 Iodine solution 0.05 M

Iodine I	12.69 g
Potassium iodide de KI	18 g
Water q.s.p.	1000 ml

Weigh exactly 12.69 g of iodine, then 18 g of potassium iodide and introduce into a 1000 ml graduated flask with about 200 ml of distilled water. Allow the dissolution to operate in cold conditions with the flask being sealed. Add about 500 ml of distilled water, then shake to absorb the iodine in a vapour state and complete to 20°C with distilled water, the volume to 1000 ml of solution. Mix and store in a coloured glass flask with a glass stopper.

2.3 Sodium thiosulphate solution 0.1 M

The 0.1 M sodium thiosulphate solution contains for 1000 ml:

Sodium thiosulphate $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$	24.82 g
---	---------

Weigh exactly 24.82 g of sodium thiosulphate and introduce into a 1000 ml graduated flask containing about 600 ml of boiled distilled water. Shake to dissolve and complete to 20°C with boiled distilled water, the volume to 1000 ml of solution. Mix. Store away from light. Control the titre of this solution using the 0.05 M iodine solution.

3. TECHNIQUE

Using a tube with a handle, put about 0.50 g of potassium iodide in the recipient inside the flask; (it is convenient to make a circular mark on the tube corresponding to the salt's weight so as not to have to weigh each dosage). Caution has to be taken so as not to introduce iodide on the external part of the flask. Then introduce the measured volume of the solution of the product to be measured, dissolved in

neutral or alkaline water, in the external part of the flask, then 25 ml of potassium bromate solution 0.016 M measured with a pipette, and 2 g of pure potassium bromide. Rinse the sides with water to come to a total volume of about 100 ml, then add 5 ml of concentrated hydrochloric acid (R); quickly close the flask with the stopper, the joint being humid with distilled water; by a circular movement homogenise the content and allow to stand the prescribed time. Shake the flask *vigorously* so as to put the potassium iodide in contact with the liquid so as to enable the vapour bromine to react; open the flask while rinsing the joint and the stopper with a spray of distilled water, and determine iodine using 25 ml of sodium thiosulphate solution 0.1 M; titrate the excess of sodium thiosulphate with the iodine solution 0.05 M in the presence of starch paste;

Let n be the volume used:

Quantity of bromine (in mg) set by the substance to be dosed = $n \times 0.008$

**DETERMINATION OF CADMIUM BY
ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1. PRINCIPLE

The cadmium is determined in solid oenological products after mineralisation by wet process or directly for liquid oenological products or put in a solution.

The determinations are performed by atomic absorption without a flame (electro-thermal atomisation in a graphite oven).

2. APPARATUS

2.1 Instrumental parameters (given as an example)

Spectrophotometer equipped with an atomiser with a graphite tube.

wave length: 228.8 nm

hollow-cathode lamp (cadmium)

width of slit: 1 nm

intensity of the lamp: 3 mA

correction of continuum by the Zeeman effect

graphite oven with a tantalised platform

(tantalisation procedure of the platform described above)

adjusting the oven for an analysis:

step	temperature (°C)	time (s)	gas flow rate (/ mn)	type of gas	reading of signal
1	100	35	3.0	argon	no
2	500	10	3.0	argon	no
3	500	45	1.5	argon	no
4	500	1	0.0	argon	no
5	2250	1	0.0	argon	yes
6	2250	1	0.0	argon	yes
7	2500	2	1.5	argon	no
8	1250	10	3.0	argon	no
9	75	10	3.0	argon	no

2.2 Adjustments of the automatic sampler (given as an example)

	volumes injected in μl		
	solution of Cd at 8 $\mu\text{g/l}$	blank	matrix modifier
blank	0	10	2
calibration N° 1 at 8 $\mu\text{g/l}$	1	9	2
calibration N° 2 at 16 $\mu\text{g/l}$	2	8	2
calibration N° 3 at 24 $\mu\text{g/l}$	3	7	2
calibration N° 4 at 32 $\mu\text{g/l}$	4	6	2
Sample to be dosed	5	5	2

3. REAGENTS

Demineralised water

Pure nitric acid for analysis at 65%

Anhydrous palladous chloride (59% in Pd)

Magnesium nitrate with 6 water molecules (ultra pure)

Ammonium dihydrogenophosphate

Matrix modifier: palladous chloride and magnesium nitrate mixture (dissolve 0.25 g of PdCl_2 and 0.1 g of $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in 50 ml of demineralised water) or ammonium dihydrogenophosphate at 6% (dissolve 3 g of $\text{NH}_4\text{H}_2\text{PO}_4$ in 50 ml of demineralised water).

Cadmium reference solution at 1 g/l, commercial or prepared as follows: dissolve 2.7444 g $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ in a solution of HNO_3 0.5 M, adjust to 1 l with HNO_3 0.5 M.

Cadmium solution at 10 mg/l: place 1 ml of the reference solution in a 100 ml graduated flask, add 5 ml of pure nitric acid and complete to volume with demineralised water.

Cadmium solution at 0.8 g/l: place 4 ml of the diluted solution in a 50 ml graduated flask, add 2.5 ml of pure nitric acid and complete to volume with demineralised water.

Calibration range at 0, 8, 16, 24 and 32 $\mu\text{g/l}$ of cadmium.

4. PREPARATION OF SAMPLES

No preparation is necessary for liquid oenological products or in solution form; solid products are mineralised by wet process.

The blank solution is made up of a pure nitric acid solution for analysis at 1%.

5. PROCEDURE

Each calibration solution is passed right after the blank solution. Perform 2 successive absorbance readings and establish the calibration curve.

Calculate the cadmium content of the samples while taking into account the test sample of different dilutions.

**DETERMINATION OF CALCIUM BY
ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1. PRINCIPLE

The calcium is directly determined in the liquid oenological product (or in the mineralisation solution) suitably diluted by atomic absorption spectrometry by air-acetylene flame after the addition of spectral buffer.

2. APPARATUS

Instrumental parameters (given as an example)

Atomic absorption spectrophotometer

Reducing air-acetylene flame

Hollow-cathode lamp (calcium)

wave length: 422.7 nm

width of slit: 0.2 nm

intensity of the lamp: 5 mA

No correction of non specific absorption.

3. REAGENTS

3.1 demineralised water

3.2 calcium reference solution at 1 g/l, commercial or prepared as follows: dissolve 5.8919 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ in a solution of HNO_3 0.5 M, adjust at 1 l with HNO_3 0.5 M.

3.3 calcium solution at 100 mg/l:

place 10 ml of the reference solution in a 100 ml graduated flask and 1 ml of pure nitric acid.

complete to volume with demineralised water

3.4 concentrated hydrochloric acid (R): 35% minimum

3.5 lanthanum solution at 25 g/l:

weigh 65.9 g lanthanum chloride ($\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$) in a 250 ml cylindrical vase, transfer to a 1000 ml graduated flask with demineralised water; add to the test tube 50 ml of concentrated hydrochloric acid (R); after solubilisation, allow to cool, complete to volume with demineralised water.

3.6 set of calibration solutions: 0, 2, 4, 6, 8 mg/l of calcium

place successively 0, 1,0, 2,0, 3,0 and 4,0 ml of the solution at 100 mg/l of calcium in 5, 50 ml graduated flasks, add 10 ml of lanthanum solution at 25 g/l, complete to volume with demineralised water.

4. PREPARATION OF SAMPLES

4.1 Case of liquid or solution oenological products

In a 50 ml graduated flask place 10 ml of the lanthanum solution and a volume of sample as after having being completed to volume with demineralised water; the concentration is below 8 mg/l.

4.2 Case of solid oenological products

Proceed with mineralisation by dry process;

Put in each solution of the set the same quantity of acid used for putting cinders in solution or mineralisation (see chapter "Mineralisation").

Take up cinders and 2 ml of concentrated hydrochloric acid (35% minimum) in a 100 ml flask; add 20 ml of lanthanum solution at 25 g/l and complete to volume with demineralised water.

Perform a blank test in the same conditions.

5. PROCEDURE

Pass each solution of the set in ascending order of the concentration of calcium.

For each solution, perform 2 absorbance readings when they are perfectly stabilised (integration time of signal: 10 seconds).

Pass each sample twice and calculate the calcium content.

SEARCH FOR CHLORIDES
(OENO 18/2003)

In a 160 × 16 mm test tube, place the volume prescribed of the solution obtained by the means indicated in each monography; add 5 ml of diluted nitric acid (R); complete to 20 ml and add 0.5 ml of silver nitrate solution at 5% (R).

Compare the opalescence or any cloudiness to the control sample prepared with 0.5 ml of hydrochloric acid at 0.10 g per litre (0.05 mg of HCl) with 5 ml of diluted nitric acid (R), and adjust to 20 ml with distilled water. Add 0.5 ml of silver nitrate solution at 5% (R). This tube contains 50 µg of HCl.

**DETERMINATION OF CHROME BY
ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1. PRINCIPLE

The chrome is determined by atomic absorption spectrophotometer without flame.

2. APPARATUS

2.1 Experimental parameters (given as an example)

Atomic absorption spectrophotometer
wave length: 357.9 nm
hollow-cathode lamp (Chrome)
width of slit: 0.2 nm
intensity of the lamp: 7 mA
correction of continuum by the Zeeman effect
introduction in hot conditions of the samples in the

graphite oven

measurement of the signal: peak height
time of measurement: 1 second
number of measurements per sample: 2
pyrolytic graphite tube:
pyrolytic graphite oven containing a platform L'Vov

tantalised

tantalisation of platform (see above)
inert gas: argon - hydrogen mixture (95%; 5%)
parameters for oven:

step	temperature (°C)	time (s)	gas rate flow (l / mn)	type of gas	reading of signal
1	85	5	3.0	argon + hydrogen	no
2	95	40	3.0	argon + hydrogen	no
3	120	10	3.0	argon + hydrogen	no
4	1000	5	3.0	argon + hydrogen	no
5	1000	1	3.0	argon + hydrogen	no
6	1000	2	0.0	argon + hydrogen	no
7	2600	1.2	0.0	argon + hydrogen	yes
8	2600	2	0.0	argon + hydrogen	yes
9	2600	2	3.0	argon + hydrogen	no
10	75	11	3.0	argon + hydrogen	no

2.2 Adjustments of the automatic sampler
(given as an example)

	volumes injected in µl		
	chrome solution at 50 µg/l	blank	matrix modifier
blank	0	17	3
calibration N° 1 at 50 µg/l	5	12	3
calibration N° 2 at 100 µg/l	10	7	3
calibration N° 3 at 150 µg/l	15	2	3
sample to be measured	5	12	3

3. REAGENTS

3.1 pure demineralised water for analysis

3.2 pure nitric acid for analysis at 65%

3.3 anhydrous palladous chloride (59% in Pd)

3.4 pure hexahydrated magnesium nitrate for analysis

3.5 ammonium dihydrogenophosphate

3.6 matrix modifier: mixture of palladium chloride and magnesium nitrate (dissolve 0.25 g of PdCl₂ and 0.1 g of Mg(NO₃)₂·6H₂O in 50 ml of demineralised water) ammonium dihydrogenophosphate at 6% (dissolve 3 g of NH₄H₂PO₄ in 50 ml of demineralised water).

3.7 reducing agent: L-ascorbic acid in solution at 1% m/v.

3.8 chrome reference solution at 1 g/l, commercial or prepared as follows: dissolve 7.6952 g of Cr(NO₃)₃·9H₂O in a solution of HNO₃ 0.5 M, adjust at 1 l with HNO₃ 0.5 M

3.9 chrome solution at 10 mg/l: place 1 ml of the reference solution in a 100 ml graduated flask, add 5 ml of nitric acid at 65% and complete to volume with demineralised water.

3.10 set of calibration solutions: 0, 50, 100 and 150 µg/l of chrome (see table: adjustments of the automatic sampler).

4. PREPARATION OF SAMPLES

4.1 Case of liquid or solution oenological products

The preparations are performed manually or automatically by the diluter by following the data from the table "adjustments of the automatic sampler".

4.2 Case of solid oenological products

Proceed with mineralisation by wet process. Do a blank test.

5. PROCEDURE

Pass each solution of the set in ascending order of the concentration of chrome;

Pass each sample twice and calculate the chrome content while taking into account the test sample.

SULPHURIC CINDERS
(OENO 18/2003)

The sulphuric cinders result from the calcination after being in contact with air after being attacked by sulphuric acid.

Heat a silica or platinum crucible of low form for 30 min until red; allow to cool in a vacuum dessicator and tare the crucible. Place the exactly weighed test sample in the crucible and wet it with a sufficient quantity of concentrated sulphuric acid (R) diluted beforehand by an equal volume of water. Heat until dry evaporation, then in a muffle oven, first carefully until red without exceeding the temperature of $600^{\circ}\text{C} \pm 25^{\circ}\text{C}$. Maintain calcination until the black particles disappear, allow to cool, add 5 drops of sulphuric acid diluted to half to the residue, then evaporate and calcinate as previously until constant weight; weigh after cooling in the desiccator.

Calculate the rate of sulphuric cinders referring to 100 g of substance.

TOTAL CINDERS

The total cinders result from the calcination of the product after contact with air.

Heat a silica or platinum crucible of low form for 30 min until red. Allow to cool in a vacuum dessicator and tare the crucible. Dispose homogenously the exactly weighed test sample in the crucible. Desiccate for an hour in the incubator at 100°C - 105°C . Incinerate in the muffle oven, first carefully to avoid that the sample catches fire, then until red at a temperature of $600^{\circ}\text{C} \pm 25^{\circ}\text{C}$. Maintain the calcination until the black particles disappear. For 30 min allow to cool in a vacuum desiccator. Weigh. Continue the calcination until constant mass.

If the black particles persist, take up the cinders in hot distilled water. Filter these cinders on an ashless filter paper (porosity $10\text{ }\mu\text{m}$). Incinerate the filter and residue until constant mass. Group the new cinders with the filtrate. Evaporate the water. Incinerate the residue until constant mass.

Calculate the rate of total cinders by referring to 100 g of substance.

**DETERMINATION OF COPPER BY
ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1. PRINCIPLE

The copper is determined by atomic absorption spectrometry by flame by using the method of measured additions.

2. APPARATUS

Instrumental parameters: (given as an example)

Atomic absorption spectrophotometer

flame: oxidant air-acetylene

wave length: 324.7 nm

hollow-cathode lamp (copper)

width of slit: 0.5 nm

intensity of the lamp: 3.5 mA

no correction of non specific absorption.

3. REAGENTS

3.1 pure demineralised water for analysis

3.2 pure nitric acid for analysis at 65%

3.3 reference solution copper at 1 g/l, commercial or prepared as follows: dissolve 3.8023 g of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ in a solution of HNO_3 0.5M, adjust at 1 l with HNO_3 0.5M.

3.4 copper solution at 10 mg/l: place 2 ml of the reference copper solution in a 200 ml graduated flask, add 2 ml of nitric acid at 65% and complete to volume with demineralised water.

Adjust apparatus using a calibration solution at 0.4 mg/l (2 ml of the copper solution at 10 mg/l in a 50 ml graduated flask, complete to volume with pure demineralised water for analysis).

4. PREPARATION OF SAMPLES (METHOD OF MEASURED ADDITIONS)

- Addition of 0.2 mg/l of copper:
place 5 ml of liquid oenological product or mineralisate of oenological product obtained by dry process in a flask and add 100 µl of the copper solution at 10 mg/l
- Addition of 0.4 mg/l of copper:
place 5 ml of liquid oenological product or mineralisate in a flask and add 200 µl of the copper solution at 10 mg/l
- dilution of the sample
Dilution of the sample: the dilution is only necessary if the copper content is more than 0.5 mg/l of copper.

5. PROCEDURE

- For each sample, pass in order:
- blank solution (demineralised water)
 - sample with 0.2 mg/l of copper
 - sample with 0.4 mg/l of copper
 - sample without addition
- the results are obtained automatically or by manual graph.

**ANALYSES OF GAS CONTROL BY
GASEOUS CHROMATOGRAPHY
(OENO 18/2003)**

1. PRINCIPLE

The gases are controlled by chromatography in gaseous phase using a "molecular sieve" type column and detection by catharometer or flame ionisation.

2. SAMPLING

Either use

- a stainless steel flask for sampling gas
- a Teflon sampling bag for gas.

3. INJECTION METHOD

Use of a unheated gas valve with a 250 µl ring.

4. SEPARATION OF LIGHT GASES, H₂, O₂, N₂, CO, CH₄.

4.1 Column (for example)

Phase: Molecular sieve Chromosorb 101, Porapak Q
diameter of particles 5µm
granulometry: 80 to 100 mesh

Dimensions: length: 2 m, internal diameter: 2 mm.

