Title

Production of Processed Commodities from Transgenic Event FG72 Soybeans and the Non-transgenic Counterpart (2009)

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Test Guideline

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Sponsor

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B) or (C).

Company: Bayer CropScience

Company Representative: Isabelle Coats

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US Registration Manager
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The above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

APPROVALS PAGE

Study Director/Author	William J. Kowite William J. Kowite, Ph.D. Senior Scientist BioAnalytics	Oct. 6, 2009 Date
Study Director Management	Princy Jesud Princy Jesudason, Ph.D. Manager, BioAnalytics-RTP	Oct 5, 09 Date
Sponsor	Donna Mitten Donna Mitten Global Regulatory Affairs Manager	22 Sept 2009 Date

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SUMMARY

Production of Processed Commodities from Transgenic Event FG72 Soybeans and the Non-transgenic Counterpart (2009)

Samples of processed commodities or fractions (FRAC) were prepared from soybeans obtained from 2008 field production HT08SOY001. The soybeans were grown by M.S. Technologies, LLC near Adel, Iowa, USA. The samples were derived from the non-transgenic counterpart soybeans (Jack) and the transgenic event FG72 soybeans that were sprayed with isoxaflutole and glyphosate herbicides during the growth cycle and FG72 soybeans that were not sprayed with the above herbicides. The FG72 transformation event contains the stably integrated gene *2mepsps* which encodes the 2mEPSPS protein, and the *hppd* gene which encodes the HPPD protein. The 2mEPSPS and HPPD proteins confer tolerance to the herbicides glyphosate and isoxaflutole (IFT), respectively.

Processing of the soybeans was performed by GLP Technologies of Navasota, Texas, USA, and was completed on July 15, 2009. Samples of soybean grain (seeds), untoasted and toasted meal, hulls, protein isolate, crude oil, refined oil, deodorized and bleached oil and crude lecithin were obtained and shipped to the BioAnalytics Laboratories of Bayer CropScience in Research Triangle Park, NC, USA.

STUDY IDENTIFICATION

Study Initiated:	20 March 2009
Experimental Termination Date:	15 July 2009
Study Director/Facility Address:	William J. Kowite, Ph.D. Bayer CropScience P.O. Box 12014 2 T. W. Alexander Drive Research Triangle Park, NC 27709 USA
Processing Facility Address and Processing Investigator:	Dick Dusek GLP Technologies 22723 State Highway 6 South Navasota, TX 77868 USA

1. INTRODUCTION

Soybean plants containing the transgenic event FG72 were grown in the field in 2008 by M.S. Technologies, LLC, under study number HT08SOY001, along with the non-transgenic counterpart "Jack". The FG72 transformation event contains the stably integrated gene *2mepsps* which encodes the 2mEPSPS protein, and the *hppd* gene which encodes the HPPD protein. The genes were introduced by direct gene transfer. The 2mEPSPS and HPPD proteins confer tolerance to the herbicides glyphosate and isoxaflutole (IFT), respectively. Large quantities of soybean grain (seeds) were harvested from the field plots to provide samples for processing.

The objective of this study, DQ09B002, was to convert samples of soybean grain into processed commodities or fractions (FRAC). The processed samples were produced for use in additional studies conducted by Bayer CropScience.

2. ORIGIN OF SOYBEAN GRAIN

The soybeans used in this processing study were obtained in 2008 from field trial 01 of field production HT08SOY001. The field production was performed in Dallas County in Iowa by M.S. Technologies, LLC, 22555 Laredo Trail, Iowa, USA. One plot of the soybean plants containing the transgenic event FG72 was sprayed with isoxaflutole and glyphosate herbicides. A separate plot of transgenic event FG72 soybeans and the non-transgenic plot of the counterpart "Jack" were not sprayed with these herbicides. The field production data were reported in Bayer CropScience study report HT08SOY001 (DART Number M-353428-02-1).

