Centre for Horticulture & Plant Sciences
School of Science, Food & Horticulure

Mr Greg Seymour
General Manager
Australian Mushroom Growers Association

Dear Greg

Conclusions and recommendations on mushroom food safety validation study

I am giving below my conclusions and recommendations based on the study carried out to verify and validate the absence of *Listeria monocytogenes, Escherichia coli*, and *Salmonella* in substrate (compost) prepared for cultivation of mushrooms. This study, commissioned by the Australian Mushroom Growers Association, was carried out by Complete Food Safety in collaboration with myself.

- Background

There appears to be a concern among some within the food chain that mushroom substrate (compost) could be a source of human pathogens and that this could lead to contamination of fresh mushrooms and thus be a food safety risk. Consequently, some food safety authorities have placed mushrooms at a high-risk category. The perception that mushrooms should be classified in that manner has been brought about by a lack of understanding of the processes involved in substrate preparation.

In order to dispel this perception, the Australian Mushroom Growers Association commissioned a study to verify and validate the absence of *Listeria monocytogenes, Escherichia coli*, and *Salmonella* in mushroom substrate after phase 2 pasteurisation.

Preparation of mushroom substrate consists of two phases. During phase 1, the substrate is produced in bulk (custom-made) and supplied to mushroom growers who carry out phase 2. The objectives of phase 2 are pasteurisation and final conditioning of the substrate.

The basic ingredients in mushroom substrates are poultry manure, wheat straw and gypsum. Nutrient supplements such as cottonseed hull and cottonseed meal are also added as required. The use of poultry manure was the reason for the concern that mushroom substrate could be a source of pathogenic microorganisms.

- Substrate (compost) samples

Three sources where mushroom substrate is produced were included in the study. These sources supplied, between them, substrates to 10 mushroom farms. At each farm, 2 batches of substrate after completion of phase 2 pastuerisationr were collected at random and in triplicate for microbial analysis. A total of sixty samples of substrate were collected and sub-samples were tested for the presence of *Listeria monocytogenes, Escherichia coli* and *Salmonella*. Details of the method of collection of samples and, the tests used for microbial analysis are described in the report from Complete Food Safety.

- Results of microbial analysis of mushroom substrates

The test results showed that the levels of *E. coli* in the mushroom substrates were below the lowest detection level of $< 3\ g^{-1}$. *Listeria monocytogenes* and *Salmonella* were not detected per 25 g of the substrates.

- Discussion

Mushrooms are recognised universally as a safe food product. This assertion is also true in the local context since, as far as I know, there are no documented evidence of food-borne illness attributed to mushroom consumption in Australia. The scant reports (e.g. Bean and Griffin 1990; Brunner and Wong 1992) of mushroom-related food poisoning from overseas are related to post-processing contamination such as botulinum toxin rather than of the mushroom itself.

The present study has shown that the levels of *Listeria monocytogenes, Escherichia coli* and *Salmonella* in mushroom substrates were below the levels of detection used in the tests. These findings are not surprising because the substrate is subjected to temperatures well above the thermal death points of human pathogens during both phases 1 and 2 (see Figs. 1 and 2). Temperature during phase 1 reaches $70 - 80^0$ C. It is also likely that the high levels of NH_3 (2500 – 6000 ppm) formed during phase 1 could have a synergistic effect on the elimination of harmful microorganisms. During phase 2, the substrate is pasteurised at 60^0 C for 2 hours.

A fundamental aspect of mushroom cultivation is that the substrate should be free of undesirable microorganisms. This is a pre-requisite for optimum production of mushrooms. Without adherence to high standards of hygiene, mushroom production will be economically unsustainable.

- Conclusions

The study commissioned by the Australian Mushroom Growers' Association has validated the fact that levels of *Listeria monocytogenes, Escherichia coli*, and *Salmonella* in substrates (compost) used for mushroom cultivation are below the levels of detection used in the tests. The test results prove that the standard procedure used by all growers for the prepartion of mushroom substrates is efficient in the elimination of human pathogens such as *Listeria monocytogenes, Escherichia coli*, and *Salmonella*. The results prove further that mushrooms grown in such substrate (compost) do not pose a food safety risk. Consequently, mushrooms should be placed in the low-risk category in terms of food safety.