4.2 Vector Gas

Helium (He), flow: 3 ml/mn

4.3 Oven temperature: 40°C isotherm

4.4 Detector: Catharometer, Intensity 190 µA

5. SEPARATION OF LIGHT HYDROCARBONS

5.1 Column (for example)

Wide bore

Phase: apolar, diameter of particles: 5 µm

Length: 30 m, internal diameter: 0.53 mm

5.2 Vector gas

Nature: Helium, Flow: 3 ml/mn

Oven temperature 35°C to 200°C rise: 10°C/mn

5.3 Detector: Flame ionisation, temperature 220°C.

SEARCH FOR HEAVY METALS
(OENO 18/2003)

1. Principle of the method

Heavy metals react with the thiol function to form sulphurs. The coloration that results is compared to a standard.

2. Reagents

2.1 Ammonium acetate,

2.2 Lead nitrate (II),

2.3 Glycerol,

2.4 Methanol,

2.5 Sodium hydroxide, solution at 1 mole NaOH /l,

2.6 Hydrochloric acid at 37%,

2.7 Thioacetamide reagent (R):

2.8 Standard lead solution:

2.8.1 Lead solution at 1000 µg/ml: dissolve 1.598 g of lead nitrate(II) in water and complete to 1000 ml.

2.8.2 Lead solution at 10 µg/ml. Add 10 ml of the solution 2.8.1 and complete to 1000 ml. To be prepared just before use.

2.9 Buffer solution, pH = 3.5: dissolve 6.25 g of ammonium acetate in 6 ml of water, add 6.4 ml of hydrochloric acid (2.6) and dilute with water until 25 ml.

3. Procedure

3.1 Test solution: pour 5 ml of buffer solution (2.9), 25.0 g of sample and about 15 ml of water into a 50 ml graduated flask. Complete with water up to the reference mark.

3.2 Coloured solutions:

3.2.1. Sample solution: mix 12.0 ml of test solution (3.1) and 2.0 ml of buffer solution (2.9) in a test tube.

3.2.2. Comparative solution: mix 2.0 ml of test solution (3.1), 2.0 ml of buffer solution (2.9), 0.5 ml of standard lead solution (2.8.2), 4.5 ml of water and 5.0 ml of methanol in a test tube.

INTERNATIONAL CENOLOGICAL CODEX
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3.2.3. Control solution: mix 12.0 ml of test solution (3.1), 2.0 ml of buffer solution (2.9) and 0.5 ml of standard lead solution (2.8.2) in a test tube.

3.2.4 Comparison of colorations:
add 1.2 ml of thioacetamide reagent (2.7) in the 3 test tubes (3.2.1 to 3), mix and wait 2 minutes. Compare the coloration vertically in the light of day.

- the sample solution must not be darker than the comparative solution.
- the control solution must not be lighter than the comparative solution.

4. Results:

The conditions described in 3.2.4 are obtained if the heavy metal content is less than 10 mg/l expressed in lead and with a precision of 1 mg/l.

**DETERMINATION OF IRON BY
ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1. PRINCIPLE

The iron is determined by atomic absorption spectrophotometry by flame.

2. APPARATUS

2.1 Instrumental parameters: (given as an example)

atomic absorption spectrophotometry
flame: oxidant air-acetylene
hollow-cathode lamp (iron)
wave length: 248.3 nm
width of slit: 0.2 nm
intensity of the lamp: 5 mA
no correction of non specific absorption.

3. REAGENTS

3.1 pure demineralised water for analysis

3.2 iron solution at 1 g/l, commercial or prepared as follows:
dissolve 7.2336 g of $\text{Fe}(\text{NO}_3)_2 \cdot 9\text{H}_2\text{O}$ in a solution HNO_3 0.5 M adjust at 1 l avec HNO_3 0.5 M.

3.3 iron solution at 100 mg/l

place 10 ml of the reference iron solution in a 100 ml graduated flask, complete with demineralised water pure for analysis

3.4 set of calibration solution: 2, 4, 6, 8 mg/l of iron

place successively 1.0, 2.0, 3.0 and 4.0 ml of the solution at 100 mg/l of iron in 4, 50 ml graduated flasks; complete to volume with pure demineralised water for analysis

Perform a blank without iron in the same conditions.

4. PREPARATION OF SAMPLES

4.1 Case of liquid or solution oenological products

Each sample is diluted with demineralised water in order to have a concentration of iron between 0 and 8 mg/l.

4.2 Case of solid oenological products

Proceed with mineralisation by dry process.
Put in each solution of the set of calibration the same quantity of acid used for putting of cinders in solution; each sample is diluted with demineralised water in order to have a concentration of iron between 0 and 8 mg/l.

5. PROCEDURE

Pass successively the calibration solutions and the blank which will be demineralised water or a water-acid solution with concentrations used for samples of solid oenological products mineralised by dry process and perhaps diluted.

**DETERMINATION OF LEAD BY
ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1. PRINCIPLE

After mineralisation of the sample in an acid medium, the lead is determined by spectrometry without flame (electro-thermal atomisation).

2. APPARATUS

2.1 Instrumental parameters: (given as an example)

Atomic absorption spectrophotometer equipped with an atomiser with a graphite tube
wave length: 283.3 nm
hollow-cathode lamp (lead)
width of slit: 0.5 nm
intensity of the lamp: 5 mA
correction of continuum: by Zeeman effect
introduction in hot conditions of the samples in the graphite oven by an automatic distributor (rinsing water contains 2 drops of Triton per litre)
measurement of signal: peak height
time of measurement: 1 second
number of measurements per sample: 2
pyrolytic graphite tube
pyrolytic graphite oven containing a platform of L'Vov tantalised
(tantalisation of a platform: see above).
parameters for oven

temperature (°C)	time (s)	gas flow rate (l / min)	type of gas	Reading of signal
150	20.0	3.0	argon	no
150	35.0	3.0	argon	no
800	15.0	3.0	argon	no
800	30.0	3.0	argon	no
800	2.0	0.0	argon	no
2250	0.8	0.0	argon	yes
2250	1.0	0.0	argon	yes
2500	1.0	1.5	argon	no
1200	9.0	3.0	argon	no
75	10.0	3.0	argon	no

2.2 Adjustments of the automatic sampler
(given as an example)

	volumes injected in μl		
	lead solution at 50 $\mu\text{g} / \text{l}$	blank	matrix modifier
blank	0	10	2
calibration N° 1	1	9	2
calibration N° 2	2	8	2
calibration N° 3	3	7	2
calibration N° 4	4	6	2
calibration N° 5	6	4	2
Sample to be measured	10	0	2

3. REAGENTS

3.1 Pure demineralised water for analysis

3.2 Pure nitric acid for analysis at 65%

3.3 Ammonium dihydrogenophosphate

3.4 Matrix modifier: ammonium dihydrogenophosphate at 6%.

Introduce 3 g of ammonium dihydrogenophosphate in a 50 ml graduated flask, dissolve and complete to volume with demineralised water.

Lead reference solution at 1 g/l commercial or prepared as follows: dissolve 1.5985 g of pure $\text{Pb}(\text{NO}_3)_2$ for analysis in a solution of HNO_3 0.5 M, adjust at 1 l avec HNO_3 0.5 M.

Lead solution at 10 mg / l: place 1 ml of the reference lead solution at 1 g/l in a 100 ml graduated flask; add 1 ml of nitric acid at 65% complete to volume with pure demineralised water for analysis.

Lead solution at 0.1 mg/l: place 1 ml of the lead solution at 10 mg/l in a 100 ml graduated flask, add 1 ml of nitric acid at 65%; complete to volume with pure demineralised water for analysis.

Set of calibration solutions: 0, 50, 100, 150, 200, 300 $\mu\text{g}/\text{l}$ of lead.

The automatic distributor cycle allows to directly inject these quantities of lead on the platform from the lead solution at 0.050 mg/l.

4. PREPARATION OF SAMPLES

The liquid or solution samples must have concentrations between 0 and 300 µg/l of lead.

The solid samples will be mineralised by wet process (attack by nitric acid).

The blank is made up of pure water for analysis containing 1% of nitric acid at 65%.

5. PROCEDURE

The calibration curve represents the variations of absorbencies depending on the concentrations enabling to calculate the lead content of the samples.

**DETERMINATION OF MERCURY BY THE GENERATION OF VAPOUR
AND ATOMIC FLUORESCENCE SPECTROMETRY
(OENO 18/2003)**

1 – FIELD OF APPLICATION

This method is applied to the analysis of mercury in oenological products in the concentration range of 0 to 10 µg/l.

2 – DESCRIPTION OF THE TECHNIQUE

2.1. Principle of the method

2.1.1. Mineralisation by the wet process of the oenological product to be analysed.

2.1.2. Reduction of the permanganate not consumed by hydroxylamine hydrochloride.

2.1.3. Reduction of mercury(II) into metal mercury by tin chloride (II).

2.1.4. Entrainment of mercury by a current of argon at room temperature.

Detreming mercury in the state of monoatomic vapour by atomic fluorescence spectrometry, with the wave length at 254 nm: the mercury atoms are excited by a mercury vapour lamp; the atoms thus excited reemit fluorescent radiation that enables to quantify the mercury present using a photonic detector placed at 90° in relation to excitation beam; detection by atomic fluorescence enables to obtain good linearity and eliminates memory effects.

2.2. Principle of the analysis (figure n° 1)

The peristaltic pump draws up the tin chloride (II) solution, the blank (demineralised water containing 1% nitric acid) and the mineralised sample or calibration.

The metal mercury is entrained in the gas-liquid separator by a current of argon.

After going through the membrane of a dessicator, the mercury is detected by fluorescence.

Then the gaseous current goes through a potassium permanganate solution in order to trap the mercury.

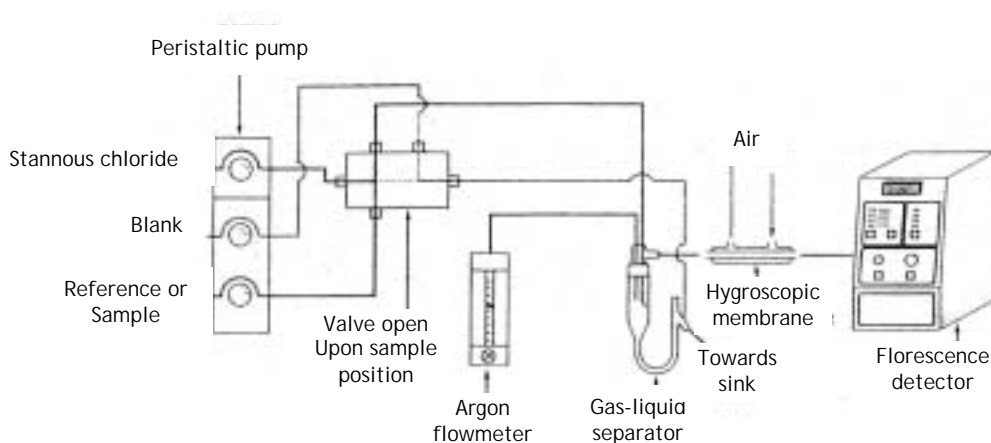


Figure n°1. Analytic Chain for dosage of mercury

3 – REAGENTS AND PREPARATION OF REAGENT SOLUTIONS

3.1. Ultra-pure demineralised water

3.2. Ultra-pure nitric acid at 65%

3.3. Blank: demineralised water (3.1.) containing 1% nitric acid (3.2.)

3.4. Nitric acid solution 5.6 M: introduce 400 ml of nitric acid (3.2.) into a 1000 ml flask; complete to volume with demineralised water (3.1.).

3.5. Sulphuric acid ($d = 1.84$)

3.6. Sulphuric acid solution 9 M: introduce 200 ml of demineralised water (3.1.) in a 1000 ml flask, then 500 ml of sulphuric acid (3.5.); after cooling, complete to volume with demineralised water (3.1.).

3.7. Potassium permanganate KMnO_4

3.8. Potassium permanganate solution at 5%: dissolve with demineralised water (3.1.), 50 g of potassium permanganate (3.7.) in a 1000 ml flask; complete to volume with demineralised water (3.1.).

3.9. Hydroxylamine hydrochloride $\text{NH}_2\text{OH}, \text{HCl}$

3.10. Reducing solution: weigh 12 g of hydroxylamine hydrochloride (3.9.) and dissolve in 100 ml of demineralised water (3.1.).

3.11. Tin chloride II ($\text{SnCl}_2, 2 \text{H}_2\text{O}$)

3.12. Concentrated hydrochloric acid

3.13. Tin (II) chloride solution: weigh 40 g of tin chloride (3.11.) and dissolve in 50 ml of hydrochloric acid (3.12.); complete to 200 ml with demineralised water (3.1.).

3.14 Mercury reference solution at 1 g/l prepared by dissolution of 1.708 g of $\text{Hg}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$, in 1 l of HNO_3 solution at 12% (m/n).

3.15. Mercury calibration solution at 10 mg/l, containing 5 % of nitric acid and prepared from the reference solution at 1 g/l (3.14).

3.16. Mercury solution at 50 µg/l: place 1 ml of the solution at 10 mg/l (3.14.) in a 200 ml flask; add 2 ml of nitric acid (3.2.); complete to volume with demineralised water (3.1.).

4 – APPARATUS

4.1. Glassware:

4.1.1. graduated flasks 100, 200 and 1000 ml (class A)

4.1.2. graduated pipettes 0.5; 1.0; 2.0; 5; 10 and 20 ml (class A)

4.1.3. precautions: before use, the glassware must be washed with nitric acid at 10%, left in contact for 24 hours, then rinsed with demineralised water.

4.2. Mineralisation apparatus (see Compendium of international methods of analysis of wines and musts)

4.3. Thermostatic heating mantle

4.4. Peristaltic pump

4.5. Cold vapour generator

4.5.1. gas-liquid separator

4.6. Dessicator (hygroscopic membrane) covered by an air current (supplied by a compressor) and placed before the detector

4.7. Spectrofluorimeter:

4.7.1. mercury vapour lamp, adjusted to the wave length of 254 nm

4.7.2. specific atomic fluorescence detector

4.8. PC:

4.8.1. software that adjusts the parameters of the vapour generator and atomic fluorescence detector and allows calibration and the analysis of results.

4.8.2. printer that archives results

4.9. Bottle of neutral gas (argon)

5. PREPARATION OF THE SET OF CALIBRATION SOLUTIONS AND SAMPLES

5.1. Set of calibration solutions: 0; 0.25; 0.5 and 1.0 µg/l

Introduce 0; 0.5; 1.0; 2.0 ml of the mercury solution at 50 µg/l (3.15.) in 4 100 ml flasks; add 1% nitric acid (3.2.); complete to volume with demineralised water (3.1.).

5.2. Samples

Mineralise the samples by wet process. The test sample is introduced into the round-bottomed flask in borosilicate glass placed on a disc with a hole. The neck is inclined.

Add 5 ml of concentrated sulphuric acid (R) and 10 ml of concentrated nitric acid (R) and gently heat. When the mixture starts to turn brown, add a small quantity of nitric acid while continuing to heat and so forth until the liquid remains colourless and that the atmosphere of the flask fills with white smoke of SO₃. Allow to cool, take 10 ml of distilled water and heat again to allow the nitrous fumes to escape until the release of the white smoke. This operation is repeated; after a third time, boil an instant, cool, stabilise with several drops (about 10) of potassium permanganate (aqueous sol.) at 5% (m/m) and add water to the liquid to reach 40 ml.

Filter on filters without cinders. Introduce 10 ml of filtrate into a 50 ml flask. Add potassium permanganate (3.8.) until persistence of coloration. Solubilise the precipitate (MnO₂) with the reducing solution (3.10.). Complete to volume with demineralised water (3.1.).

Do a blank test with demineralised water.

6 – PROCEDURE

6.1. Analytical determination

Turn on the fluorimeter; the apparatus is stabilised after 15 minutes.

The peristaltic pump draws up the blank solution (3.3.), the tin chloride (II) solution (3.13.) and the calibrations or samples (5.1.) or (5.2.).

Check if there is a bubbling in the gas-liquid separator.

Present successively the calibration solutions (5.1.); start the programming of the vapour generator. The computer software sets up the calibration curve (percentage of fluorescence depending on the concentration of mercury in µg/l).

Then present the samples (5.2.).

6.2. Self-check

Every five determinations, an analytical blank solution and a calibration are analysed in order to correct a possible drift of the spectrofluorimeter.

7 – EXPRESSION OF RESULTS

The results are given by the computer software and are expressed in p.p.b. (or µg/l).

The concentration of mercury in oenological products is calculated according to the test sample and the dilution of the mineralisate. It is expressed in µg/kg.

8 – CONTROL OF RESULTS

The quality control is performed by placing, after the set of calibration solutions and all five samples, a reference material whose mercury content is known with certainty.

A control card is set up for each reference material used. The control limits are set at: $\pm 2S_R$ intra (S_R intra: standard deviation for reproducibility).

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**MINERALISATION METHODS OF SAMPLES BEFORE
DETERMINATION BY ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1. MINERALISATION BY DRY PROCESS

Method applicable for determining the following elements: calcium, magnesium, sodium, iron, copper, zinc.