Soybean grain was obtained from each of the plots of field production HT08SOY001. The grain was stored at M.S. Technologies in Adel, Iowa. Portions of the sprayed and unsprayed transgenic event FG72 soybeans and non-transgenic "Jack" soybeans were shipped from Adel, IA by M.S. Technologies to the processor, GLP Technologies, in Navasota, Texas on April 29, 2009 under USDA Interstate Movement Notification 09-069-105n. The soybeans were received at GLP Technologies on May 1, 2009.

3. PROCESSING SUMMARY

Processing of the soybeans into toasted meal was performed by GLP Technologies, with Dick Dusek serving as the Processing Principal Investigator. The samples were processed at:

GLP Technologies
22723 State Highway 6 South
Navasota, TX 77868
USA
Tel: +001 (936) 825-2180 or +001 (936) 825-2184
Fax: +001 (936) 825-7929
E-mail: ddusek@glptech.net

After receipt at the processing facility, the soybeans were placed into frozen storage. The processing of the soybean samples took place between June 23, 2009, and July 14, 2009. The non-transgenic "Jack" grain was processed first, followed by the unsprayed transgenic event FG72 grain, and finally the glyphosate and IFT herbicide-sprayed transgenic event FG72 grain.

Processing of each Regimen was done in cleaned equipment using simulated industry standard protocols. The equipment and facilities were reported to have been thoroughly cleaned and inspected before and after the processing of each sample.

The whole soybeans were cleaned by screening prior to processing. The soybeans were cracked in a roller mill and then aspirated to separate the hulls from kernel material. The kernel material was moisture-conditioned, heated and flaked. A portion of the flakes was solvent extracted with hexane and used to produce soy isolate via production of defatted soy flour. The rest of the flakes were extruded while being steam injected and were then dried and ground. The dried collets were extracted with hexane to produce untoasted and toasted meal fractions. The miscella, consisting of crude oil in hexane, was used to produce crude oil, crude lecithin, refined oil, and bleached, deodorized oil.

One protocol deviation and one facility SOP deviation were reported for the processing. Neither of the deviations produced any adverse affect upon the processing or sample integrity. Processed samples collected are summarized in Table 1. More detailed procedures, observations and material balances are included in the Processing Report (Appendix 1).

Table 1 Sample Information Summary

Sample	Regimen A	Regimen B	Regimen C	Total	Sample Wt (grams) Delivered
	Sample Code: HT08SOY001-01-				
Soybean Seed	01	02	03	3	500
(Grain used for processing)					
	Sample Code: DQ09B002 -				
Hulls (H)	AH	BH	CH	3	300
Meal (M)	AM	BM	CM	3	700
Toasted Meal (T)	AT	BT	CT	3	700
Protein Isolate (P)	AP	BP	CP	3	153 - 165
Crude Oil (C)	AC	BC	CC	3	300
Refined Oil (R)	AR	BR	CR	3	300
Deodorized, Bleached Refined Oil (D)	AD	BD	CD	3	734 - 740
Crude Lecithin (L)	AL	BL	CL	3	190 - 230
Total Samples	9	9	9	27	

4. ANALYTICAL SUMMARY

The processed samples, including the soybean seeds used in the processing, were analyzed for DNA content to confirm the presence or absence of the FG72 transgenic event, in support of analytical studies involving these samples. All samples, except for the oil and lecithin matrices, were confirmed to contain the FG72 event DNA in Regimens B and C and did not contain the FG72 event DNA in Regimen A. No detectable DNA was found in the oil and lecithin matrices, and fortification of these matrices with the FG72 DNA confirmed that the method would have found it if the FG72 DNA were present. Certificate of Analysis BTS002109 documents the findings and is archived at Bayer CropScience in Research Triangle Park, NC.

5. RESULTS AND DISCUSSION

Samples of soybean grain were processed into the required fractions by GLP Technologies. The samples listed in Table 1 were divided into two parts, and the duplicate sets of frozen samples were shipped to the BioAnalytics Laboratories of Bayer CropScience in Research Triangle Park, NC, USA on July 14, 2009 and July 15, 2009. The sample weights delivered, as shown in Table 1, are listed for each set of the duplicate samples. Shipments were carried out by Federal Express priority overnight shipping in the presence of dry ice. Each set of samples was logged in and placed in frozen storage upon arrival at the BioAnalytics Laboratories. The processing was reported to be routine and is summarized in the processing report of Appendix 1.