- Recommendations

I recommend that mushrooms should be considered as a safe food product on the basis of evidence obtained in the present study which showed that substrate (compost) prepared for mushroom cultivation is free of pathogenic microorganisms. I also recommend that mushrooms should be removed from a high-risk category and placed in a low-risk category in terms of food safety.

Literature cited:

Bean, N.H. and Griffin, P.M. (1990). Foodborne disease outbreaks in the United States, 1973-1983: Pathogens, vehicles and trends. *Journal of Food Protection* **53**:804-817.

Brunner, K.G. and Wong, A.C. (1992). *Staphylococcus aureus*. Growth and enterotoxin production in mushrooms. *Journal of Food Science* **57**:700-703.

Yours Sincerely

Dr. N. G. (Tan) Nair MSc., Ph.D.
Senior Lecturer

Figure 1 - Mushroom Substrate Preparation – Phase 1

Flow Chart

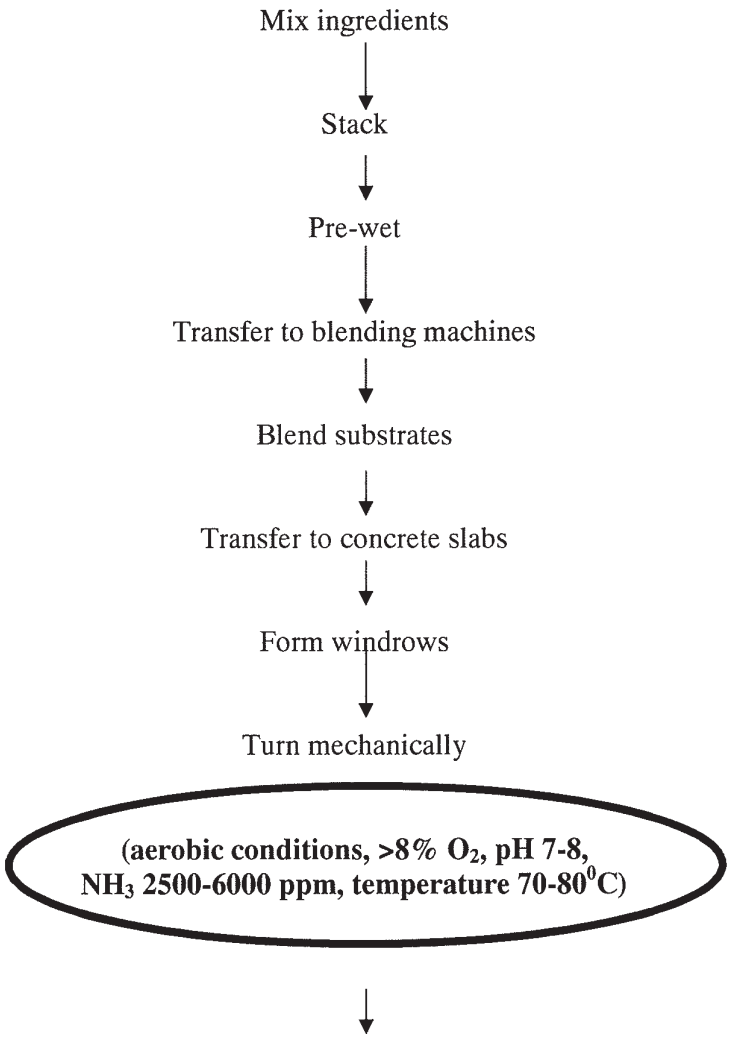
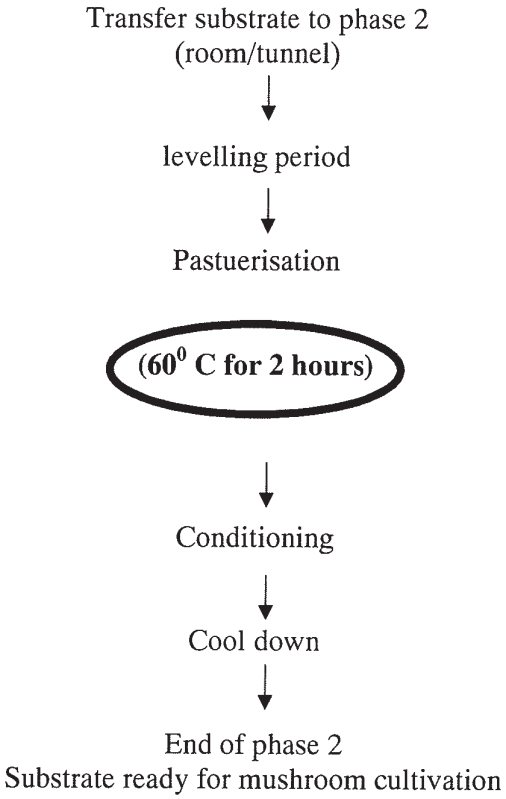