1.1 Obtaining cinders

Weigh with precision 5 g of oenological product (or 1 g in the case of products rich in mineral matters), in a platinum or silice capsule cleaned and tared beforehand.

Gently burn the sample with the flame of a Bunsen burner under a hood.

Put the capsule in a muffle oven at $525^{\circ}\text{C} \pm 25^{\circ}\text{C}$ for 12 hours.

Take up the residue with a few ml of demineralised water.

Evaporate water over a water bath at 100°C .

Replace the capsule containing the sample in the oven.

The mineralisation is over when the cinders are white.

1.2 Putting the cinders in a solution

The cinders are solubilised with 2 ml of concentrated hydrochloric acid (R), bring to volume at 100 ml with demineralised water

Complementary dilutions:

Re-dilute the cinders solution in hydrochloric acid in order to be compatible with the sensitivity of the apparatus; see separately the method of each cation.

For the determination of calcium and magnesium, add lanthanum chloride during this dilution.

Do a blank test.

2. MINERALISATION BY WET PROCESS

Method applicable for determining the following elements: arsenic, cadmium, lead in oenological products containing water.

2.1 Case of aqueous products

Weigh with precision in a 50 ml polypropylene tube 3 grammes of pulverised oenological product, add 5 ml of nitric acid at 65%; close

with a screw cap; leave 12 hours at room temperature then after unscrewing the cap place the tube in a water bath at 90°C for 3 hours under a hood; allow to cool; adjust the volume to 20 ml with demineralised water; shake; filter on an ashless filter paper (if necessary).

Do a blank test in the same conditions.

2.2 Case of dry products

The mineralisation is similar as for aqueous products but by using a test sample of 0.5 gramme of oenological product.

**DETERMINATION OF NICKEL BY
ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1. PRINCIPLE

The nickel is directly determined by atomic absorption spectrometry without flame (electro-thermal atomisation).

2. APPARATUS

2.1 Instrumental parameters: (given as an example)

Atomic absorption spectrophotometer equipped with an atomiser with a graphite tube.

wave length: 232.0 nm

hollow-cathode lamp (nickel)

width of the slit: 0.2 nm

intensity of the lamp: 4 mA

correction of continuum by the Zeeman effect

introduction in hot conditions of the samples in the graphite oven with an automatic distributor

rinsing water contains 2 drops of Triton per litre.

measurement of signal: peak height.

Time of measurement: 1 second.

pyrolytic graphite tube:

pyrolytic graphite oven containing a platform of

L'Vov tantalised.

tantalisation of a platform: see above.

inert gases: argon and argon + hydrogen mixture (95%: 5%).

INTERNATIONAL CENOLOGICAL CODEX
Nickel

parameters for oven:

Parameters for oven for determining nickel

step n°	temperature (°C)	time (s)	gas flow rate (l/min)	type of gas	reading of signal
1	85	5.0	3.0	argon	no
2	95	40.0	3.0	argon	no
3	120	10.0	3.0	argon	no
4	800	5.0	3.0	argon	no
5	800	1.0	3.0	argon	no
6	800	2.0	0	argon	no
7	2 400	1.1	0	argon + hydrogen	yes
8	2 400	2.0	0	argon + hydrogen	yes
9	2 400	2.0	3.0	argon	no
10	75	11.0	3.0	argon	no

2.2 Adjustment of automatic sampler (given as an example)

- Parameters of automatic sampler

	volume injected in µl		
	solution of Ni at 50 µg/l	blank	matrix modifier
blank		17	3
calibration 1	5	12	3
calibration 2	10	7	3
calibration 3	15	2	3
sample	5	12	3

3. REAGENTS

3.1 Pure demineralised water for analysis

3.2 Pure nitric acid for analysis at 65%

3.3 Anhydrous palladium chloride (59% in Pd)

3.4 Pure hexahydrated magnesium nitrate for analysis

3.5 Ammonium dihydrogenophosphate

3.6 Matrix modifier: mixture of palladium chloride and magnesium nitrate (dissolve 0.25 g of PdCl₂ and 0.1 g of Mg(NO₃)₂·6H₂O (3.4) in 50 ml of demineralised water) ammonium dihydrogenophosphate at 6% (dissolve 3 g de NH₄H₂PO₄ in 50 ml of demineralised water), (3.1).

3.7 L-ascorbic acid

3.8 Analytical blank solution: L-ascorbic acid solution at 1% (m/v).

3.9 Nickel reference solution at 1 g/l (1000 µg/ml) off the shelf or prepared as follows: dissolve 4.9533 g of $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in a solution of HNO_3 0.5 M, adjust at 1 l with HNO_3 0.5 M.

4. PROCEDURE

Nickel solution at 10 mg/l: place 1 ml of the reference solution (3.8) in a 100 ml graduated flask, add 5 ml of nitric acid (3.2); complete to volume with demineralised water.

Nickel solution at 50 µg/l: place 1 ml of the nickel solution at 10 mg/l in a 200 ml graduated flask, 10 ml of nitric acid (3.2) and complete with demineralised water.

Set of calibration solution: 0, 50, 100 and 150 µg/l of nickel.

The automatic distributor cycle enables to perform this calibration on the platform from a nickel solution at 50 µg/l.

5. PREPARATION OF SAMPLES

5.1 Case of liquid or solution samples

No preparation or sample dilution is necessary; the samples are placed directly in the cups of the automatic injector.

5.2 Case of solid samples

The solid samples are mineralised by dry process.

6. DETERMINATIONS

The calibration graph (absorbance depending on the concentration of nickel) gives the concentration of nickel in the samples.

**DETERMINATION OF POTASSIUM
BY ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1. PRINCIPLE

The potassium is determined by mineralisation by dry process by atomic absorption spectrometry.

The addition of a spectral buffer (cesium chloride) to avoid the ionisation of the potassium is necessary.

2. APPARATUS

2.1 Glassware

100 and 200 ml graduated flasks (class A)
1, 2, 4 and 10 ml graduated pipettes (class A)
100 ml cylindrical vase

2.2 Instrumental parameters (given as an example)

atomic absorption spectrophotometer
oxidant air-acetylene flame (flow rate-air: 3 l/min, flow rate-acetylene: 1.8 l/min.)
Hollow-cathode lamp (potassium)
wave length: 769.9 nm
width of the slit: 0.5 nm
intensity of the lamp: 7 mA
no correction of non specific absorption.

3. REAGENTS

3.1 Pure demineralised water for analysis

3.2 Cesium chloride (CsCl)

3.3 Cesium chloride solution at 5% in cesium: Dissolve 6.330 g of cesium chloride in 100 ml of demineralised water.

3.4 Potassium reference solution at 1 g/l commercial or prepared as follows: dissolve 2.5856 g KNO_3 in water, adjust to 1 l.

3.5 Diluted potassium solution at 100 mg/l: Place 10 ml of the potassium reference solution at 1 g/l in a 100 ml graduated flask and 1 ml of pure nitric acid; complete to volume with pure demineralised water for analysis.

3.6 Set of calibration solution at 0, 2, 4, 6 and 8 mg of potassium per litre:

In a series of 100 ml graduated flasks, introduce 0; 2.0; 4.0; 6.0; 8.0 ml of the potassium solution at 100 mg/l ; add 2 ml of the cesium chloride solution to all the graduated flasks; adjust the volume to 100 ml with pure demineralised water for analysis.

The calibration solutions prepared contain 1 g of cesium per litre.

PREPARATION OF SAMPLES

4.1. Liquid or solution oenological products

In a 50 ml graduated flask, place 1 ml of the cesium chloride solution at 5% and a volume of a sample as is after having completed to volume with demineralised water; the concentration of potassium to be measured is below 8 mg/l.

4.2. Solid oenological products

Proceed with mineralisation by dry process (take cinders in 2 ml of hydrochloric acid in a 100 ml flask, add 2 ml of cesium chloride at 5% and complete to volume with demineralised water).

Perform a blank test with demineralised water.

5. DETERMINATIONS

Present successively the calibration solutions.

Perform an absorbance reading for 10 seconds; perform two measurements.

Set up the calibration curve (absorbance depending on the concentration in mg/l of potassium).

Then present the samples, perform an absorbance reading for 10 seconds; perform two measurements.

Calculate the concentration of potassium in the oenological products in mg/kg.

GRAPE SUGAR:

**DETERMINATION OF SACCHAROSE BY HPLC
(OENO 18/2003)**

1. PRINCIPLE

The samples diluted or put in solution are analysed by high performance liquid chromatography: Separation on column of grafted silica NH_2 and detection using a differential refractometer.

2. APPARATUS AND ANALYTICAL CONDITIONS (for example)

2.1 Chromatograph

- Grafted silica column NH_2 (length 20 cm, internal diameter 4 mm granulometry 5 μm)
- A pumping system
- An auto-sampler (maybe)
- Microfiltres with porosity 0.45 μm
- Differential refractometry detector

2.2 Chromatographic conditions (given as an example)

The water used is deionised and microfiltered.

The acetonitrile is of HPLC quality

The composition of the mobile phase is the following:

- If the column is new: acetonitrile/water (75/25)
- When the fructose - glucose resolution starts to deteriorate, the mobile phase is then a acetonitrile/water 80/20 mixture.

The flow is 1 ml/min.

3. REAGENTS AND CALIBRATION SOLUTIONS

3.1 Preparation of the reference solution

The chemicals used for the reference solution preparation are of "pure for analysis" quality.

The composition of this solution is about 10 g/l for each sugar (fructose, glucose and saccharose).

The reference solution is prepared every two weeks (maximum) and stored in the refrigerator in the 100 ml graduated flask used for the preparation.

**DETERMINATION OF SELENIUM BY
ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1. PRINCIPLE

After mineralisation of the sample by wet process, the selenium is determined by atomic absorption spectrometry without flame (electro-thermal atomisation in the graphite oven).

2. APPARATUS

2.1 Glassware

Graduated flasks 50, 100 ml (class A)
Graduated pipettes 1, 5 and 10 ml (class A)
Polypropylene tubes 50 ml with screw top.

2.2 Instrumental parameters: (given as an example)

Atomic absorption spectrophotometer equipped with an atomiser

with a graphite tube.

wave length: 196.0 nm

hollow-cathode lamp (selenium)

width of slit: 1.0 nm.

intensity of the lamp: 10 mA

correction of continuum by the Zeeman effect

introduction in hot conditions of the samples in the graphite oven with an automatic distributor (rinsing water contains 2 drops of Triton per litre).

measurement of signal: peak height

time of measurement: 1 second

number of measurements per sample: 2

Pyrolytic graphite tube:

Pyrolytic graphite oven containing a platform of L'Vov tantalised.

tantalisation of a platform: see given procedure beforehand.

inert gas: argon.

INTERNATIONAL CENOLOGICAL CODEX
Selenium

parameters for oven: table I

Table I - Parameters for oven for determining selenium

step	temperature (°C)	time (s)	gas flow rate (l/min)	type of gas	reading of signal
1	85	5	3.0	argon	no
2	95	40	3.0	argon	no
3	120	10	3.0	argon	no
4	1 000	5	3.0	argon	no
5	1 000	1	3.0	argon	no
6	1 000	2	0	argon	no
7	2 600	0.8	0	argon	yes
8	2 600	2	0	argon	yes
9	2 600	2	3.0	argon	no

2.3 Automatic sampler parameters (table II)
(given as an example)

Table II - Parameters de automatic sampler.

	volumes injected in µl		
	solution	blank	matrix modifier
blank		17	3
calibration n°1 50 µg/l	5	12	3
calibration n°2 100 µg/l	10	7	3
calibration n°3 150 µg/l	15	2	3
sample	15	2	3

3. REAGENTS

3.1 Pure demineralised water for analysis

3.2 Pure nitric acid for analysis at 65%

3.3 Anhydrous palladium chloride (59% in Pd)

3.4 Pure hexahydrated magnesium nitrate for analysis

3.5 Ammonium dihydrogenophosphate

3.6 Matrix modifier: mixture of palladium chloride and magnesium nitrate (dissolve 0.25 g of PdCl₂ and 0.1 g of Mg(NO₃)₂·6H₂O in 50 ml of demineralised water) ammonium dihydrogenophosphate at 6% (dissolve 3 g de NH₄H₂PO₄ in 50 ml of demineralised water).

3.7 Selenium reference solution at 1 g/l, off the shelf or prepared as follows: dissolve 1.4052 g SeO_2 in a solution of HNO_3 0.5 M, adjust at 1 l avec HNO_3 0.5 M.

3.8 Selenium solution at 10 mg/l: place 1 ml of the reference solution at 1 g/l in a 100 ml graduated flask; add 5 ml of nitric acid at 65%; complete to volume with pure demineralised water for analysis

3.9 Selenium solution at 50 $\mu\text{g/l}$: place 0.5 ml of the selenium solution at 10 mg/l, 5 ml of nitric acid at 65% in a 100 ml graduated flask; complete to volume with pure demineralised water for analysis.

3.10 Set of calibration solutions: 0, 50, 100 and 150 $\mu\text{g/l}$ of selenium.

The automatic distributor cycle enables to perform this calibration on the platform from the selenium solution at 50 $\mu\text{g/l}$.

4. PREPARATION OF SAMPLES

Weigh with precision a test sample of 1 to 3 g in the graduated tube; add 5 ml of nitric acid at 65%; close with the screw cap; leave 12 hours at room temperature; place the tube in a water bath at 90°C for 3 hours (the caps are unscrewed during the heating); allow to cool; adjust the volume to 20 ml with pure demineralised water for analysis.

5. DETERMINATIONS

Set up the calibration graph (absorbance depending on the concentration in $\mu\text{g/l}$ of selenium); determine the concentration of selenium in the samples.

Calculate the concentration of selenium in the mineralisate, then in the sample in $\mu\text{g/kg}$.

**DETERMINATION OF SODIUM BY
ABSORPTION ATOMIC SPECTROMETRY
(OENO 18/2003)**

1. PRINCIPLE

The sodium is determined after mineralisation by dry process by atomic absorption spectrometry.

The addition of a spectral buffer (cesium chloride) to avoid ionisation of sodium is necessary.

2. APPARATUS

2.1 Glassware

Graduated flasks 50 and 100 ml (class A)

Graduated pipettes 2.0; 5.0; 10.0 ml (class A)

Automatic pipette 1000 µl

Cylindrical vase 100 ml.

2.2 Instrumental parameters: (given as an example)

Atomic absorption spectrophotometer

oxidant air-acetylene flame (rate-air: 3.1 l/mn; rate-acetylene: 1.8 l/mn)

wave length: 589.0 nm

hollow-cathode lamp (sodium)

width of slit: 0.2 nm

intensity of the lamp: 5 mA

no correction of non specific absorption

3. REAGENTS

3.1 Pure demineralised water for analysis

3.2 Pure nitric acid for analysis at 65%

3.3 Cesium chloride solution at 5% in cesium:

Dissolve 6.330 g of cesium chloride in 100 ml of pure demineralised water for analysis.

3.4 Sodium reference solution at 1 g/l commercial or prepared as follows: dissolve 3.6968 g NaNO₃ in water, adjust at 1 l.

3.5 Diluted sodium solution at 10 mg/l:

Place 1 ml of the reference solution at 1 g/l in a 100 ml graduated flask, 1 ml of nitric acid at 65%, complete to volume with pure demineralised water for analysis.

3.6 Set of calibration solutions 0; 0.25; 0.50; 0.75;

1.00 mg of sodium per litre:

In a series of 100 ml graduated flasks, place 0; 2.5; 5.0; 7.5; 10 ml of the diluted sodium solution; in all the graduated flasks add 2 ml of the cesium chloride solution and adjust the volume at 100 ml with pure demineralised water for analysis.

The calibration solutions prepared contain 1 g of cesium per litre; they are stored in polyethylene flasks.

PREPARATION OF SAMPLES

4.1. Liquid or solution oenological products

In a 50 ml graduated flask, place 1 ml of the cesium chloride solution at 5% and a volume of sample after having been completed to volume with demineralised water, the concentration of sodium to be measured is below at 1 mg/l.

4.2. Solid oenological products

Proceed with a mineralisation by dry process (take up the cinders in 2 ml of hydrochloric acid in a 100 ml flask, add 2 ml of cesium chloride at 5% and complete to volume with demineralised water).

Perform a blank test with demineralised water.

5. DETERMINATIONS

Present successively calibration solutions.

Perform an absorbance reading for 10 seconds; perform two measurements.

Set up the calibration curve (absorbance depending on the concentration in mg/l of sodium).

Then present the samples; determine the concentration of sodium of the diluted samples in mg/l.

Calculate the concentration of sodium in the oenological products in mg/kg.

The dosages of air-acetylene flame are performed manually.

SEARCH FOR SULPHATES
(OENO 18/2003)

In a 160 × 16 mm test tube, place the volume prescribed of the solution obtained by the means indicated in each monography; add 1 ml of diluted hydrochloric acid (R); adjust to 20 ml with water and add 2 ml of barium chloride solution at 10% (R).