6. CONCLUSIONS

Samples of processed transgenic event FG72 soybeans (sprayed with glyphosate and IFT herbicides, and unsprayed) and non-transgenic counterpart "Jack" soybeans were prepared in this study in suitable quantity and quality for use in planned analytical studies.

7. ARCHIVING

The protocol, final report, processing and analytical reports and supporting documentation are archived under study number DQ09B002 in the Archives of Bayer CropScience, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA.

Appendix 1 Processing Report

SPONSOR:

Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, North Carolina 27709

STUDY DIRECTOR/TESTING FACILITY:

William J. Kowite, Ph.D.
Bayer CropScience
2 T.W. Alexander Drive
Research Triangle Park, North Carolina 27709

PROCESSING REPORT:

Soybean: Small-Scale Processing

STUDY TITLE:

Production of Processed Commodities from Transgenic Event FG72 Soybeans and the Non-Transgenic Counterpart (2009)

AUTHOR:

Dick L. Dusek
GLP Technologies
Navasota, Texas

Dick Dusek
Signature

Aug. 19, 2009
Date

PROCESSING FACILITY:

GLP Technologies
22723 State Highway 6 South
Navasota, Texas 77868

STUDY IDENTIFICATION:

Study Number: DQ09B002
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STUDY TITLE: Production of Processed Commodities from Transgenic Event FG72 Soybeans and the Non-Transgenic Counterpart (2009)

SPONSOR: Bayer CropScience
Research Triangle Park, North Carolina 27709

STUDY DIRECTOR: William J. Kowite, Ph.D.
Bayer CropScience
Research Triangle Park, North Carolina 27709

PROCESSING PRINCIPAL INVESTIGATOR: Dick L. Dusek
GLP Technologies
Navasota, Texas

PROCESSING, DATA RECORDING & SHIPPING TECHNICIANS: Timothy R. Adams, Dick L. Dusek, Theodore F. Dusek Jr., K. Todd Hausman, Joseph M. Gibson, Tye A. Holloway, C. Lee Jordan, and Kristin E. Roberts.

SAMPLE RECEIPT DATE: May 1, 2009

PROCESSING START DATE: June 23, 2009

PROCESSING COMPLETION DATE: July 14, 2009

FRACTION SHIPMENT DATE: July 14, 2009
July 15, 2009 (Duplicates)

INTRODUCTION:

Soybean samples received from MS Technologies in Adel, Iowa were processed into commercially representative fractions. These fractions were shipped to Bayer CropScience in Research Triangle Park, North Carolina.

TEST SYSTEM: [From protocol]

Soybeans produced by MS Technologies in 2008

OBJECTIVE:

The objective of GLP Technologies was to produce and collect commercially representative processed fractions from soybean samples grown in the field.

METHODS & MATERIALS:

Receipt of Test Commodities:

Three ambient soybean samples (Raw Agricultural Commodity (RAC)/seed) were received from Justin Mason at MS Technologies in Adel, Iowa. United Parcel Service (UPS) delivered the samples on May 1, 2009. After receipt and inventory, the samples were placed into frozen storage.

Storage Conditions:

GLP Technologies SOP E.2 "Storage of Samples in Freezers" requires that freezer temperatures be maintained at or below 10 degrees Fahrenheit with the exception of the defrost cycle and removal and placement of samples in the freezers. Recorded in the data are the times and dates for removal or placement of samples/fractions in freezers or coolers.

Sample/Fraction Handling:

Samples were handled in a manner that minimizes the possibility of contamination. It is this facility's policy to use only containers and utensils washed with detergent and rinsed with water. Additionally, all equipment used in processing of these samples was cleaned prior to processing and between processing of each sample.

Processing Methods:

Soybean samples were identified and processed in the following order: HT08SOY001-01-01 (Regimen A), HT08SOY001-01-02 (Regimen B), and HT08SOY001-01-03 (Regimen C).

