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Title:Soxtec Reference Method for Oil in Oilseeds and Commodities		
Lab: USDA,GIPSA,FGIS,TSD,ARTS	Program: Oil Reference Program	
Revision: 4	Replaces: 3	Effective: 09/16/02

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"CONTROLLED DOCUMENT"

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Changes From Previous Revision

1. In section 3. References:
   Added *SPC for the rest of Us*.

2. In section 5. Equipment:
   (u) Added Boerner grain divider.
   (v) Added Polyethylene sample bags, 6" x 13" x 6 mil, and 8.75" x 17.75" x 6 mil.
   (w) Added Poly bag impulse sealer.

3. In section 6. Materials:
   (g) Added soybean bulk sample.
   (h) Added corn bulk sample.
   (i) Added sunflower bulk sample.

4. In section 7. Instrument Parameters:
   Moved to 7.1 and Changed title of section 7 to Quality Control Procedures.
   7.1 through 7.11 moved to 7.1.a through 7.1.k respectively.

5. Added section 7.2 Soybean, Corn, and Sunflower Lab Control Samples

6. In section 10.1 Data Entry:
   Clarified that the oil results are calculated and uploaded to LIMS through Excel.

7. In section 10.2 Acceptance Criteria:
   Completely reworked to describe the development of a control chart and the rules for using it.
   (b) Moved to c.

Table of Contents

1. Purpose and Scope of Application

The purpose of this Working Instruction (WI) is to establish the operational parameters, methodology, and requirements for the quality assurance and acceptability of data in the determination of crude oil in corn, sunflower seeds, soybeans, and processed commodity samples.

2. Analyst Qualifications and Responsibilities

a) The analyst will receive on-the-job training in the conduct of this WI from a technician who has prior experience and training in using the WI, and/or from the Supervisory Chemist (or Project Leader).

b) The analyst will follow the WI as written. The Supervisory Chemist (or Project Leader) is responsible for ensuring this WI is followed, and modified as necessary or appropriate. All revisions to this WI must be approved by the Branch Chief of the Analytical, Reference and Testing Services Branch, prior to implementation.

c) The analyst is responsible for checking that the difference between replicate analyses of a sample meets the tolerances specified in this WI. The analyst is also responsible for repeating the analysis on any sample that exceeds the tolerance.

3. References

- American Association of Cereal Chemists (AACC) Methods 30-20, 30-25, and 30-26.
- American Oil Chemists Society (AOCS) Ac 3-44, Ai 3-75.
- National Institute of Standards and Technology (NIST).
- Kenyon, A.S., Black, J.C., & Layloff, T.P. (1995) *J. Assoc. Off. Anal. Chem.* **78**, 1109-1111.
- Pitt, H., 1993: *SPC for the Rest of Us*. Addison-Wesley, 419 pp.

4. Safety and Hazardous Waste

Refer to the Technical Services Division Chemical Hygiene Plan.

5. Equipment

a) Analytical balance accurate to 0.1 mg and equipped with an electronic interface for data transmission.

b) Computer and software, equipped with a barcode wand, electronic data interface, Microsoft® Excel, and Laboratory Information Management System (LIMS).

c) Desiccator cabinet with shelves and desiccant tray.

d) Tecator Soxtec HT solvent extraction unit.

e) Tecator Soxtec service unit.

f) Metal extraction cups and cup holders.

g) Thimble supports and holder.

h) Thimble handler.

i) Thimble stands.

j) Forced-draft oven.

k) Reference weight set, NIST Class S or ASTM Class 1, including 1g, 10g, and 50g weights.

l) Explosion-proof fume hood.

m) Solvent dispenser.

n) Udy Cyclone Mill, equipped with a 2-mm round-hole screen for grinding soybeans and corn.

o) Stein M-2 Mill.

p) Mikro-Samplmill equipped with a 0.039” round screen for grinding soybeans for the AOCS Smalley series, or a 0.035” x 0.5” herringbone screen for grinding sunflower seeds.

q) Recirculating chiller unit.

r) NIST-traceable digital thermometer.

s) Wide-mouth pint or quart plastic sample bottles such as Seedburo No. 630 and 635

t) 50ml graduated cylinder with 1 ml increments.

u) Boerner grain divider

v) Polyethylene sample bags, 6" x 13" x 6 mil, and 8.75" x 17.75" x 6 mil.

w) Poly bag impulse sealer.