Compare the opalescence or any cloudiness to the control sample prepared with 1 ml of solution at 0.100 g of sulphuric acid per litre (i.e. 0.10 mg of H₂SO₄,) with 1 ml of diluted hydrochloric acid (R) and water until volume of 20 ml and 2 ml of barium chloride solution (R). This tube contains 100 µg of H₂SO₄.

TANTALISATION OF PLATFORMS OF L'Vov IN GRAPHITE
(OENO 18/2003)

PREPARATION OF TANTALUM SOLUTION AT 6% (m/v)
ACCORDING TO THE ZATKA PROCESS

Three grammes of tantalum powder are put in a 100 ml Teflon ® cylindrical vase.

Add 10 ml of hydrofluoric acid diluted to a half, 3 g of dehydrated oxalic acid and 0.5 ml of hydrogen peroxide at 30 vol.

Heat carefully to dissolve the metal.

Add a few drops of hydrogen peroxide as soon as the reaction slows down; when the dissolution is complete, add 4 g of oxalic acid and 30 ml of water.

The acid is dissolved and the solution is brought to 50 ml with ultra pure demineralised water.

Store this solution in a plastic flask.

TREATMENT OF GRAPHITE PLATFORMS

The platform is placed inside the graphite tube or used pyrolytic graphite tube. It is set to the unit of atomisation of the spectrophotometer.

A volume of 10 µl of tantalum solution is injected on the platform using an automatic distributor of samples;

Put the tantalum solution in the blank's position on the sample holder.

The temperature cycle is set according to the following programme:

drying at 100°C for 40 seconds

mineralisation at 900°C for 60 seconds

atomisation at 2600°C for 2.5 seconds

argon is used as an inert gas.

REFERENCE:

Zatka, Anal. Chem., vol 50, n° 3, March 1978.

DETERMINATION OF TOTAL NITROGEN
(OENO 18/2003)

1. APPARATUS

1.1 The apparatus used for separating NH_3 is either a distillation apparatus with a rectifying column or a distillation apparatus under a current of steam (diagram) made up of:

A 1 l flask **A** of borosilicate glass used as a boiler with a stopcock funnel for filling. It can be heated by a gas or electric furnace.

An adapter **C** which gathers the spent liquid from the bubbler **B**.

A bubbler **B** of 500 ml with an inclined neck; the supply tube must reach the lowest part of the flask. The out-going tube has an anti-entrainment ball that makes up the top part of the bubbler. A stop-cock funnel **E** allows to introduce the liquid to be treated and alkaline lye.

A cooler 30 to 40 cm long, vertical, with a ball with fine dowel bush on the tip.

A 250 ml conical flask for the distillate.

1.2 Mineralisation flask, 300 ml ovoid-shaped flask with a long neck.

2. REAGENTS

Concentrated sulphuric acid (R).

Mineralisation catalyser (R).

Sodium hydroxide solution at 30% (m/m) (R).

Boric acid solution at 4% (R).

Hydrochloric acid solution 0.1 M.

Mixed-based indicator with methyl red (R) and methylene blue.

The boiler must contain acidulated water by 1 per 1 000 of sulphuric acid. It is advisable to boil this liquid before any operation, with the drain cock P open to let the CO_2 escape.

3. PROCEDURE

In the mineralisation flask, introduce the test sample containing 4 to 50 mg of nitrogen. Add 5 g of mineralisation catalyser (R) and 10 ml of concentrated sulphuric acid (R), if the quantity of dry organic matter to be mineralised is below 500 mg. Increase these quantities if a higher quantity of organic matter must be used.

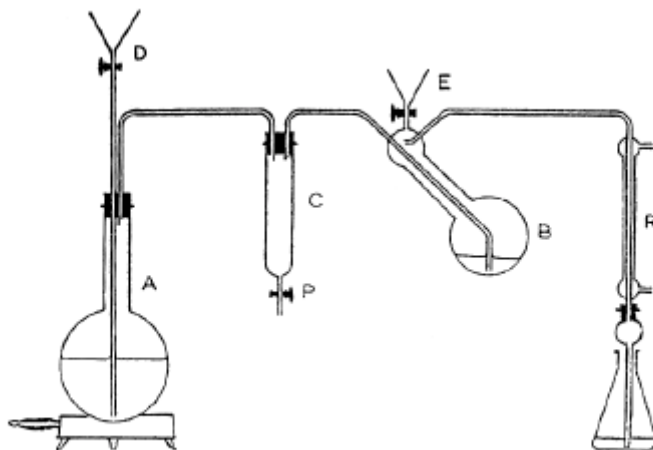
INTERNATIONAL OENOLOGICAL CODEX
Total Nitrogen

Heat in an open flame under a hood. The neck of the flask is maintained inclined until the solution becomes colourless and the walls of the flask are clear of carbonised products.

After cooling, dilute with 50 ml of water and cool; introduce this liquid in the bubbler **B** with the funnel **E**, then add 40 to 50 ml of sodium hydroxide solution at 30% (R) in order to obtain frank alkalisation of the liquid. Entrain the ammoniac with the vapour by gathering the distillate in 5 ml of boric acid solution (R) placed beforehand in a receiving conical flask with 10 ml of water, with the tip of the ampoule plunged into the liquid. Add 1 or 2 drops of mixed-based indicator and gather 70 to 100 ml of distillate.

Titrate the distillate with the hydrochloric acid solution 0.1 M until the indicator turns pink violet.

1 ml of 0.1 M hydrochloric acid solution corresponds to 1.4 mg of nitrogen.



Apparatus for the distillation of ammoniac
in a current of steam (PARNAS and WAGNER)

The cocks **P** and **E** can be replaced by a plastic
pipe fitting with a Mohr pinch-clamp cock.

**DETERMINATION OF ZINC BY
ATOMIC ABSORPTION SPECTROMETRY
(OENO 18/2003)**

1 .PRINCIPLE

The zinc is determined directly by atomic absorption spectrometry by flame.

2. APPARATUS

Instrumental parameters: (given as an example)

atomic absorption spectrometer

oxidant air-acetylene flame

wave length: 213.9 nm

hollow-cathode lamp (zinc)

width of slit: 0.5 nm

intensity of the lamp: 3.5 mA

correction of the non specific absorption with a deuterium lamp.

3. REAGENTS

3.1 Pure demineralised water for analysis

3.2 Pure nitric acid for analysis at 65%

3.3 Zinc reference solution at 1 g/l commercial or prepared as follows: dissolve 4.5497 g of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in a solution of HNO_3 0.5 M, adjust at 1 l with HNO_3 0.5 M.

3.4 Zinc solution at 10 mg/l:

place 1 ml of the zinc reference solution in a 100 ml graduated flask, 1 ml of nitric acid (3.2) and complete to volume with pure demineralised water for analysis.

3.5 Set of calibration solution: 0.2; 0.4; 0.6; 0.8; 1.0 mg/l:

place successively 1, 2, 3, 4, 5 ml of the zinc solution at 10 mg/l in 5, 50 ml graduated flasks, complete to volume with pure demineralised water for analysis.

4. PREPARATION OF SAMPLES

The liquid or solution samples must have concentrations between 0 and 1 mg/l of zinc.

The solid samples are mineralised by dry process.

The blank solution is made up of pure water for analysis containing 1% of nitric acid at 65%.

5. PROCEDURE

Pass successively the blank, the calibration solutions and the samples of oenological products.

The absorbency readings are performed for 10 seconds and the measurements are duplicated.

The concentrations of zinc in the samples are obtained from absorbency values.

METHODS OF MICROBIOLOGICAL ANALYSIS
BACTERIOLOGICAL CONTROL
ANALYSIS COMMON TO ALL MONOGRAPHS
(Oeno 17/2003)

1. Preliminary rehydration of active dry yeasts (ADY)

- weigh 1 g of ADY under sterile conditions;
 - add 100ml of sterile water at room temperature (20°C) under sterile conditions;
- slowly homogenise using a rod and a magnetic stirrer for 5 min;
- stop stirring and allow to stand for 20 minutes at a temperature of 25°C-30°C;
 - homogenise again at room temperature for 5 minutes;
 - take 10 ml under sterile conditions and then proceed with micro-biological controls on the homogenised reference solution.

2. Preliminary rehydration of bacteria

- under sterile conditions weigh 1 g of lactic bacteria,
- under sterile conditions add 100 ml of sterile water at room temperature (25°C),
- homogenise using a magnetic plate for 5 min,
- leave for 20 minutes at room temperature (20°C),
- homogenise for 5 minutes at room temperature (20°C),
- take 10 ml under sterile conditions and proceed with micro-biological controls.

3. Determine number of viable yeasts

3.1 - YM agar medium (MALT WICKERHAM)

Composition:

Bacteriological agar	15 g
Yeast extract	3 g
Malt extract	3 g
Peptone	5 g
Glucose	10 g
Water	q.s.f. 1000 ml

Prior to use, the medium is autoclaved at 120°C for 20 minutes.
After inoculation, the dishes are incubated at 25°C under anaerobic conditions for 48 to 72 hours.
Count the number of CFU and refer to the weight of the dry matter.

3.2 - YMS agar medium

Composition:

Agar	20 g
Glucose	20 g
Yeast extract	5 g
Malt extract	3 g
Peptone	2 g
Malic acid	4g
Grape juice	100 ml
Vitamin complex*	1%
Water	q.s.f. 1000 ml

Prior to use, the medium is autoclaved at 120°C for 20 minutes.
After inoculation, the dishes are incubated at 25°C under anaerobic conditions for 48 to 72 hours.
Count the number of CFU and refer to the weight of the dry matter.

* Vitamin complex (inositol 25 mg, biotin 0.02 mg, Ca pantothenate 4 mg, folic acid 0.002 mg, nicotinamide 4 mg, paraminobenzoic acid 2 mg, pyridoxine hydrochloride 4 mg, riboflavin 2 mg, thiamine 10 mg, water q.s.f. 1000)

3.3 – OGA medium

Composition:

Autolytic yeast extract	5 g
Glucose	20 g
Bacteriological agar agar	15 g
Water	q.s.f. 1000 ml

Autoclavage at 120°C for 20 min.

After inoculation, aerobiosis incubation at 25°C for 48 to 72 hours.
Count the number of CFU and refer to the weight of the dry matter.

4 – Counting of yeasts of a different species of the *Saccharomyces* strain according to the lysine test

Lysine test

The yeasts are cultivated in the medium with lysine whose composition is the following:

Agar	20 g
L-lysine monohydrochloride	5 g
Glucose	1 g
Bromocresol purple	0.015 g
Water	q.s.f. 1000 ml
Adjust	pH 6.8 ± 0.2

After inoculation, the dishes are incubated at 25°C for 48 to 72 hours. Count the number of CFU and refer to the weight of the dry matter.

5. Determination of the number of viable lactic bacteria.

5.1 - MTB/s agar medium

Composition:

Glucose	15 g
Peptone	8 g
Yeast extract	5 g
Casein hydrolysate	1 g
Tomato juice	20 ml
Na acetate	3 g
NH ₄ citrate	2 g
Malic acid	6 g
Mg sulphate	0.2 g
Mn sulphate	0.035 g
Tween 80	1 ml
TC minimal Eagl vitamin	10 ml
after sterilisation	
adjust to pH 5.0 and add	
Agar	2%
Water	q.s.f. 1000 ml

Potassium sorbate (400 mg/l in liquid medium) or
Add directly to the Petri dish 0.2 ml of pimarcine hydroalcoholic solution
at 25% m/v

Sterilisation at 120°C for 20 minutes

Anaerobic incubation to contrast moulds at 25°C for 8 to 10 days.

5.2 - Milieu Man, Rogosa and Sharpe (MRS)

The bacteria are cultivated in a MRS medium (Man, Rogosa, Sharpe 1960) and the composition is as follows:

Agar agar	15 g
Bacto-peptone	10 g
Meat extract	10 g
Yeast extract	5 g
Sodium acetate	5 g
K ₂ HPO ₄	2 g
Trisodium citrate	2 g
MgSO ₄ at 100 mg	2.5 ml
MnSO ₄ at 20 mg	2 ml
Tween 80	1 ml
DL malic acid	5 g
Concentrated tomato juice*	20 ml
Glucose	20 g
Adjust (HCl or NaOH)	pH 4.8
Distilled water	q.s.f. 1000 ml

Autoclave at 120°C for 20 min.

Potassium sorbate (400 mg/l in liquid medium) or

Add directly to the Petri dish 0.2 ml of pimarinic hydroalcoholic solution at 25% m/v.

Anaerobic incubation at 25°C for 8 to 10 days.

*tomato juice is used to improve lactic bacterial growth.

preparation: take canned tomato juice containing at least 7 g/l of NaCl
(maxi 9 g/l)

centrifuge at 4000 g for 20 min;

gather the clear juice and filter through paper filter;

autoclave at 110°C for 20 min.

6. Counting mould

Czapeck-Dox/s gelose medium

Composition:

Agar agar	15 g
Saccharose	30 g
NaNO ₃	3 g
K ₂ HPO ₄	1 g
MgSO ₄	0.5 g
KCl	0.5 g
FeSO ₄	0.01 g
Potassium sorbate	0.4 g
Water	q.s.f. 1000 ml
Adjust	pH 3.5

Sterilisation at 120°C for 20 min.

Add directly to the Petri dish 0.1 ml a 0.25% penicillin solution in pure alcohol.

Aerobic incubation at 20°C for 10 days.

7. Count of acetic bacteria

Act/s agar

Composition:

Bacteriological agar agar	20 g
Yeast extract	5 g
Casein amino acids	5 g
Glucose	10 g
Adjust to	pH 4.5
Water	q.s.f. 1000 ml
Sterilisation	

Aerobic incubation at 25°C for 7 days

Potassium sorbate (400 mg/l in liquid medium) or

Add directly to Petri dish 0.2 ml of pimarcine hydroalcoholic solution at 25% m/v.

8. Count of Salmonella

8.1. Principle

The sample undergoes a pre-enrichment phase in peptoned buffered water for 16 to 20 hours at 37°C. Then the aliquot part of this mixture is inoculated for culture. This contains a specific medium and 2 special tubes (made up of 2 parts) and is incubated 24 hours at 41°C. *Salmonella* migrates from the bottom (selective medium) to the top part of the tube (indicator medium). The presence of *Salmonella* is indicated by a change in colour of this solution.

8.2. Apparatus and analytical conditions

Preparation for culture is carried out in the sterile zone ensured by the Bunsen burner. The soiled material is submitted for destruction by autoclave for 1 hour at 120°C or by total immersion in a bleaching agent for at least 18 hours (See cleaning procedure).

- Sterile glass test tube in 125 ml
- Sterile stomacher bag
- Closing Barrette
- Stomacher
- Sterile glass tubes 16x160 mm.
- Cottoned glass test tubes 20x220
- 2 ml sterile plastic pipettes graduated by 0.1 ml
- 10 ml sterile plastic pipettes graduated by 0.1 ml
- Tube shaker

Method for culture to be rehydrated.

2 ml sterile needle with plastic sterile syringe.

- Tweezer forceps
- Wrench for unscrewing tubes A and B for culture method
- Clean glass slide
- Sterile cottoned Pasteur pipettes
- Monosaccharide
- Oven at 41°C ± 1°C
- Oven at 37°C ± 1°C
- Bunsen burner

8.3. Reagents

Sterile peptoned water (SPW)
Sterile distilled water (SDW)
Sterile 500 ml sealed flask filled with 125 ml of SPW
Sterile 500 ml sealed flask filled with 225 ml of SPW
Special medium for *Salmonella*: SRTEM
Novobiocin disk (1.8 mg of novobiocin)
Hektoën agar agar (see DOMIC-08)
API 20E gallery
Agar agar tubes TSAYE inclined
Sterile NaCl at 8.5 g/l solution
Anti-*Salmonella* serum

8.4. Procedure

8.4.1 Preparation of reference suspension

This differs according to nature of products and dilution rate.
Add a test portion of 25 grams or millilitres of the product in a stomacher bag to a nine fold greater amount of peptoned water.
Close the bag by heat welding or using a barrette.
Grind in a stomacher for 1 minute.

8.4.1.1 Pre-enrichment phase in a non selective liquid medium:

Incubate the reference suspension for 16 to 20 hours at 37°C ± 1°C.

8.4.1.2 Enrichment in selected liquid mediums

Preparation of culture measures

- unscrew the lid of the culture container;
- add SDW up to line 1 as marked on the container.

Note: The base of tubes A and B must be located under water level.

- adjust the needle to the syringe and check that the syringe plunger is pushed in (absence of air);
- vertically introduce the needle to the syringe in the rubber disc in the centre of the stopper in tube A (blue stopper). Check that the needle is visible under the stopper;
- carefully withdraw the syringe up until the liquid reaches line 3 on the container.