Figure 2 - Mushroom Substrate Preparation – Phase 2

Flow Chart



MUSHROOM FOOD SAFETY

VALIDATION PROJECT

PREPARED FOR

AUSTRALIAN MUSHROOM GROWERS ASSOCIATION

COMPILED BY:

MICHAEL HARRISON

COMPLETE FOOD SAFETY

DECEMBER, 2001

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Executive Summary

The objective of the mushroom food safety project is to verify and validate the absence of *Listeria monocytogenes, Escherichia coli*, and *Salmonella* in mushroom substrate (compost) after pasteurisation.

Food standards are continually changing around the world and, they are also being regularly updated in Australia. Mushrooms are unique among horticultural crops in being an indoor crop whose production efficiency is directly related to the standard of hygiene at all stages of cultivation. Consequently, the Australian mushroom industry is pro-active in maintaining high standards of hygiene in cultivation practices.

The Australian Mushroom Growers Association commissioned a study to verify and validate the absence of *Listeria monocytogenes, Escherichia coli*, and *Salmonella* in mushroom substrate after pasteurization during phase 2.

The substrates for analysis came from three different sources; two were from bulk phase 1 producers (Elf and Singleton) and one was from a mushroom grower who prepares phases 1 and 2 in the farm (self-made). Substrates from five growers supplied by Elf and from four growers supplied by Singleton were included in the analysis

The results showed that the levels of *Escherichia coli* in the mushroom substrates were below the lowest detection level of $<3\ g^{-1}$. *Listeria monocytogenes* and *Salmonella* were not detected per 25 g of the mushroom substrates. This finding is not surprising since one of the key stages in the technology of mushroom substrate production is a pasteurisation process, which is carried out at 60^0 C for 2 hours.

The study commissioned by the Australian Mushroom Growers' Association has validated the fact that levels of *Listeria monocytogenes, Escherichia coli*, and *Salmonella* in substrates (compost) used for mushroom cultivation were below the levels of detection used in the tests. Mushroom substrate (compost) therefore, does not pose a food safety risk.

Aim

The objective of the project is to verify and validate the absence of *Listeria monocytogenes, Escherichia coli*, and *Salmonella* in mushroom substrate (compost) after pasteurisation.

Introduction

Food standards are continually changing around the world and, they are also being regularly updated in Australia. Mushrooms are unique among horticultural crops in being an indoor crop whose production efficiency is directly related to the standard of hygiene at all stages of cultivation. Consequently, the Australian mushroom industry is pro-active in maintaining high standards of hygiene in cultivation practices.

Preparation of mushroom substrate (compost) consists of two phases. During phase 1, the substrate is produced in bulk (custom-made) and supplied to mushroom growers who carry out phase 2 (pasteurisation and final conditioning). Mushroom growers rarely carry out both the phases in substrate preparation. There are currently two phase 1 mushroom substrate producers in Sydney.