6. Materials

a) Hy-flo super cel (Celite, diatomaceous earth).

b) Petroleum ether, ACS.

c) Cellulose thimbles, 26x60mm, double thickness.

d) Desiccant: silica gel, grade 44, 3-4 mesh; or molecular sieve, type 4A, grade 513; or magnesium perchlorate anhydrous, reagent grade.

e) Absorbent cotton, fat free.

f) Humidity indicating cards.

g) Bulk sample of soybeans, minimum size: 1500 grams

h) Bulk sample of corn, minimum size: 1500 grams

i) Bulk sample of sunflower seeds, minimum size: 1500 grams

7. Quality Control Procedures

7.1 Instrument Parameters

IMPORTANT! Read and understand the manufacturer's operating manual before using the following equipment.

If any piece of calibrated equipment (Soxtec service unit, recirculating chiller unit, or forced draft oven) is found to be out of the acceptable range during the monthly routine verification, identify three samples, tested during the previous three days on which analyses were done prior to checking the equipment, and analyze them again using equipment that has been verified as being in tolerance with the corresponding standard(s). If the results of the repeated samples agree with the original results by the tolerance listed under 10.2 Acceptance Criteria, record this information in the equipment log – no further action is required. If the repeated results do not agree with the originals within the allowed tolerance, record this information in the equipment log, determine which customer(s) submitted samples for analysis during the time period in question, and alert them of the discrepancy.

7.1.a Soxtec Solvent Extraction Unit

1. The condenser valves should be tight enough to prevent leaking but still be able to be turned.

2. The extraction mode levers should move freely.

3. The unit's handle should lock when in the down position

4. The unit is connected to the recirculating chiller and service units via tubing and clamps.

7.1.b Soxtec Service Unit

1. Silicone oil is used to transfer heat from the service unit to the heating block of the extraction unit. A floating switch protects the service unit from overheating due to the oil level being too low, and an adjustable thermostat protects the unit from overheating should the main thermostat malfunction. If either of these safety systems cuts out, a corresponding lamp on the front panel will light, accompanied by an audible warning signal. To bring the unit back

into operation, correct the fault and press the reset button.

2. Once a month verify the accuracy of each service unit's thermocouple by comparing it's reading to a NIST-traceable thermometer. Remove the service unit from operation if its temperature reading differs from the NIST-traceable thermometer by more than 3 °C and cannot be adjusted to agree.

7.1.c Analytical Balance

1. Refer to the balance-operating manual for information regarding the operation and calibration of the balance.

2. Leave the power to the balance on at all times to ensure that the balance is at temperature equilibrium with the surroundings. If power is interrupted, the balance must be allowed one hour to return to equilibrium before a weighing is made (unless it is known that the interruption was brief, e.g., less than ten minutes, in which case the balance will reach equilibrium more quickly -- see Kenyon et al.).

3. Locate the balance in an area that is as free as possible of drafts and temperature fluctuations, preferably on a stone balance table with vibration-damping pads separating the table top from the legs.

4. The balance must be cleaned, serviced, and calibrated with NIST-certified weights twice a year by a qualified service engineer.

5. Calibrate the balance at the beginning of the workweek, and anytime the response seems questionable (see balance operating manual for instructions on calibrating the balance).

6. Make sure all balance doors are closed before reading weights.

7. On the first work day of the week, weigh the 1, 10, and 50 gram weights from a set of NIST class S or ASTM Class 1 weights to check the balance for drift or other malfunction.

    a. Record the weights and the date in the maintenance logbook.

    b. Gross deviation from the known weights (i.e., a milligram or more) indicates a malfunction. Remove the balance from service and have it repaired by a qualified service engineer. Record the action taken in

the logbook.

c. A small deviation from the known weights that is continuously in the same direction on all three weights, and that grows larger over time, is indicative of a drift error. Remove the balance from service and have it repaired by a qualified service engineer. Record the action taken in the logbook.

7.1.d Reference Weight Set

Recertify the calibration of the reference weights once a year by sending them to a facility equipped to provide the service using NIST-traceable standards.

7.1.e Udy Grinder

Disassemble and clean the grinder after grinding every sample.

1. To clean the grinder, turn it off, remove the lid, and vacuum or dust all parts, including the cover, impeller, grinding ring, screen, separator, cyclone, and filter assembly. Vacuum or dust all other accessible surfaces.

2. The grinding ring and the screen in the grinder must be replaced after not more than 5,000 samples have been ground. The grinding ring and the screen must be replaced at the same time.

3. Replace the grinding screen separately if it becomes damaged before the scheduled maintenance.

4. When a belt is replaced, always replace both belts. After replacing the belts, make certain the round sleeve that houses the motor is replaced properly. The air vents at the base of the sleeve must be toward the back of the grinder. Maintain a record of all grinder service and maintenance.

5. Some grinders have a switch for reversing the grinder motor. Always operate the grinder with the motor switch in the forward position.