Moisture content of the incoming soybean was determined. If the moisture content was greater than 13.5%, samples were dried in a Steelman Industries oven at 130-160°F to a moisture content of 10-13.5%. None of the samples required drying. Following drying (if necessary), samples were cleaned by aspiration and screening. Aspiration was not necessary due to the condition of the soybean samples. Samples were screened using a Hance Corporation cleaner to separate large and small foreign particles (screenings) from the soybean sample.

Cleaned whole soybeans were fed into an A. T. Ferrell roller mill to crack the hull and liberate the kernel. After hulling, the material was passed through the Kice aspirator to separate hull and kernel material. Resulting fractions were hulls and kernel.

Kernel material was moisture conditioned to 13.5% and allowed to temper for a minimum of twelve hours. Moisture adjusted kernel material was heated to 160-175°F

in a Marion mixer and flaked in an A. T. Ferrell flaking roll with a gap setting of 0.008-0.013". After flaking, a portion of the flakes were removed and taken to solvent extraction. The solvent extracted flakes were used for production of soy isolate.

Flakes used in toasted and untoasted meal production were extruded in a Readco/Teledyne continuous processor, where they were turned into collets by direct steam injection and compression. Collets exited the processor at 200-250°F. After extrusion, the collets were dried in a Steelman Industries oven at 150-180°F for 30-40 minutes and then ground in the C. S. Bell mill. Flakes and collets were placed in separate stainless steel batch extractors and submerged in 120-140°F hexane (solvent). After 30 minutes, the miscella (crude oil and hexane) was drained and fresh hexane was added to repeat the cycle two more times. Final two washes were for 15 minutes each.

After the final draining, extracted flakes were desolventized using ambient air. Following desolventization, flakes were ground in a Glen Mills grinding mill. Ground material was screened with a Great Western sample sifter equipped with a 62 mesh sieve. Material passing through the screen was defatted soy flour and was used to produce protein isolate. Protein isolate was produced using a freeze drying method.

Following the final draining, collets were desolventized in a Reliance Industries paddle mixer. Collets were heated to 210-220°F and removed from the mixer. An untoasted meal fraction was collected and a portion of the remaining meal was toasted using a small scale procedure. Material was moisture conditioned to 12.0%, heated 220-240°F in a Presto electric cooker. Material was held for 30-60 minutes in the temperature range. After toasting, the product was cooled to room temperature and screened with a No. 8 screen(~1/8 inch). Resulting fraction through the screen was toasted soybean meal.

Miscella was passed through a laboratory vacuum evaporator unit to separate the crude oil and hexane. Crude oil was heated to 195-205°F for hexane removal and filtered.

Crude oil was de-gummed by adding a weighed amount of water (2.0-3.0%) and mixing with the crude oil for 30-60 minutes at a temperature of 158-168°F. Following mixing the degummed oil and crude lecithin were separated by centrifugation and filtering. Resulting fractions were degummed oil and crude lecithin.

Percent free fatty acid (FFA) was determined for the degummed oil. Based on the FFA, a weighed amount of crude oil and 12° Baumé sodium hydroxide was placed in a water bath at 68-75°F and mixed for 90 minutes at high RPM, and then for 20 minutes at low RPM and 145-153°F. Neutralized oil was then centrifuged. Refined oil was decanted

and filtered. Resulting fractions were alkali refined oil and soapstock. Soapstock was discarded.

Refined oil was heated to 104-122°F, activated bleaching earth added (1.0% by weight of oil), and placed under vacuum. Temperature was increased to 185-212°F and held for 10 to 15 minutes. After reducing the temperature, the bleached oil was filtered. Resulting fractions were bleached oil and spent bleaching earth.

Bleached oil was steam bathed for 28-32 minutes under vacuum and temperature held between 428-446°F. During the cooling period a 0.5% citric acid solution was added (1 ml per 100 grams of oil deodorized). Resulting fractions was deodorized oil (RBD oil) and deodorizer distillates.