The basic ingredients in mushroom substrate are poultry manure, wheat straw and gypsum. Nutrient supplements such as cottonseed hull and cottonseed meal are also added as required. The use of poultry manure has caused concern in some within the food chain that mushroom substrate could be a source of pathogenic microorganisms. They fear that this could lead to contamination of fresh mushrooms and thus be a food safety risk. However, this perception is due to a lack of understanding of the processes involved in the preparation of mushroom substrate. For instance, the elimination of undesirable microorganisms from the substrate during pasteurisation is a pre-requisite for optimum production of mushrooms. Therefore only pasteurised substrate is used for mushroom cultivation.

The Australian Mushroom Growers Association commissioned a study to verify and validate the absence of *Listeria monocytogenes, Escherichia coli*, and *Salmonella* in

mushroom substrate after pasteurization during phase 2. The results of this study are reported here.

Materials and Methods

Mushroom substrate: Mushroom substrates that have been fully processed after phases 1 and 2 and ready for use in mushroom cultivation was used for microbial analysis. The substrates for analysis came from three different sources; two were from bulk phase 1 producers (Elf and Singleton) and one was from a mushroom grower who prepares phases 1 and 2 in the farm (self-made). Substrates from five growers supplied by Elf and from four growers supplied by Singleton were included in the analysis (Table 1).

Table 1 – Sources of mushroom substrate sampled for microbial analysis

Sources of substrate	Numbers of samples from each source*
Elf	5
Singleton	4
Self-made	1
Total	10

* each source represents substrate from one mushroom farm

Sampling procedure: Samples were collected aseptically using sterile tools. They were collected at random from wooden trays arranged in stacks within the pasteurisation rooms. The top, middle and bottom trays from the front, middle and end of the rooms were sampled. Ten samples were collected from each farm and they were transported to the laboratory for analysis with minimum delay. Services of Weston Food Laboratory, an accredited facility with NATA registration, were used for microbial analysis.

Microbial analysis : The substrates were tested for the presence of *Listeria monocytogenes, Escherichia coli*, and *Salmonella*. The George Weston Food (GWFB) methods used for testing were:

Escherichia coli: GWFB 6.1 adapted from the following standards
AS 1766.2.3 – 1992
Examination of specific organisms – coliforms and E. Coli

Salmonella: GWFB 8.2 adapted from the following standards
AOAC Official Method of Analysis 3rd Supplement (1997) to the 16th Edition
AS 1766.2.5 – 1991

Listeria: GWFB 9.1 adapted from the following standards
AS / NZS 1766.2.16.1:1998
AOAC Official Method of Analysis No. 999.06

The following sample rinsing method was used:

1. Combine 10 samples from each farm, by transferring the contents into a large sterile blender bag and shake it around to obtain one homogeneous composite sample.

2. Weigh aseptically 3 x 100gr of the composite sample into 3 sterile blender bags.

3. Aseptically add 0.1% peptone diluent (1:1 dilution) to each sample bag and shake the contents in the bag.

4. Using the rinse/ diluent as the sample (1ml of rinse = 1 gram of sample), proceed to the appropriate GWFB* test methods for examination of specific microorganisms.

The detection levels for the microorganisms were as follows:

E. coli MPN – lowest detection = <3 g^{-1}

L. monocytogenes detection in 25 g

Salmonella sp. detection in 25 g

Results

The results showed that the levels of *Escherichia coli* in the mushroom substrates were below the lowest detection level of <3 g^{-1}. *Listeria monocytogenes* and *Salmonella* were not detected per 25 g of the mushroom substrates (Table 2).

Farm Number	Date Sampled	E. coli M.P.N. / g GWFB 6.1	Salmonella sp. / 25 g GWFB 8.2	Listeria monocytogenes / 25 g GWFB 9.1
5	23/10/01	<3 / g	ND / 25g	ND / 25g
7	23/10/01	<3 / g	ND / 25g	ND / 25g
8	23/10/01	<3 / g	ND / 25g	ND / 25g
9	23/10/01	<3 / g	ND / 25g	ND / 25g
1	24/10/01	<3 / g	ND / 25g	ND / 25g
2	24/10/01	<3 / g	ND / 25g	ND / 25g
3	26/10/01	<3 / g	ND / 25g	ND / 25g
4	26/10/01	<3 / g	ND / 25g	ND / 25g
5	30/10/01	<3 / g	ND / 25g	ND / 25g
8	30/10/01	<3 / g	ND / 25g	ND / 25g
9	30/10/01	<3 / g	ND / 25g	ND / 25g
3	1/11/01	<3 / g	ND / 25g	ND / 25g
4	2/11/01	<3 / g	ND / 25g	ND / 25g
6	5/11/01	<3 / g	ND / 25g	ND / 25g
7	6/11/01	<3 / g	ND / 25g	ND / 25g
1	7/11/01	<3 / g	ND / 25g	ND / 25g
2	7/11/01	<3 / g	ND / 25g	ND / 25g