Note: Do not draw up liquid into the syringe.

This operation should take approximately 5 seconds.

- Repeat this operation with tube B (red stopper);
- Close the stopper from the culture container tightly;
- Press the side of the recipient on a tube shaker and maintain at least 5 seconds.

Note: the liquid in tubes A and B must be shaken vigorously.

- Let the culture at least 5 minutes;
- Unscrew the culture container's stopper and pour in the SRTEM medium until the level reaches line 2 as marked on the container;
- Add a novobiocin disc using a tweezer forceps;
- Remove the stoppers from tubes A (blue) and B (red) using a wrench, then dispose of the stoppers.

Note: avoid touching the tubes and the inside wall of the container.

Inoculation of culture container

- Homogenise the pre-enriched culture;
- Identify the culture container. Write down the analysis number on the lid.
- Unscrew the lid.
- Using a 2 ml pipette introduce 1 ml of pre-enriched culture in the culture container.
- Tighten the lid on the culture container.
- Write down the incubation time and date.
- Incubate 24 hours \pm 30 min at 41°C \pm 1°C in a strictly vertical position.

8.4.2 Reading and interpretation

This is carried out by observing the top part of tubes A and B through the container walls.

The possible presence of *Salmonella* is characterised by modifications in indicator medium colour located in one or both of the top parts of the tubes:

REACTION	TUBE A	TUBE B
Positive :	All degrees of black colouring	All degrees of red or black colouring
Negative :	Absence of black colouring	Absence of red or black colouring

Tubes showing a positive reaction are subjected to selective agar isolation.

- Dry boxes of Hektoën agar in an incubator at $46^{\circ}\text{C} \pm 1^{\circ}\text{C}$ until the drops on the surface of the medium disappear completely (lid removed and agar surface facing down).
- Take a wire hoop from the positive middle indicator and inoculate it into 5 ml of SPW, in a 16x160 mm sterile glass tube in order to dilute the culture.
- Proceed as such with each positive tube.
- Identify the dish and write down on the lid the number of the analysis and the letter of the tube being confirmed.
- Homogenise the culture and take a wire hoop.
- Isolate the Hektoën agar on the surface to enable the development of isolated colonies.
- Incubate 24 hours at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
- Select at least 2 isolated colonies considered to be typical.

8.4.3. Confirmation

8.4.3.1 Biochemical tests

- Identify the different colonies by using specific miniaturised galleries

(API 20^E gallery) by referring to the recommendations of the manufacturer.

- Incubate 24 hours at 37°C ± 1°C.
- At the same time inoculate: an agar to confirm the purity of the strain.

1 agar TSAYE inclined for serological typing.

- Incubate 24 hours at 37°C ± 1°C.
- Read the API20E gallery following the manufacturer's indications.
- Compare the profile obtained to the standard profiles given by the manufacturer.
- Store TSAYE agar in the refrigerator until utilisation.

8.4.3.2 Serological tests:

Tests are conducted if the strain profile corresponds to *Salmonella* following the recommendations defined by the manufacturer from cultures obtained on agar and after eliminating self-agglutinating strains.

Elimination of self-agglutinating strains:

- Place a drop of 8.5 g/l saline solution on a perfectly clean glass slide.
- Disperse a little bit of the culture removed from the nutritive agar to obtain a homogeneous and cloudy solution using a Pasteur pipette.
- Oscillate the slide for 30 to 60 seconds.
- On a black background using a magnifying glass: if any observation reveals more or less distinct clusters, the strain is considered as being self-agglutinating and should not be subjected to serological typing.

8.5. Results

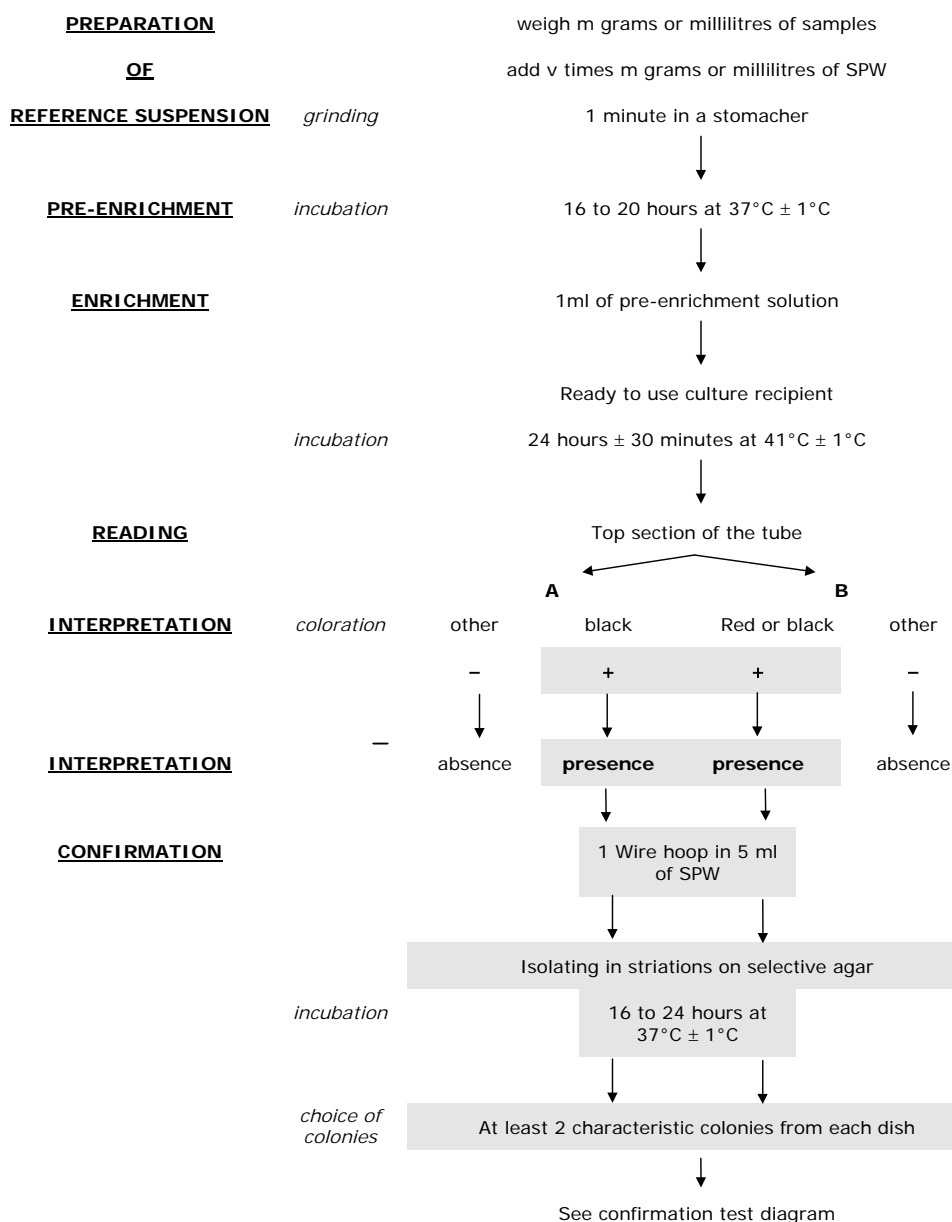
According to the results based on the interpretation of biochemical and serological testing, the results are expressed as follows:

- Presence of *Salmonella* in m number of grams or ml of product.
- Absence of *Salmonella* in m number of grams or ml of product.

INTERNATIONAL ŒNOLOGICAL CODEX

Bacteriological Control

Diagram of procedures



INTERNATIONAL OENOLOGICAL CODEX

Bacteriological Control

Test confirmation diagram

CHOICE OF COLONIES

PURIFICATION

incubation

BIOCHEMICAL IDENTIFICATION

incubation

SEROLOGICAL IDENTIFICATION

subculture

serology

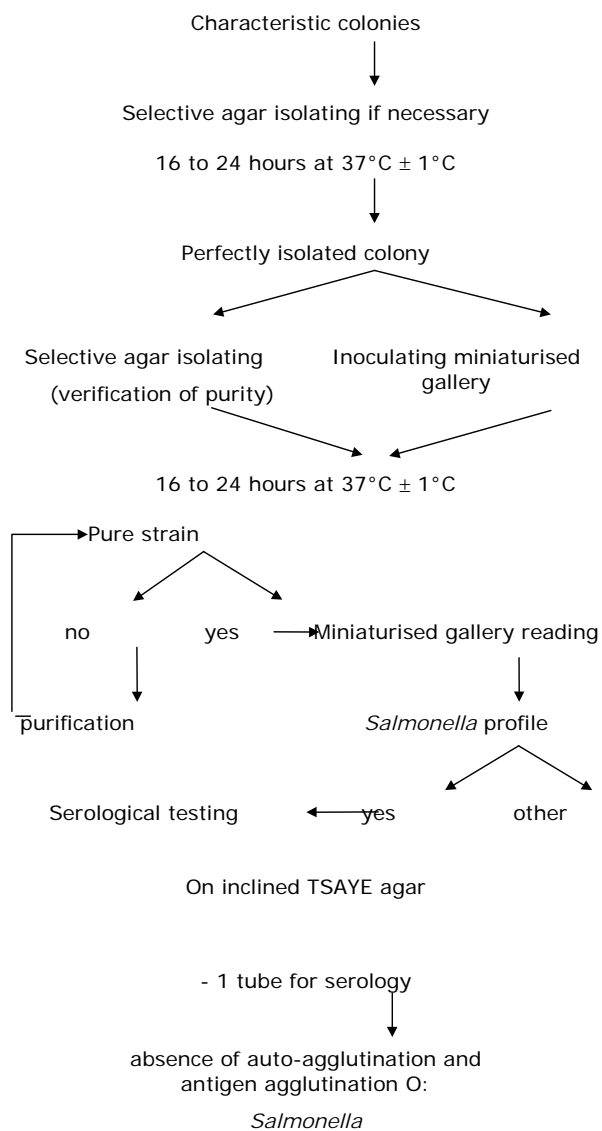


Diagram of biochemical and serological interpretations

Biochemical reactions	Self-agglutination	Serological reactions	Interpretation
Typical	no	"O" positive antigen	<i>Salmonella</i>
Typical	no	Negative reactions	Sent to an authorised centre for determination of the serological type
Typical	yes	Not carried out	

9. Count of *Escherichia coli* by the counting of colonies obtained at 44°C

9.1. Principle

Inoculating rapid E. *coli* agar in depth is carried out in a Petri dish for each of the dilutions chosen. Following a 24 hour incubation at 44°C, all characteristic colonies which appear are counted.

9.2. Apparatus and analytical conditions

Cultures are carried out in a sterility zone ensured by the usage of a Bunsen burner.

Plastic sterile Petri dishes with a diameter of 90 millimetres
Sterile 16x60 cottoned glass test tubes
Tube holder
2 ml plastic sterile pipettes with 0.1 ml graduations
Water bath at 100°C ± 2°C
Water bath at 47°C ± 2°C
Tube shaker
Oven at 44°C ± 1°C
Bunsen burner
Colony counter

9.3. Reagents

Sterile diluent for decimal dilutions: tryptone salt (TS)
16x160 pre-filled sterile tubes with 9ml of sterile TS
Rapid *E. coli* cooling agar (R.E.C)

9.4. Procédure

9.4.1 Agar agar medium

- Melt R.EC agar in a boiling water bath. Avoid overheating.
- Never use a culture medium above 50°C.
- For immediate usage, keep agar in the water bath at 47°C ± 2°C.
- Do not cool over 8 hours.
- For a deferred usage maintain the cooling agar in an oven at 55°C ± 1°C.

- The melted culture medium not used within 8 hours will not re-solidify for another usage.

9.4.2 Culture

- Homogenise each dilution before inoculation in Petri dishes and before carrying out decimal dilutions.
- Transfer 1 ml from the reference solution and/or the retained decimal dilutions in the respective Petri dishes. Change the pipette after each dilution.
- Introduce at least 20 minutes after inoculum, 15 to 20 ml of R.EC maintained in the water bath at $47^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- Slowly homogenise by shaking.
- Let solidify on the bench (lid up).
- Pour 4 to 5 ml of R.EC maintained at $47^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- Let solidify on a bench (lid up).
- Return the dishes and incubate in an oven 24 hours \pm 2 hours at $44^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

9.4.3 Count

Dishes containing between 15 and 150 characteristic colonies of two successive solutions are retained for counting.

If the dish inoculated with 1 ml of first dilution contains characteristic colonies and fewer than 15, it will be retained for counting.

Characteristic colonies are counted using a counter or are counted manually after 24 hours \pm 2 hours of incubation.

9.5 Results

9.5.1 General case

The dishes contain between 15 and 150 characteristic colonies for two successive dilutions.

9.5.1.1 Method of calculation

The two dishes retained have between 15 and 150 characteristic colonies. The number N of counted micro-organisms at 44.5°C per millilitre (ml) or by gram (g) of product is obtained by calculating the weighted mean on 2 dishes retained.

$$N = \frac{\sum c}{1,1d}$$

$\sum c$: sum of characteristics counted on 2 dishes retained

d : rate of dilution corresponding to first dilution

9.5.1.2 Expression of results

- Round off the number N to 2 significant digits
- Express to the tenth power
ex.: $1.6 \cdot 10^3$ / g or ml

9.5.2 Estimation of small numbers

If the dish inoculated with 1 ml of the 1st retained solution for analysis contains at least 15 characteristic colonies, express the result as follows:

$$N = c \frac{1}{d}$$

c : sum of characteristic colonies counted

d : rate of dilution

If the dish inoculated with 1 ml of the 1st retained solution for analysis does not contain any colonies, express the result as follows:

$$N = < 1 \frac{1}{d} \text{ micro-organism per g or ml}$$

d : rate of dilution

10. Count of Staphylococci with a positive coagulase by the counting and confirmation of colonies obtained at 37°C

10.1. Principle

Decimal dilutions and inoculation on the surface of 1 Baird Parker agar drawn previously in a Petri dish with each of the dilutions retained, are carried out simultaneously from the sample (liquid product) or from the reference solution (other products).

After an incubation of 48 hours at 37°C the characteristic and/or non characteristic colonies are counted and then confirmed by the coagulase test.

10.2. Apparatus and analytical conditions

Cultures are carried out in a sterility zone ensured by the usage of a Bunsen burner.

- Sterile glass 16x160 cottoned test tubes
- Sterile plastic precipitating tubes with plastic stoppers
- Tube holder
- 2 ml plastic sterile pipettes with 0.1 ml graduations
- Sterile plastic spreader
- Sterile Pasteur pipettes
- Tube shaker
- Incubate at 37°C ± 1°C
- Bunsen burner
- Colony counter

10.2.1 Reagents

- Sterile diluent for tryptone salt (TS) decimal dilutions.
- 16x160 sterile tubes pre-filled with 9ml of sterile TS.
- Baird Parker agar pre-poured in a Petri dish.
- Tubes pre-filled with 5ml brain heart bouillon (sterile).
- Plasma of lyophilised rabbit rehydrated at the time of use.

10.2.2 Procedure

10.2.2.1 Culture

- Dry the agar plates in an incubator at 46°C ± 1°C until the droplets on the surface of the environment have completely disappeared (cover is removed and the agar surface is turned downwards).

- Homogenise each dilution prior to inoculation of the surface of agar plate surface before carrying out decimal dilutions.
- Place 0.1 ml of reference solution and/or the retained decimal dilutions on the agar surface while changing the pipette after each dilution.
- Carefully spread the inoculum as quickly as possible using a spreader without touching the edges of the plate.
- Leave the plates with the lids closed for 15 minutes at room temperature.
- Incubate 48 hours \pm 2 hours at 37°C \pm 1°C

10.2.2.2 Counting

Dishes containing less than 150 characteristic and/or non-characteristic colonies on two successive dilutions are retained, but one of them must include at least 15 colonies. The characteristic and/or non-characteristic colonies are counted either manually or by using a counter.

Characteristic colonies after 48 hours \pm 2 hours of incubation:

- Black or grey, shiny or convex with at least a 1 mm in diameter and a maximum of 2.5 mm in diameter outlined with lightening and precipitation halos.

Non-characteristic colonies after 48 hours \pm 2 hours of incubation:

- Black and shiny with or without a white edge with lightening or precipitation halos absent or barely visible.
- Grey without light zones.

10.2.2.3 Confirmation

Remove 3 characteristic colonies or 3 colonies of each type (characteristic or non-characteristic) and submit them to the coagulase test.

Coagulase test:

a) Bouillon culture:

- Take part of the selected colony using a Pasteur pipette sterilised with the Bunsen burner flame and inoculate into a brain heart bouillon.
- Repeat this manipulation for other selected colonies.