This processing procedure is outlined in form H.202 "Soybean Processing Material Balance" and is described in detail in SOP G.2 Revision 04, "Soybean: Batch Processing Method".

Comparison to Industrial Practice:

Soybeans were processed in a way that simulates industrial practice as closely as possible. Because of compliance monitoring requirements and sample size, the samples were processed by batch rather than continuous, as in commercial operation.

Processing Results:

Soybean samples were processed to produce hull material, untoasted soybean meal, toasted soybean meal, crude oil, crude lecithin, refined oil, refined-bleached-deodorized (RBD) oil, and protein isolate. An unprocessed whole soybean (RAC) sample was collected prior to processing. All fractions were collected in duplicate.

Other Circumstances Pertaining to Study:

Due to contamination of protein isolate samples from Regimen A and Regimen B, an additional protein isolation was necessary for both samples. Isolate produced from the initial process was discarded.

The following protocol deviation was reported to the Study Director via email:

1. Protocol states "Equipment will be cleaned and inspected thoroughly prior to the beginning of processing and also will be cleaned sufficiently between samples in order to minimize the risk of cross-contamination. Pre-process verification (per facility SOP) is not documented prior to processing Regimen A in the equipment log for the Great Western sifter used to sieve flour on June 26, 2009. Pre-process verification ensures that a visual inspection of the machine was done

and that it was clean and operational. Cleaning between samples (requested by protocol) is not documented in the data for Regimen B and C for the same piece of equipment.

The following facility SOP deviation was reported to the Study Director via email:

1. After degumming, the degummed oil is cooled to room temperature, centrifuged, and filtered. For all samples, the degummed oil was placed in walk-in cooler "C1" after degumming.

Fraction Shipment:

Frozen processed fractions packed in dry ice were shipped to William Kowite at Bayer CropScience in Research Triangle park, North Carolina via Federal Express priority overnight delivery on July 14 and 15, 2009. Duplicate factions were shipped on July 15, 2009. A "Shipment of Fractions (Chain of Custody)" and "Fraction Shipment and Packing List" forms accompanied each shipment.

CONCLUSIONS:

Commercially representative processed soybean fractions were produced and collected from the whole soybean samples received from MS Technologies.

DATA ARCHIVAL:

Record Transfer and Retention:

This processing report and raw data has been transferred to the Study Director, William J. Kowite, Ph.D. at Bayer CropScience.

GLP Technologies will archive the following study specific data:

-copy of the sponsor processing protocol
-exact copy of the processing report (main body)
-exact copy of the sample material balance
-exact copy of the original raw processing data (includes communication logs, calculations, and deviation forms, when applicable)
-exact copy of personnel records (names and initials of personnel with processing study duties)
-exact copy of receiving record(s)
-exact copy of shipping record(s)
-exact copy of shipping bills of lading