10	7/11/01	<3 / g	ND / 25g	ND / 25g
6	12/11/01	<3 / g	ND / 25g	ND / 25g
10	14/11/01	<3 / g	ND / 25g	ND / 25g

Discussion

The levels of *Listeria monocytogenes, Escherichia coli*, and *Salmonella* in the substrates (compost) used for mushroom cultivation were below the levels of detection used in the tests. This finding is not surprising since one of the key stages in the technology of mushroom substrate production is a pasteurisation process, which is carried out at 60^0 C for 2 hours. It has been well established by several investigators in the past that the thermal death points for these microorganisms are < 60^0 C at an exposure time of 2 hours.

Limits of Growth of *E. Coli, Salmonella, and L. Monocytogenes*

Temperature ^{0}C

Organism	Minimum	Optimum	Maximum
E. Coli	Ca 7-8	35 - 40	Ca 44 – 46
Salmonella	5.2	35 – 43	46.2
L. Monocytogenes	-0.4	37	45

(ICMSF:1996)

Elimination of undesirable microorganisms from the substrate by pasteurisation is a pre-requisite for optimum production of mushrooms. Without such adherence to high standards of hygiene, mushroom production will be economically unsustainable. Therefore mushroom substrate has never been a food safety risk. This is in contrast with that of milk where appropriate legislation was brought in to safeguard the food safety of milk consumption by the public.

The object of pasteurisation is to destroy undesirable organisms in the compost whilst causing as little damage as possible to the compost. Growers have a narrow margin to move because if the temperature is too high, it can kill not only harmful, but also beneficial micro-organisms, and this leads to other problems, such as ammonification.

If minimum conditions for destroying undesired organisms are not achieved the farmer will find that there will be problems with the crop due to the growth of the undesired organisms. Most literature on mushroom farming and pasteurisation of the compost

focuses on temperature control and promotion of the environment where the growth of mycelium will thrive, at a temperature high enough to knock out the harmful bacteria.

The samples tested in this project, demonstrated that there was no presence of the microbes, and under normal conditions these microbes should be killed during pasteurisation. Factors that are extremely important in the control of food safety include temperature control and hygiene, and these are extremely important when it comes to growing mushrooms. Without correct temperature control and hygiene on the mushroom farm it is difficult to grow mushrooms successfully.

Conclusion

The study commissioned by the Australian Mushroom Growers' Association has validated the fact that levels of *Listeria monocytogenes, Escherichia coli*, and *Salmonella* in substrates (compost) used for mushroom cultivation were below the levels of detection used in the tests. Mushroom substrate (compost) therefore, does not pose a food safety risk.

References

International Committee on Microbiological Standards for Foods. **Micro-Organisms in Foods. 5. Characteristics of Microbial Pathogens**. James & James (Science Publishers) Ltd. & Chapman & Hall. London. 1996.

Seamons, Colleen

From: Greg Seymour Bigpond <gregseymour1@bigpond.com>
Sent: Tuesday, 12 July 2011 8:14 AM
To: submissions
Subject: AMGA submission to FSANZ in response to the Consultation Paper : Improving Food Safety for Fresh Produce.
Attachments: AMGA_Submission_-_FSANZ_-_July_2011.docx; AMGA Substrate_Micro_Test__Rpt +Letter 2001.docx

G'day,

Please find attached the AMGA submission to FSANZ in response to the Consultation Paper : Improving Food Safety for Fresh Produce.

Cheers,
Greg
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