- Identify the tubes by sample number and its dilution with a blue marker for characteristic colonies and a green marker for non-characteristic colonies.
- Incubate at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 20 to 24 hours $\pm 2\text{H}$.

b) Testing for free coagulase:

- Add 0.5 ml of culture obtained in brain heart bouillon to 0.5 ml of rehydrated rabbit plasma in a sterile precipitating tube and identify as follows.
- Repeat this procedure for each bouillon culture.
- Incubate 4 to 6 hours at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
- Check for the presence of coagulum or examine the tube after 24 hours ± 2 hours of incubation.

10.2.3 Results

Coagulase is considered positive when it occupies $\frac{3}{4}$ of the initial volume of the liquid.

10.2.3.1 General case

The plates contain a maximum of 150 characteristic and/or non-characteristic colonies.

Calculation procedure:

- Number of *Staphylococci* with positive coagulase for each plate: a

$$a = \frac{b^c}{A^c} \times c^c + \frac{b^{nc}}{A^{nc}} \times c^{nc}$$

A^c is the number of spotted characteristic colonies

A^{nc} is the number of spotted non-characteristic colonies;

b^c is the number of characteristic colonies of positive *Staphylococci* coagulase;

b^{nc} is the number of non-characteristic colonies of positive coagulase *Staphylococci*

c^c Is the total number of characteristic colonies of positive coagulase *Staphylococci* for the plate retained;

c^{nc} Is the total number of non-characteristic colonies of positive coagulase *Staphylococci* positive for the plate retained.

Round off the number to the nearest whole number.

- *Number of positive coagulase Staphylococci in trials: N*

The weighted average, calculated as follows from two successive retained solutions:

$$N = \frac{\sum a}{1,1 \times F} \times 10 \text{ positive coagulase Staphylococci by g or ml}$$

$\sum a$: sum of positive coagulase Staphylococci colonies identified on 2 retained plates

F : rate of dilution corresponding to the 1st retained dilution.

Expression of results:

- round off the number N to the two largest whole digits
- express to the tenth power

ex.:	Amount obtained	Amount rounded off	Result
	36364	36000	3.6 10 ⁴

10.2.3.2 Estimation of small numbers:

If the plate inoculated with 0.1 ml of the first dilution retained for analysis contains less than 15 colonies, the result will be expressed as follows:

$$N = a \frac{1}{d} \times 10 \text{ positive coagulase Staphylococci per g or ml}$$

a : number of positive coagulase Staphylococci identified.

d : rate of dilution for the first dilution retained for analyse.

If the dish inoculated with 0.1 ml of the first dilution retained for analysis contains no positive coagulase Staphylococci the result shall be expressed as follows:

$$N < \frac{1}{d} \times 10 \text{ no positive coagulase Staphylococci per g or ml}$$

d : Rate of dilution from the first retained dilution for analysis.

11. Coliform count by counting colonies obtained at 30°C

11.1. Principle

Inoculation in deeply in crystal violet to neutral red (VRBL) lactose bile agar was carried out in Petri dishes for each of the dilutions retained. After incubation for 24 hours at 30°C, the characteristic colonies were counted.

11.2. Apparatus and analytical conditions

Cultures are carried out in a sterile environment as ensured by a Bunsen burner.

- Plastic sterile Petri dishes with a diameter of 90 millimetres
- Sterile glass 16 x 160 cottoned tubes
- Tube holder
- 2 ml plastic sterile pipettes graduated at 0.1 ml
- Water bath at 47°C ± 2°C.
- Tube shaker
- Incubate at 30°C ± 1°C
- Incubate at 55°C ± 1°C
- Bunsen burner
- Colony counter

11.3. Reagents

- Sterile diluent for decimal dilutions: tryptone salt (TS)
- 16 × 160 sterile tubes pre filled with 9ml of sterile TS
- Cooled crystal violet and neutral red lactose bile agar (VRBL).

11.4. Procedure

11.4.1 Agar medium

- Once prepared, keep the VRBL agar cooled in the water bath at 47°C ± 2°C (for immediate usage).
- Never use a culture medium at a temperature higher than 50°C.
- Do not cool over 8 hours.
- For a deferred usage, keep agar cooled in an incubator at 55°C ± 1°C.
- Melted culture mediums unused within 8 hours, shall never re-solidify for later usage.

11.4.2 Culture

- Homogenise each dilution before inoculating in Petri dishes prior to carrying out decimal dilutions.
- Transfer 1 ml of reference solution and/or decimal dilutions retained in respective Petri dishes with pipettes changed after each dilution
- Introduce up to 20 minutes after the inoculum 15 to 20 ml of VRBL maintained in the water bath at $47^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- Slowly homogenise by shaking.
- Let solidify on laboratory bench (lid upwards).
- Pour approximately 5 ml of VRBL maintained in the water bath at $47^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
- Let solidify on laboratory bench (lid upwards).
- Turn over dishes and incubate immediately 24 hours \pm 2 hours at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

11.4.3 Count

Dishes containing less than 150 characteristic or non-characteristic colonies based on two successive dilution are retained, but one of them must contain at least 15 characteristic colonies.

If only the dish inoculated with 1 ml of the 1st dilution contains under 15 characteristic colonies, then the dish will be retained for counting.

Characteristic colonies are counted manually or by using a counter.

Characteristic colonies after 24 hours \pm 2 hours of incubation

- violet colonies surrounded sometimes by a red area (bile precipitation)
- diameter ≥ 0.5 mm

11.5. Results

11.5.1 General case

Dishes containing less than 150 characteristic or non-characteristic colonies, based on two successive dilutions with one containing at least 15 characteristic colonies.

Method of calculation:

Number N of micro-organisms counted at 30°C per millilitre (ml) or by gram (g) of product is obtained by calculating the weighted average of 2 retained dishes.

$$N = \frac{\sum c}{1,1d}$$

$\sum c$: sum of characteristic colonies counted of 2 retained dishes

d : dilution rate corresponding to the 1st dilution

Expression of results:

- round off the number N to the 2 largest digits
 - express to the tenth power
- ex: $1.6 \cdot 10^3$ / g or ml

11.5.2 Estimation of small numbers

If the dish inoculated with 1 ml of the 1st dilution retained for analysis contains less than 15 characteristic colonies, the result will be expressed as follows:

$$N = c \frac{1}{d}$$

c : sum of characteristic colonies counted

d : rate of dilution

If the dish inoculated with 1ml of the 1st dilution retained for analysis contains no colonies then the result will be expressed as follows:

$$N = < 1 \frac{1}{d} \text{ micro-organisms per g or ml}$$

d : rate of dilution.

Chapter III

**Reagents and
Titrated Solutions**

List of reagents and titrated solutions¹
Mention (R)²
(Oeno 19/2003)

Acetic	crystallisable acid 98-100% diluted acid (10% m/m) neutral lead acetate (see Lead) potassium acetate (see Potassium) sodium acetate (see Sodium) uranyl and magnesium acetate
Starch	Paste (aqueous solution at 5 g/l)
Ammonium	concentrated hydroxide solution (20% NH ₃ , d(20/4)=0.92 diluted hydroxide solution (10 g concentrated solution/100 g) Aqueous hydroxide solution about 5 M chloride in solution at 20% (m/m) citrate in solution oxalate in solution at 4% (m/m) persulphate in solution at 15% (m/m)
Aniline	reagent
Silver	Nitrate (99.5%) nitrate solutions at 5% (m/m) (R1) Nitrate solution at 1% (m/m) (R2) ammonia nitrate solution
Barium	BaCl ₂ ·2H ₂ O solution at 10% (m/m) chloride
Bore	boric acid, H ₃ BO ₃ 99% concentrated boric acid solution at 4% (m/v)
Bromine	Br ₂ (d(20/4)=3.12) bromine water

¹ this list does not contain the titrated acid solutions, sodium hydroxide, iodine, silver nitrate, etc.

² The composition of reagents "(R As)" is indicated for determining arsenic.

INTERNATIONAL OENOLOGICAL CODEX
Reagents and titrated solutions

Bromophenol	tetrabromophenolsulfonephtaleine (blue) alcoholic solution (blue)
Bromothymol	dibromothymolsulfonephtaleine (blue) alcoholic solution (blue)
Bromocresol	tetrabromo-m-cresol-sulfonephtaleine (green) (green) alcoholic solution methyl red and (green) in solution (mixed indicator)
Calcium	acetate aqueous solution at 25% (m/v) chloride saturated solution chloride solution at 20% (m/v) hydroxide (milk of lime) saturated sulphate solution
Mineralisation catalyser	
Chloramine T	solution at 1% (m/v)
Chlorine	concentrated hydrochloric acid at 35% (d(20/4)=1.19) hydrochloric acid diluted at 30% (v/v) hydrochloric acid diluted at 10% (m/m) hydrochloric acid diluted at 10% (v/v) potassium dichromate (see Potassium)
Chrome	potassium dichromate (see Potassium)
Chromotropic acid	sodium salt sodium salt solution
Citric acid	Monohydrated 99% aqueous solution at 21% (m/m) aqueous solution at 20% (m/v) aqueous solution at 10% (m/v) aqueous solution at 5% (m/v) aqueous solution 0.003 M hydrochloric solution solution adjusted to pH 3

INTERNATIONAL CENOLOGICAL CODEX
Reagents and titrated solutions

Cobalt	chloride $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ aqueous solution at 5% (m/m)
Copper	sulphate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ sulphate solution at 1 g of copper per litre sulphate solution at 0.01 g of copper per litre copper sulphate ammonia solution (II) alkaline copper reagent
Dichlorophenolindo phenol	sodium salt of 2,6-dichlooro-N-(4- hydroxyphenyl)-1,4-benzoquinone monoimine dihydrate aqueous solution at 0.5 g per litre
Diphenylcarbazine	1,5-diphenylcarbonodihydrazide at 0.5 g per litre of alcoholic solution at 95% vol.
Dithizone	1,5-diphenylthiocarbazone solution at 0.5 g/l in chloroform extemporaneous preparation
Iron	iron sulphate (II) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 99% iron sulphate (II) solution at 5% (m/m) iron sulphate (II) and ammonium $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ 98.5% iron sulphate (II) solution and ammonium at 10% (m/m) iron sulphate (III) $\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$ solution at 0.01 g of iron (III) per litre
Formaldehyde	aqueous solution at 35% (m/m)
Basic fuchsine	Mixture of rosaniline hydrochloride and pararosaniline hydrochloride Solution bleached by sulphur dioxide
Hydrazine	dihydrochloride aqueous solution
Hydrogen peroxide	concertrated solution at 30% (m/m) (=110 volumes) diluted solution 3% (m/m) (10 vol.)
Iodine	99.5% iodine solution

INTERNATIONAL OENOLOGICAL CODEX
Reagents and titrated solutions

Sodium indigo-sulphonate	(see sodium)
Mixed indicator	(see methyl red)
Magnesium	chloride $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 99% magnesian mixture
Mercury	mercury oxide (II), yellow mercuric oxide 99% mercury sulphate (II) solution
Metaphenylene-diamine	(see m-phenylenediamine)
Methyl (red)	(see red methyl)
Methyl orange	sodium 4(dimethylamino)azobenzene-4-sulfonate. Methyl orange alcoholic solution at 1% (m/v)
Molybdenum	reagent (see Nitric)
Naphthol	β -naphthol (2-naphtol) solution at 5% (m/m)
Nitric	concentrated acid 63% acid diluted at 10% (m/m) nitromolybdic reagent nitro-vanadomolybdic reagent lead nitrate (see Lead)
Eriochrome black T	Biting black 11 solution at 0.2% (m/v) in triethanolamine
Oxalic acid	acid $\text{C}_2\text{O}_4\text{H}_2 \cdot 2 \text{H}_2\text{O}$ 99% aqueous solution at 5% (m/m)
m-Phenylenediamine	dihydrochloride $\text{C}_6\text{H}_8\text{N}_2 \cdot 3 \text{HCl}$ 99%
Phenol (red)	(see phenol red)
Phenolphthalein	phenolphthalein solution at 1% in alcohol (m/v)

INTERNATIONAL OENOLOGICAL CODEX
Reagents and titrated solutions

Phosphorus	concentrated phosphoric acid (orthophosphoric acid) 85% $d(20/4)=1.7$ Diluted solution of phosphoric acid at 50% (m/m) Diluted solution of phosphoric acid at 25% (m/v) Dihydrogenophosphate (see Potassium)
Lead	neutral lead acetate $C_4H_6O_4Pb \cdot 3H_2O$ aqueous solution at 10% (m/m) (in water free from carbon dioxide) nitrate $Pb(NO_3)_2$ 99% lead nitrate aqueous solution at 1 g of lead per litre lead nitrate aqueous solution at 0.01 g of lead per litre
Potassium	acetate $C_2H_3KO_2$ 99% aqueous solution at 5% (m/m) anhydrosulphite $K_2S_2O_5$ (disulphite) 94% free from selenium potassium anhydrosulfite aqueous solution at 2% (m/m) cyanide KCN 98% aqueous solution at 10 g per 100 ml potassium cyanide aqueous solution at 1 mg of hydrocyanic acid per litre dichromate $K_2Cr_2O_7$ 99% aqueous solution at 10% (m/m) aqueous solution at 1 g of chromium per litre aqueous solution at 0.01 g of chromium per litre dihydrogenophosphate H_2KPO_4 99% aqueous solution at 0.05 g of phosphorous per litre hexacyanoferrate (II) $K_4Fe(CN)_6 \cdot 3H_2O$ 98% aqueous solution at 5% (m/m) hydroxide KOH 85% aqueous solution at 40% (m/m); $d(20/4) = 1.38$ iodide KI 99% iodine potassium iodide solution permanganate $KMnO_4$ 99%

INTERNATIONAL CENOLOGICAL CODEX
Reagents and titrated solutions

	aqueous solution at 5% (m/m)
	aqueous solution at 3% (m/m)
	aqueous solution at 2% (m/m)
	aqueous solution at 1% (m/m)
	aqueous solution at 0.5% (m/m)
	aqueous solution at 0.2% (m/m)
	potassium permanganate phosphoric solution
	saturated aqueous solution
	thiocyanate KSCN 99%
	aqueous solution at 5% (m/m)
Pyridine-pyrazolone	reagent
Quinine	sulphate $C_{40}H_{48}N_4O_4 \cdot H_2SO_4 \cdot 2 H_2O$ 99%
	quinine sulphate sulphuric solution at 0.1 mg
	per litre of sulphuric acid 0.05 M
Rosaniline	hydrochloride (see fuchsine)
	aqueous solution at 0.1 g per 100 ml
Methyl red	acid 4-dimethylamino-2-phenylazobenzoic
	red methyl alcoholic solution
	methyl red mixed indicator
Phenol red	phenolsulfonephtaleine 98%
	phenol red solution
Selenium	dioxide SeO_2 99%
	aqueous solution at 100 mg of selenium per
	litre
Sodium	acetate $C_2H_3NaO_2 \cdot 3 H_2O$
	aqueous solution at 10% (m/m)
	borate (tetraborate) $Na_2B_4O_7 \cdot 10 H_2O$ 99%
	saturated aqueous solution
	decahydrate carbonate $Na_2CO_3 \cdot 10 H_2O$ 99%
	aqueous solution at 25% (m/m)
	diethyldithiocarbamate $C_5H_{10}NS_2Na \cdot 3 H_2O$
	99%
	alcoholic solution at 1% (m/v)
	ethylenediaminetetracetate (disodic edetate)
	$C_{10}H_{14}N_2O_8Na_2 \cdot 2 H_2O$ 98.5%
	aqueous solution 0.01 M
	fluoride NaF 98.5 %

INTERNATIONAL OENOLOGICAL CODEX
Reagents and titrated solutions

	aqueous solution at 4% (m/m) concentrated hydroxide solution (caustic soda) at 30% (m/m); $d(20/4)=1.33$ diluted aqueous solution of sodium hydroxide at 10% (m/m) hydrogenophosphate (disodic dihydrate phosphate) $\text{HNa}_2\text{PO}_4 \cdot 2 \text{H}_2\text{O}$ 99.5% aqueous solution at 10% (m/m) pyrophosphate $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$ (diphosphate decahydrate tetrasodium) 98% aqueous solution at 1% (m/m) thiosulphate $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5 \text{H}_2\text{O}$ 99% aqueous solution at 25% (m/v) disodic indigo-sulphonate (see indigo carmine) indigo carmine solution
Hydrogen sulphide	saturated aqueous solution acid aqueous acid solution at 1 g of sulphur per litre aqueous acid solution at 0.01 g of sulphur per litre
Sulforesorcinic Sulphuric	reagent concentrated acid 95% $d(20/4)=1.83$ concentrated acid 97% (m/m) aqueous solution at 25% (m/m) aqueous solution diluted at 10% (m/m) aqueous solution diluted at 5% (m/m) acid free from nitrogen
Buffers	purified acetate (search for zinc) ammoniac pH 7.5
Tannin	definition aqueous solution at 2% (m/m) aqueous solution at 4% (m/v) aqueous solution at 10% (m/m)
Thioacetamide	reagent
Uranyl	nitrate $\text{UO}_2(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$ 99% aqueous solution at 4% (m/m)

INTERNATIONAL OENOLOGICAL CODEX
Reagents and titrated solutions

uranyl acetate $\text{UO}_2(\text{CH}_3\text{COO})_2 \cdot 2 \text{H}_2\text{O}$ 99%
magnesium and uranyl acetate and acetate
solution

Bromocresol green (see Bromocresol)

Bromocresol green and (see Bromocresol)
methyl red

Zinc Solution 1 mg per litre

REAGENTS AND TITRATED SOLUTIONS

Crystallisable acetic (acid)

ρ_{20} = 1.051; contains as a minimum 98.0% (m/m) of $C_2H_4O_2$.