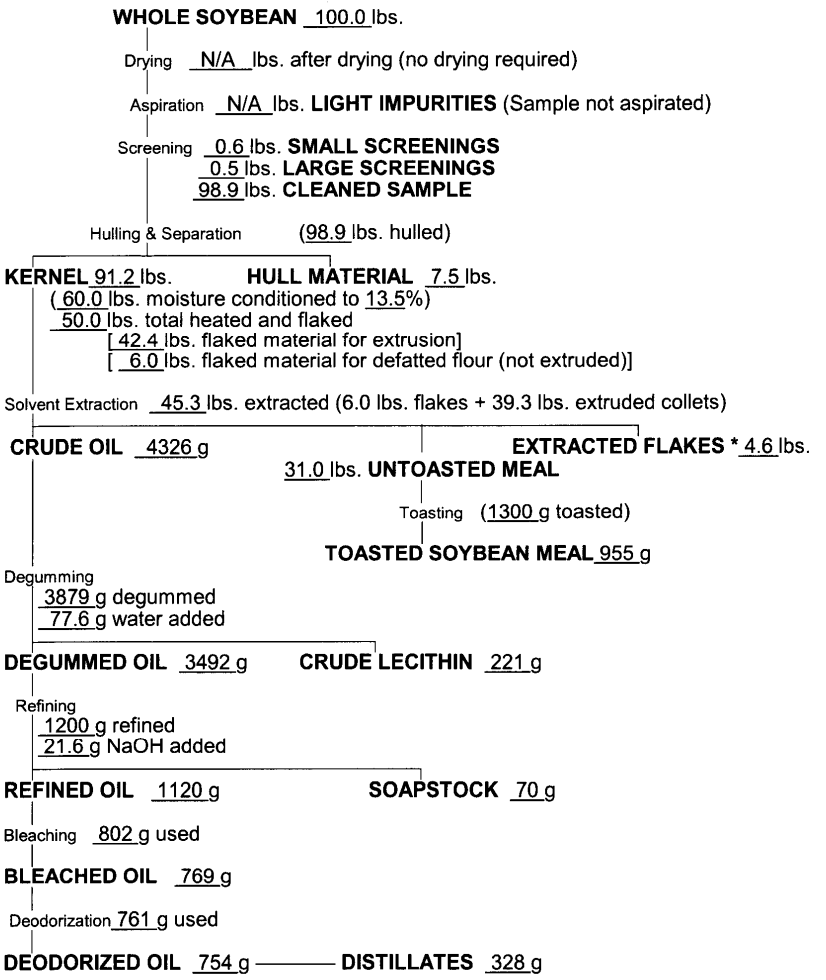
GLP Technologies will archive the following non-study specific data indefinitely:

-original freezer and cooler temperature records
-original equipment logs (includes scales, temperature recording devices, and processing equipment records)
-CVs of personnel and training records