Diluted acetic (acid)

Aqueous solution containing about 10 g of acetic acid in 100 g of reagent.

ρ_{20} = 1.0125 approximately.

Starch (paste) at 0.5% (m/v)

In a mortar, grind 2.5 g of soluble starch and 10 mg mercury iodide (II) with the necessary amount of water in order to obtain a fluid slurry. Introduce this in 500 ml of boiling water that is maintained 10 minutes. The liquid obtained is clear. Filter if necessary.

Concentrated ammonium hydroxide solution

ρ_{20} = 0.922.

Concentrated aqueous solution of ammonia gas containing about 20 g of ammonia (NH_3) in 100 g of reagent.

Diluted ammonium hydroxide solution

Aqueous solution of ammonia gas containing about 10 g of ammonia (NH_3) in 100 g of reagent.

ρ_{20} = 0.959 approximately.

Ammonium (chloride) in solution

Aqueous solution containing 20 g of ammonium chloride in 100 g of reagent.

Ammonium (citrate) in solution

Slowly pour 500 ml of concentrated ammonium hydroxide solution (R) in 400 g of citric acid in a 1000 ml graduated flask. The mass is heated and the dissolution is carried out. After cooling, complete the volume of 1000 ml with concentrated ammonium hydroxide (R).

Ammonium (hydroxide) in solution about 5 M

Dilute 460 ml of concentrated ammonium hydroxide (ρ_{20} = 0.922) with a sufficient amount of water to obtain 1 l.

Ammonium (oxalate) in solution at 4% (m/m)

Aqueous solution containing 4 g of diammonium oxalate in 100 g of solution.

Ammonium (persulphate) in solution at 15% (m/m)

Aqueous solution containing 15 g of ammonium persulphate for 100 g of solution.

Aniline

$C_6H_5NH_2 = 93.1$.

The product used as a reagent must be clear and barely yellow.

$\rho_{20} = 1.020$ to 1.023 .

During distillation, 95% as a minimum must pass between 183°C and 185°C .

Silver (nitrate) in solution at 5% (m/m)

Aqueous solution containing 5 g of desiccated silver nitrate for 100 g of reagent.

Silver (nitrate) in solution at 1% (m/m)

Aqueous solution containing 1 g of desiccated silver nitrate for 100 g of reagent.

Silver (nitrate) in ammonia solution

Ammonia solution prepared with 10 g of desiccated silver nitrate for about 100 g of reagent.

In 30 g of distilled water, dissolve 5 g of desiccated silver nitrate. Pour into this solution, drop by drop with caution, the diluted ammonium hydroxide solution (R) until nearly total redissolution of the precipitated silver oxide. Complete to 50 ml, filter and store the reagent away from light in a flask with a glass stopper.

Barium (chloride) in solution at 10% (m/m)

Aqueous solution containing 10 g of $BaCl_2 \cdot 2H_2O$, for 100 g of reagent.

Concentrated boric (acid) in solution at 40 g per litre

This acid must be pure, entirely soluble in water (insoluble residue below 50 mg for 1 kg) and must not turn brown during incineration (absence of organic matters).

The aqueous solution at 40 g for 1 l of solution must be neutral to methyl orange. The orange coloration of this indicator must be obtained with less than 3 ml of hydrochloric acid solution 0.1 M for 1 l of this solution at 40 g per litre.

Boric acid that does not respond to these test trials can be purified by hot filtration of a boiling, saturated boric acid solution (at about 350 g per litre of water) and crystallisation by cooling.

Prepare a solution of 40 g of this concentrated acid for 1 l of solution.

Bromine (water)

Bromine saturated aqueous solution containing about 3.5 g of bromine for 100 ml at 20°C.

Bromophenol blue in solution

Alcohol solution at 95% vol. containing 0.04 g of bromophenol blue in 100 ml in total.

Bromothymol blue in solution

Alcohol solution at 95% vol. containing 0.04 g of bromothymol blue in 100 ml in total.

Calcium (acetate) at 25% (m/v)

Calcium acetate aqueous solution at 25 g for 100 ml.

Calcium (acetate) in solution pH 6

In a cylindrical vase place:

- calcium carbonate	10 g
- acetic acid	12 g
- water	100 ml

Heat until dissolution, adjust the pH to 6 and adjust to 1 l.

Calcium (chloride) in saturated solution

It contains about 80 g of $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ for 100 g of solution.

Calcium (chloride) in solution at 20% (m/v)

Aqueous solution containing 20 g of crystallised calcium chloride $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ in 100 ml of reagent.

Calcium hydroxide (milk of lime) at 10% (m/m)

The calcium hydroxide suspension (milk of lime) is obtained by treating 10 g of calcium oxide (quicklime) with 90 g approximately of boiling water.

Calcium (sulphate) in saturated solution

Saturated aqueous solution; it contains about 0.2 g of CaSO_4 for 100 g.

Mineralisation catalyser

Pulverise and mix:

- selenium	2.5 g
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INTERNATIONAL OENOLOGICAL CODEX
Reagents and titrated solutions

- copper sulphate (II)	5 g
- dipotassic sulphate	100 g

Chloramine T solution at 1% (m/v)

Aqueous solution containing 1 g of chloramine T (sodium salt of *p*-toluene N-chlorosulphanomide) for 100 ml of reagent.

Concentrated hydrochloric (acid)

Aqueous solution of hydrochloric acid ($\rho_{20} = 1.18$ to 1.19) containing 35.5 to 37.25 g of hydrochloric acid (HCl) in 100 g or 100 ml.

Hydrochloric (acid) diluted at 30% (v/v)

Dilute 300 ml of concentrated hydrochloric acid ($\rho_{20} = 1.19$) with a sufficient amount of water to obtain 1 l.

This solution contains about 13 g of HCl for 100 ml.

Hydrochloric (acid) diluted at 10% (m/m) ($\rho_{20} = 1.0489$)

Aqueous solution containing 10 g of hydrochloric acid (HCl) in 100 g.

Hydrochloric (acid) diluted at 10% (v/v)

Aqueous solution of hydrochloric acid containing about 10 ml of concentrated hydrochloric acid (R) in 100 ml, i.e. about 3.6 g HCl for 100 ml.

Chromotropic (acid)

1.8-dihydroxy-3.6-naphthalene-1.6-disulphonic acid
($C_{10}H_8O_8S_2 \cdot 2H_2O = 356.3$).

White powder that turns brown in light, soluble in water. The disodium salt of this acid is generally used which is a yellow or light brown product and very soluble in water.

Chromotropic acid solution (sodium salt) at 0.05% (m/v)

Dissolve 60 mg of sodium salt of chromotropic acid in about 80 ml of water, complete to 100 ml with water. To be used within 24 hours.

Citric (acid) in solution at 21% (m/m)

Aqueous solution at 21 g for 100 g.

Citric (acid) in solution at 20% (m/v)

Aqueous solution of citric acid at 20 g for 100 ml.

Citric (acid) in solution at 10% (m/v)

Aqueous solution of citric acid at 10 g for 100 ml.

Citric (acid) in solution at 5% (m/v)

Aqueous solution of citric acid at 5 g for 100 ml.

Citric (acid) in solution 0.033 M

Solution containing exactly one tenth of the equivalent of a gramme of monohydrated citric acid per litre (i.e. 7.003 g per litre).

Citric (acid) in hydrochloric solution

Dissolve 150 g of concentrated monohydrated citric acid in 800 ml of water; add 100 ml of concentrated hydrochloric acid and add volume to 1 l.

Citric (acid) in solution to 5 g per litre adjusted to pH 3

Dissolve 5 g of citric acid in 900 ml of water. Add 8 ml of the sodium hydroxide solution 1 M and adjust to 1 l.

Cobalt (chloride) in solution at 5% (m/m)

Solution containing 5 g of cobalt chloride $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ in 100 g of reagent.

Copper (II) (sulphate) solution at 1 g and 0.01 g per litre

The aqueous solution at 1 g of copper per litre contains 3.9295 g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and 1 ml of concentrated sulphuric acid per litre. This solution is diluted to a hundredth to obtain the solution at 0.01 g of copper per litre.

Copper (sulphate) in ammonia solution

Copper sulphate $\text{Cu SO}_4 \cdot 5\text{H}_2\text{O}$	5 g
Water	500 ml
Concentrated ammonium hydroxide (R)	300 ml

Dissolve the copper sulphate in water. Add the ammonium hydroxide and homogenise.

Alkaline copper (reagent)

The titrated alkaline copper reagent contains for 1000 ml:

Copper, Cu	4.454 g
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It is obtained by mixing the two solutions:

a) Copper solution (II), (C)

Weigh exactly 35 g of copper sulphate (R) and introduce into a 1000 ml graduated flask with about 500 ml of distilled water and 5 ml of concentrated sulphuric acid (R). Shake to dissolve and complete to 20°C with distilled water until the graduated line. Mix.

b) Alkaline tartaric solution, (T)

Weigh 150 g of sodium and potassium L-tartrate (R) and introduce in a 1000 ml graduated flask containing about 500 ml of hot distilled water. Shake to dissolve. Allow to cool and add 300 ml of concentrated sodium hydroxide solution (R) non carbonated.

Complete to 20°C with distilled water the volume of 1000 ml of solution. Mix.

10 ml of the solution C with 10 ml of solution T are brought to the boil with 0.05 g of inverted sugar, 0.048 g of pure glucose and 0.0695 g of anhydrous lactose or 0.073 g of hydrated lactose.

2.6-dichlorophenolindophenol in solution

Dissolve 0.50 g of 2.6-dichlorophenolindophenol in 200 ml of water heated at 90°C. Allow to cool and complete to 1000 ml with water. Filter.

Diphenylcarbazide in solution

Solution of 0.50 g of diphenylcarbazide in 1 l of alcohol at 95% vol.

Peroxide in diluted solution

See Hydrogen (peroxide).

Iron (II) (sulphate) in solution at 5% (m/m)

Solution prepared extemporaneously with boiled distilled water containing 5 g of iron sulphate (II) $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 100 g of reagent (air oxidises it quickly).

Iron (III) (sulphate) in saturated solution

Prepare a saturated solution of iron sulphate (III) $\text{Fe}_2(\text{SO}_4)_3 \cdot 7\text{H}_2\text{O}$.

Iron (II) (and ammonium sulphate) in solution at 10% (m/m)

Aqueous solution containing 10 g of ammonium and iron sulphate (II) in 100 g of reagent.

Iron (III) (salt) in solution at 0.010 g of iron per litre

Dissolve 0.1 g of pure iron in 20 ml of water and 5 ml of concentrated H_2SO_4 (R). Heat, add 10 drops of concentrated HNO_3 (R) and bring to the boil for 10 minutes to peroxidise the iron. Adjust the volume to 1 l. Dilute 1/10.

Formaldehyde in solution

Aqueous solution with 35% (m/m) of formaldehyde.

Fuchsine bleached by sulphurous acid

8 g of potassium anhydrosulphite are dissolved in 150 ml of distilled water; add 30 ml of basic fuchsine solution at 1 per 1000 (m/v) in alcohol at 95% vol. and 55 ml of hydrochloric acid 3 M. Complete to 250 ml with distilled water. Store in a yellow flask with an emery stopper.

Hydrazine (dichlorhydrate) in solution

Hydrazine dichlorhydrate	500 mg
Water	q.s.f. 100 ml

Dissolve the hydrazine dichlorhydrate in about 80 ml of water, then adjust the volume to 100 ml.

Reagent to be prepared extemporaneously.

Hydrogen (peroxide) in solution to 3 volumes

This solution contains 9.1 g of H_2O_2 per litre; it liberates 3 times its volume of oxygen by catalytic decomposition by MnO_2 in an alkaline medium.

Iodine (solution)

Aqueous solution saturated with iodine.

Sodium indigo-sulphonate

Indigo-disulphonate sodium salt (improperly called indigo carmine): $\text{C}_{16}\text{H}_8\text{O}_8\text{S}_2\text{N}_2\text{Na}_2$

This product in solution at 10% (m/v) should turn yellow when oxidised by the potassium permanganate in a sulphuric environment; 50 ml of this solution requires 14 ml to 17 ml of potassium permanganate solution 0.02 M.

If, by permanganic oxidation, this solution does not turn yellow, it is advisable to purify the sodium indigo-sulphonate by the following process:

Put 10 g of sodium indigo-sulphonate in contact with 50 ml of concentrated sulphuric acid (R). After two days, add 100 ml of water; filter the day after. Reject the rusty-coloured filtrate. Take up the residue with 100 ml of water, reject again the filtrate. Dissolve the residue with 800 to 1000 ml of acidulated water and 5 ml of concentrated sulphuric acid (R).

Indigo carmine solution: dissolve 0.2 of indigo carmine in a mixture of 10 ml of hydrochloric acid (R) and 990 ml of sulphuric acid solution free of nitrogen (R) to 200 g per litre.

Magnesium (chloride) in solution 0.01 M

Dissolve 0.45 g of pure magnesium oxide MgO in the necessary quantity of diluted hydrochloric acid (R). Bring to a litre. Titrate this solution using a sodium ethylenediaminetetraacetate solution 0.01 M in the presence of eriochrome black T.

Magnesian (mixture)

Dissolve 82 g of magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) and 100 g of ammonium chloride in 800 ml of water. Add 400 ml concentrated ammonium hydroxide ($\rho_{20} = 0.92$) (R). Mix.

Mercury (II) (sulphate) in acid solution

Aqueous solution and mercury sulphate acid (II) HgSO_4 . In a 200 ml graduated flask, introduce 10 g of yellow mercuric oxide, 120 ml of water and 75 g of concentrated sulphuric acid (R) (40 ml). After cooling, adjust the volume to 200 ml.

Metaphenylene-diamine (hydrochloride)

Grey-mauve amorphous powder: $\text{C}_6\text{H}_8\text{N}_2 \cdot 2\text{HCl}$.

Methyl orange in solution

Solution prepared with alcohol at 90% vol. containing 1 g of methyl orange in 100 ml of reagent.

β -naphthol in solution at 5% (m/m)

Dissolve 5 g of β -naphthol in 40 ml of concentrated ammonium hydroxide solution (R) and adjust the volume to 100 ml with distilled water. Prepare extemporaneously.

Concentrated nitric (acid)

$\rho_{20/4} = 1.39$

Concentrated nitric acid contains about 63% of nitric acid (HNO_3).

Diluted nitric (acid)

$\rho_{20/4} = 1.056$

Solution containing about 10 g of nitric acid (HNO_3) in 100 g of reagent prepared with 15.8 g of nitric acid (11.35 ml) ($\rho_{20} = 1.39$) at 63 g for 100 g and 84.2 g of water.

Nitromolybdic (reagent)

Dissolve 60 g of ammonium molybdate in 200 g of warm water. Filter if necessary. Slowly pour this solution in 720 g of diluted nitric acid while constantly shaking the latter. This diluted acid is obtained by

mixing 370 g of concentrated nitric acid (R) with 350 g of water. Allow to stand 8 days. Adjust the volume to 1000 ml with distilled water. Filter or decanter.

This reagent, heated at 40°C, should not leave a deposit of precipitate.

Sensitivity: 25 µg of phosphorus for 5 ml.

Nitro-vanado-molybdic (reagent)

Prepare the following solutions

A) Solution of ammonium molybdate

Ammonium molybdate	100 g
Concentrated ammonium hydroxide (R)	10 ml
Distilled water	q.s.f. 1000 ml

B) Solution of ammonium vanadate

Ammonium metavanadate	2.35 g
Distilled water	500 ml

Slightly heat to dissolve. After complete dissolution, cool and gradually add while shaking the following mixture:

Concentrated nitric acid (R)	7 ml
Distilled water	13 ml

Complete the volume to 1000 ml with distilled water. Mix.

To obtain the nitro-vanado-molybdic reagent, mix in a 500 ml graduated flask 67 ml of concentrated nitric acid (R), 100 ml of molybdic solution (A), 100 ml of nitro-vanadic solution (B) and adjust the volume to 500 ml. Mix.

Eriochrome black T in solution

Solution containing 0.2 g eriochrome black T in 100 ml of triethanolamine.

Oxalic (acid) in solution

Aqueous solution containing 5 g of crystallised acid oxalic $C_2O_4H_2 \cdot 2H_2O$ in 100 g of reagent.

Phenolphthalein in solution

Solution prepared with alcohol at 90% vol. containing 1 g de phenolphthalein in 100 ml of reagent.

Phosphoric (acid) solution at 85% (m/m)

Aqueous solution containing 85 g of orthophosphoric acid (H_3PO_4), $\rho_{20} = 1.70$, for 100 g.

Phosphoric (acid) solution at 25% (m/v)

Aqueous solution containing 25 g of phosphoric acid (H_3PO_4), $\rho_{20} = 1.70$, in 100 ml.

Phosphoric (acid) solution at 50% (m/m)

Aqueous solution containing 50 g of orthophosphoric acid (H_3PO_4), $\rho_{20} = 1.70$ in 100 g.

Phosphate (solution at 0.05 g of phosphorus per litre)

Potassium dihydrogenophosphate

Dissolve 4.392 g of monopotassium phosphate (KH_2PO_4) in a sufficient quantity of water to obtain 1 l. This solution contains 1 g of phosphorus per litre. Dilute to the twentieth to obtain the solution at 0.05 g per litre.

Lead (nitrate) in solution at 1 g and 0.01 g of lead per litre

Dissolve 1.60 g of lead nitrate $\text{Pb}(\text{NO}_3)_2$ in a sufficient quantity of water to obtain 1 l of solution at 1 g of lead per litre. This solution is diluted to the hundredth to obtain the solution at 0.01 g of lead per litre.

Lead (neutral acetate) in solution at 10% (m/m)

Aqueous solution containing 10 g of lead acetate (II) $\text{Pb}(\text{C}_4\text{H}_6\text{O}_4) \cdot 3\text{H}_2\text{O}$ in 100 g of reagent.

Potassium sulfite

Potassium (acetate) in solution at 5% (m/m)

Aqueous solution containing 5 g of crystallised potassium $\text{KC}_2\text{H}_3\text{O}_2$ acetate in 100 g of reagent $\text{CH}_3\text{CO}_2\text{K}$.

Potassium (anhydrosulphite) $\text{K}_2\text{S}_2\text{O}_5$ (formerly potassium disulphite) free from selenium.

To search for selenium in sulphur dioxide, potassium anhydrosulphite free from selenium should be used. To check the absence of selenium, proceed with the following test trial:

Weigh 2.55 g of the potassium anhydrosulphite sample, dissolve with heat in 7 ml of distilled water and 2 ml of concentrated hydrochloric

acid (R). Allow to cool and add 3 ml of formaldehyde solute (R). Allow to stand 10 minutes. Place the tube in a water bath at 100°C and add 50 mg of the sample of pulverised potassium anhydrosulphite. The total sample is 2.60 g of potassium anhydrosulphite corresponding to 1.50 g of sulphur dioxide. A pink coloration should not develop.

Potassium (anhydrosulphite) in solution at 2% (m/m)

Aqueous solution containing 2 g of crystallised potassium anhydrosulphite in 100 g of reagent.

Potassium (cyanide) in solution at 1 mg of hydrocyanic acid per litre

Prepare an aqueous solution containing 2.44 g of KCN per litre, dilute to 1/100 to obtain the titrating solution of 1 mg of hydrocyanic acid per litre.

Potassium (dichromate) at 1 g and 0.01 g of chrome per litre

Dissolve 2.8283 g of potassium dichromate $K_2Cr_2O_7$ in a sufficient quantity of water to obtain 1 l of solution at 1 g of chrome per litre. This solution is diluted to the hundredth to obtain the solution at 0.01 g of chrome per litre.

Potassium (dichromate) in solution at 10% (m/m)

Aqueous solution containing 10 g of potassium dichromate in 100 g of reagent.

Potassium (hexacyanoferrate (II))/ potassium (ferrocyanide) in solution at 5% (m/m)

Aqueous solution containing 5 g of crystallised potassium $K_4Fe(CN)_6 \cdot 3H_2O$ hexacyanoferrate in 100 g of reagent.

Potassium (hydroxide) at 40%

Dissolve 40 g of potassium hydroxide (KOH) in a sufficient quantity of water to obtain 100 ml.

Potassium (iodide) in iodine solution

Iodine-iodide solution - aqueous iodine solution (I_2) in potassium iodide (KI).

In a tared flask with a glass stopper, introduce 2 g of iodine, 4 g of potassium iodide and about 10 g of water. Allow the dissolution to operate, then complete with water, the weight of 100 g.

Potassium (permanganate) in solution at 5% (m/m)

Aqueous solution containing 5 g of potassium permanganate (KMnO₄) in 100 g of reagent.

Potassium (permanganate) in solution at 3% (m/m)

Aqueous solution containing 3 g of potassium permanganate in 100 g of reagent.

Potassium (permanganate) in solution at 2% (m/m)

Aqueous solution containing 2 g of potassium permanganate in 100 g of reagent.

Potassium (permanganate) in solution at 1% (m/m)

Aqueous solution containing 1 g of potassium permanganate in 100 g of reagent.

Potassium (permanganate) in solution at 0.2% (m/m)

Aqueous solution containing 0.2 g of potassium permanganate in 100 g of reagent.

Potassium (permanganate) in saturated solution

Saturated aqueous solution containing about 6 g of potassium permanganate in 100 g of reagent.

Potassium (permanganate) at 5 per 1000 (m/m)

Aqueous solution containing 5 g of potassium permanganate in 1000 g of reagent.

Potassium (permanganate) phosphoric solution

Dissolve 3 g of potassium permanganate (R) in a mixture of 15 ml of phosphoric acid (R) and 70 ml of water; complete to 100 ml with water.

Potassium (thiocyanate) in solution at 5% (m/m)

Aqueous solution containing 5 g of potassium thiocyanate KSCN in 100 g of reagent.

Pyridine-pyrazolone (reagent)

Bis(1-phenyl-3-methyl-5-pyrazolone). (F. 320°C) - Dissolve 17.4 g of 1-phenyl-3-methyl-5-pyrazolone in 100 ml of alcohol at 95% vol., add 25 g of freshly distilled phenylhydrazine, bring to the boil under reflux for 4 hours. The mixture is filtered hot and the precipitate washed several times with alcohol at 95% vol.

The boiling under reflux could be prolonged beyond 4 hours if the occurrence of yellow crystals is not very abundant after this time.

Preparation of reagent pyridine-pyrazolone. – In a 100 ml graduated flask, introduce 0.150 g of 1-phenyl-3-methyl-5-pyrazolone and dissolve in 50 ml of alcohol at 95% vol. distilled on potassium hydroxide; complete to 100 ml with distilled water.

On the other hand, weigh 20 mg of bis(1-phenyl-3-methyl-5-pyrazolone), and dissolve by prolonged shaking in 20 ml of pyridine.

Mix the two resulting solutions by pouring them in a yellow glass flask wrapped in black paper. Store in the refrigerator.

Quinine (sulphate) in solution at 0.1 mg per litre of sulphuric acid 0.05 M

Dissolve 0.100 g of quinine sulphate in a sufficient quantity of sulphuric acid 0.05 M to obtain 1 l. Dilute three times 1/10 this solution with a sulphuric acid solution 0.05 M to obtain the solution at 0.1 mg of quinine sulphate per litre.

Rosaniline (hydrochloride) in solution discoloured by sulphurous acid

In a mortar, pulverise 30 mg of pure rosaniline hydrochloride, then add 30 ml of alcohol at 95% vol. The dissolution is rapid and complete. On the other hand, in a 250 ml graduated flask, dissolve 8 g of potassium anhydrosulphite in about 150 ml of distilled water. Add the alcoholic solution of hydrochloride rosaniline, then 55 ml of hydrochloric acid solution 3 M and bring to the graduation line with water. The reagent must be completely discoloured in less than an hour. It is stable for several months.

Methyl red in solution

Alcohol solution at 90% vol. containing 0.10 g of methyl red in 50 ml of reagent.

Methyl red mixed indicator:

Solution in alcohol at 90% vol. containing 0.10 g of red methyl and 0.05 g of blue methylene in 10 ml of reagent.

Phenol red in solution

Heat 0.05 g of phenol red with 2.85 ml of sodium hydroxide solution 0.05 M and 5 ml of alcohol at 90% vol. To the solution obtained, add a sufficient quantity of alcohol at 20% vol. to obtain 250 ml.

Selenium (dioxide) in solution at 100 mg of selenium per litre

Grind 2 g of pure selenium dioxide (SeO_2) and allow to stand for 24 hours in a desiccator for sulphuric acid. Weigh 1.4553 g of this dry dioxide and dissolve in a sufficient quantity of water to obtain 1 l of solution.

This solution contains 1 g of selenium per litre. Dilute 1/10 with distilled water to obtain the solution at 100 mg of selenium per litre.

Sodium (acetate) - $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ = 136.1.

The salt used as a reagent must be neutral.

Sodium (acetate) in solution at 10% (m/m)

Aqueous solution containing 10 g of sodium acetate $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ in 100 g of reagent.

Sodium (borate) in saturated solution

Saturated aqueous solution containing about 4 g of crystallised sodium borate for 100 g of solution. Sodium tetraborate $\text{Na}_2\text{B}_4\text{O}_7$.

Sodium (neutral carbonate) in solution at 25% (m/m)

Aqueous solution containing 25 g of crystallised disodic carbonate at 10 H_2O in 100 g of reagent $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$.

Sodium (diethyldithiocarbamate) in solution at 1% (m/v)

Dissolve 1 g of sodium diethyldithiocarbamate in a sufficient quantity of alcohol at 40% vol. to obtain 100 ml of solution $(\text{C}_2\text{H}_5)_2\text{NCS}_2\text{Na} \cdot 3\text{H}_2\text{O}$.

Sodium (ethylenediaminetetraacetate) in solution 0.01 M

Sodium ethylenediaminetetraacetate	4.0 g
Magnesium chloride, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	0.1 g
Water	q.s.f. 1000 ml

The titre of this solution must be checked and adjusted after titration by a calcium chloride solution 0.01 M obtained by dissolving 1 g of pure calcium carbonate in 25 g of concentrated hydrochloric acid (R) with 20 ml of water and by adjusting the volume to 1000 ml with distilled water.

Sodium (fluoride) in solution at 4% (m/m)

Aqueous solution containing 4 g of sodium fluoride (NaF) in 100 g of reagent. This solution is nearly saturated.

Sodium (hydroxide) in concentrated solution (caustic soda)

Aqueous solution with density 1.330 containing 30 g of sodium hydroxide (NaOH) in 100 g of solution.

Sodium (hydroxide) in diluted solution at 10% (m/m)

Aqueous solution containing 10 g of sodium hydroxide (NaOH) in 100 g of reagent.

Sodium (phosphate) in solution at 10% (m/m)

Aqueous solution containing 10 g of crystallised disodic phosphate in 100 g of reagent.

Sodium (pyrophosphate) at 1% (m/m)

Aqueous solution containing 1 g of crystallised tetrasodic pyrophosphate, $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$, in 100 g of reagent.

Sodium (thiosulphate) in solution at 25% (m/v)

Aqueous solution containing 25 g of sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) for 100 ml.

Hydrogen sulphide (acid) in saturated solution

Aqueous solution of saturated hydrogen sulphide acid. It contains about 3.8 g of H_2S per litre. It is alterable in air.

Hydrogen sulphide (acid) solution at 1 g of sulphur per litre and at 0.01 g per litre

Dissolve 7.5 g of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ in a sufficient quantity of water to obtain 1 l. This solution is diluted to a hundredth to obtain the solution at 0.01 g per litre (solutions rapidly oxidised by air).

Sulforesorcinic (reagent)

Dissolve 2 g of pure resorcinol in 100 ml of water and add 0.5 ml of concentrated sulphuric acid (R).

Sulphuric (acid) concentrated at 95% minimum

$\rho_{20/4} = 1.83$ to 1.84 . (H_2SO_4)

Sulphuric (acid) at 97% (m/m)

This absolutely colourless acid should not be able to be differentiated after heating at 120°C from an unheated control. It should be stored in flasks with emery stoppers. Its titre should be $97 \pm 1\%$.

Sulphuric (acid) at 25% (m/m)

$\rho_{20/4} = 1.1808$ approximately.

Aqueous solution of sulphuric acid containing about 25 g of acid H_2SO_4 in 100 g of reagent.

Sulphuric (acid) diluted at 10% (m/m)

$\rho_{20/4} = 1.0682$ approximately.

Aqueous solution of sulphuric acid containing about 10 g of acid H_2SO_4 in 100 g of reagent.

Sulphuric (acid) diluted at 5% (m/m)

Aqueous solution of sulphuric acid containing about 5 g of acid H_2SO_4 in 100 g of reagent.

Sulphuric (acid) free from nitrogen must satisfy the following test trial: nitrate. To 5 ml of water, carefully add 45 ml of sulphuric acid free from nitrogen, allow to cool to 40°C and add 8 mg of diphenylbenzidine. The solution is barely pink or pale blue.

Acetate buffer, purified, for search for zinc

Dissolve 136 g of sodium acetate in 440 ml of water, add 58 ml of concentrated acetic acid. Purify this solution by shaking with a dithizone solution at 125 mg per litre of chloroform.

Ammoniac buffer

Concentrated ammonium hydroxide	350 ml
Ammonium chloride	54 g
Distilled water	q.s.f. 1000 ml

Buffer pH 7.5

Monopotassium phosphate	94 g
Sodium hydroxide in molar solution	565 ml
Distilled water	q.s.f. 1 000 ml

Pure tannin

Tannin, called ether tannin or officinal tannin is extracted from the Aleppo gall.

It is in the form of a light mass, yellowish white, very soluble in water and alcohol at 90% vol. It is insoluble in ethylic ether. It must comply with the following test trials:

1. The aqueous tannin solution at 10% must be clear and have a very light yellow colour like white wine. The tannin solution at 10% in alcohol at 90% vol. must also be clear with hardly any colour.

A solution at 1 g of tannin in 5 g of water with its volume of alcohol at 90% vol. and half of its volume of ethylic ether, should give a clear solution (aqueous extract or alcoholic extract).

2. The officinal tannin must be combustible without leaving residue more than 0.05% (set mineral matters).

3. Desiccated at 100°C, the officinal tannin must not lose more than 12% in its weight (excess water). The anhydrous tannin content is calculated from this test trial. Its knowledge is necessary for the preparation of the solution at 4 per 1000.

Tannin in solution at 2% (m/m)

Aqueous solution containing 2 g of tannin in 100 g of reagent. It must be prepared extemporaneously.

Tannin in solution at 4% (m/v)

Dissolve a quantity of pure tannin containing 1 g of anhydrous tannin in a sufficient quantity of water to obtain 250 ml.

Tannin in solution at 10% (m/m)

Aqueous solution containing 10 g of tannin in 100 g of reagent.

Thioacetamide (reagent)

$F \cong 113^{\circ}\text{C}$

To 0.2 ml of aqueous thioacetamide solution at 40 g/l, add 1 ml of a mixture of 5 ml of water, 15 ml of sodium hydroxide 1 M and 20 ml of glycerol at 85% (m/m). Heat in a water bath at 100°C for 20 seconds. Prepare extemporaneously.

Uranyl (nitrate) in solution at 4% (m/m)

Solution containing 4 g of uranyl nitrate $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in 100 g of reagent.

Uranyl and magnesium (acetates) in hydro-alcoholic and acetic solution

Dissolve 32 g of crystallised uranyl acetate and 100 g of magnesium acetate in 300 ml of water, 20 ml of acetic acid and 500 ml of alcohol at 95% vol. by heating in a water bath at 100°C and by shaking; adjust the volume to 1 litre with water (distilled) and allow to stand 48 hours; decant or filter.

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Reagents and titrated solutions

This reagent must be stored away from light. 2.5 ml of reagent must be used per milligramme of sodium to be precipitated and per millilitre of solution to be treated.

Phosphates, arseniates and fluorides must be absent from this solution. Heavy metals, iron (II) and alkaline-earth are not bothersome.

Bromocresol green in solution

Alcohol solution at 95% vol. containing 0.04 g of bromocresol green (3',3'',5',5''tetrabromo-*m*-cresolsulfonephtaleine) for 100 ml of reagent.

Bromocresol green and methyl red in solution (mixed indicator)

Dissolve

Bromocresol green	0.04 g
Methyl red	0.06 g
in alcohol at 95% vol.	100 ml

Add 2.5 ml of sodium hydroxide solution 0.1 M.

This indicator from red (pH 4.6) turns blue-green with pH 4.9. It is violet with pH 4.75.

Zinc in solution at 1 mg per litre

Dissolve 1 g of pure zinc in the minimum concentrated hydrochloric acid (R) by gently heating. Dilute the solution to 500 ml and neutralise by adding sodium carbonate until a light precipitate appears which disappears when a few drops of hydrochloric acid are added.

Dilute successively three times 1/10 when using.

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