FORM H.202 Revision 00

SOYBEAN PROCESSING MATERIAL BALANCE

Sample # **1 (Regimen A)** Code # **HT08SOY001-01-01**

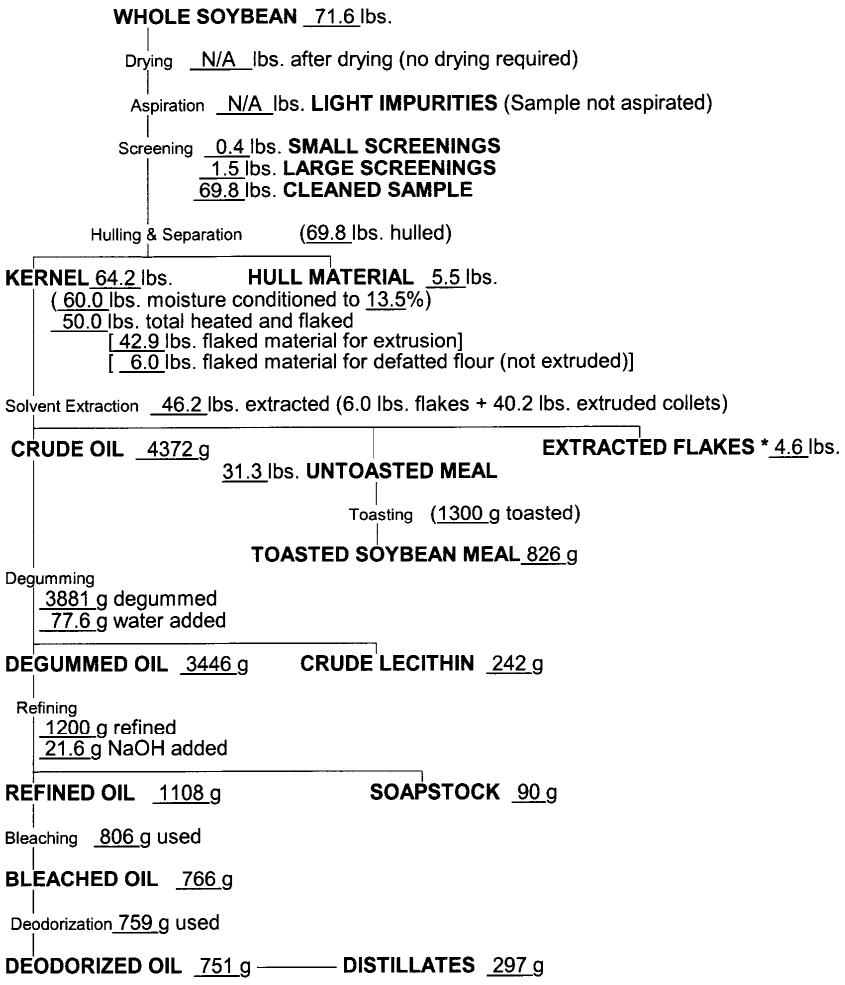


* 2.8 lbs. of defatted soy flour was produced from the defatted flakes.
600 g of flour used to produce 163 grams of **SOYBEAN ISOLATE**.

FORM H.202 Revision 00

SOYBEAN PROCESSING MATERIAL BALANCE

Sample # **2 (Regimen B)** Code # **HT08SOY001-01-02**

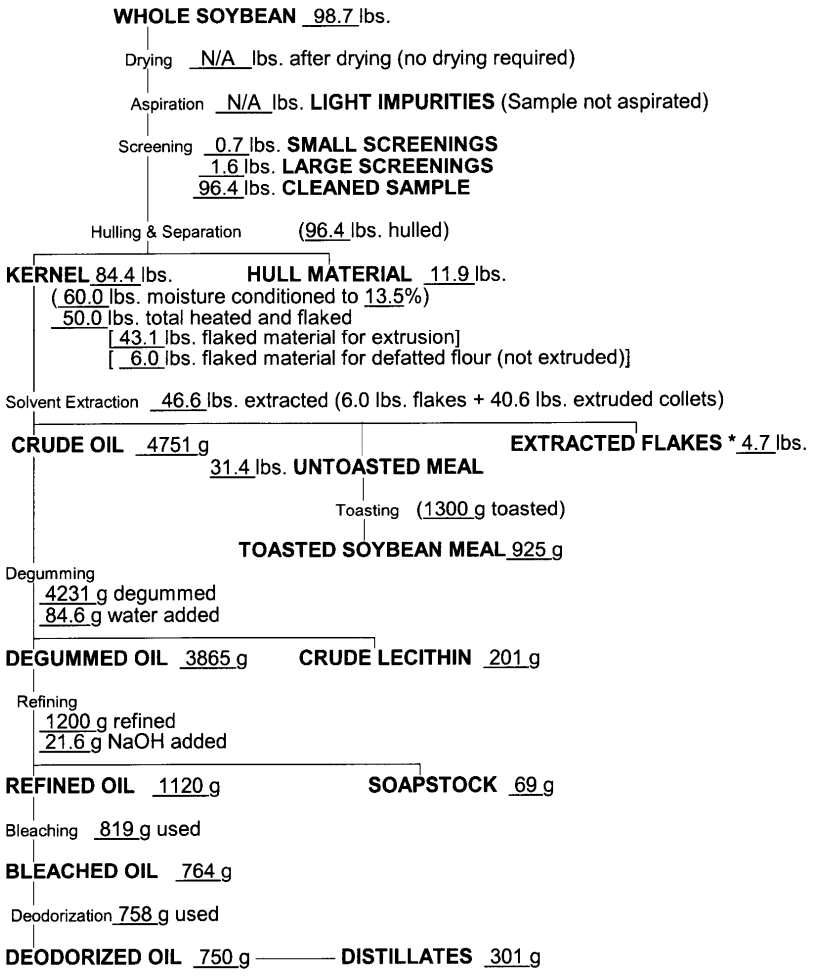


* 2.7 lbs. of defatted soy flour was produced from the defatted flakes.
600 g of flour used to produce 165 grams of **SOYBEAN ISOLATE**.

FORM H.202 Revision 00

SOYBEAN PROCESSING MATERIAL BALANCE

Sample # **3 (Regimen C)** Code # **HT08SOY001-01-03**



* 2.3 lbs. of defatted soy flour was produced from the defatted flakes.
600 g of flour used to produce 153 grams of **SOYBEAN ISOLATE**.