7.1.f Mikro Samplmill

1. Prior to using the mill, the grinding screen and all gaskets should be inspected for wear and replaced if damaged.

2. When finished grinding batches of eight or more samples, remove and clean all metallic parts, gaskets, and brushes with warm soapy water. Rinse and dry completely before reassembling.

3. Check the mill's oil level before each grinding session. The oil level should be maintained at the line marked in the sight glass while the mill is not running.
4. Change the oil once a year. Use SAE-20 grade oil.

7.1.g Explosion-proof Fume Hood

The Safety Officer will inspect the airflow rate of the fume hood once per quarter.

7.1.h Solvent Dispenser

1. The plunger should move smoothly. If it does not, wash it in warm soapy water, rinse well, and dry completely before reinstalling.

2. After every cleaning, or at least once a month, verify that the dispenser is dispensing 50 ± 1 ml with the aid of a graduated cylinder. Adjust if necessary.

7.1.i Recirculating Chiller Unit

1. If the water level drops below the required amount, the unit will generate a fault notice on the front panel. Add tap water as necessary to return the unit to operation.

2. The temperature display must be 7-15 °C during analyses. Temperatures below this range may cause a disruption of flow due to freezing, while elevated temperatures may prevent sufficient cooling in the condensers. Either condition would cause an accelerated loss of solvent from the

extraction units and, most likely, error in the analysis.

3. Once a month verify the accuracy of the unit's thermocouple by comparing it's reading to a NIST-traceable thermometer. Remove the chiller from operation if its temperature reading differs from the NIST-traceable thermometer by more than 3 °C and cannot be adjusted to agree.

7.1.j Forced Draft Oven

Once a month verify the accuracy of the oven's thermocouple by comparing it's reading to a NIST-traceable thermometer. Remove the oven from operation if its temperature reading differs from the NIST-traceable thermometer by more than 4 °C and cannot be adjusted to agree.

7.1.k Desiccator Cabinet

The relative humidity in the desiccator must be kept to ≤ 20%. Use a humidity indicating card to determine when the desiccant must be replaced or regenerated. Replace or regenerate the humidity indicating card at the same time.

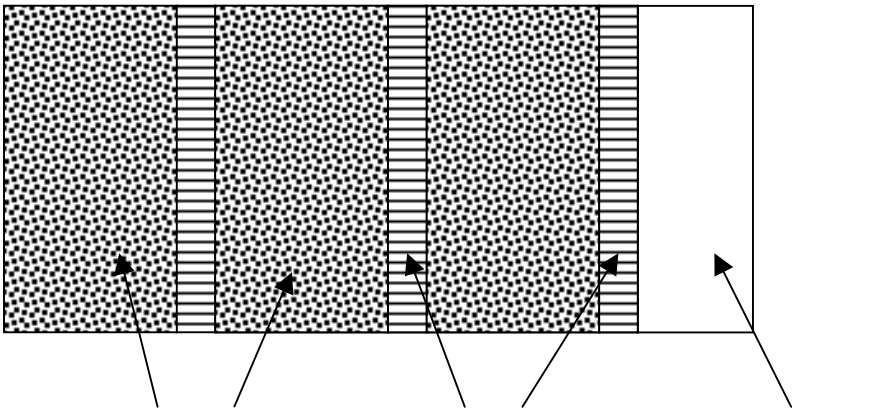
7.2 Soybean, Corn, and Sunflower Lab Control Samples

7.2.a Sample Preparation

1. Mix the bulk sample thoroughly.

2. Using the Boerner divider, cut the bulk sample into portions of approximately 30 grams each (40 grams for sunflower seeds).

3. Put the individual portions into plastic sample bags and seal. One bag can accommodate three portions as long as the bag is sealed after the addition of each successive portion as shown below:



4. Place the individually sealed portions inside a second plastic bag and seal. Reseal the outer bag each time a portion is removed for testing.

5. Keep the portions in refrigerated storage until needed.

7.2.b Parameters for Use

Note: a batch of samples is defined as all samples analyzed on the same workday.

1. One portion of an LCS must be ground and analyzed with every batch of like samples.

2. The portion(s) must be retrieved from the refrigerated storage and be allowed to come to room temperature before grinding (about one hour).

3. Although only one portion of an LCS is required per batch, up to four may be kept outside the refrigerated storage in order to prevent delays in processing the next batch of samples.

8. Sample Analysis

8.1 Sample Preparation

8.1.a Sunflower Seeds

Sunflower seeds are ground in a 50/50 mixture with celite. For accurate results it is critical that the ratio remains 50/50 throughout the analysis. Therefore, great care must be taken to get all of the mixture through the grinding mill and recovered.

1. Check the Mikro Samplmill to make sure it is clean, the screen and sealing gaskets are in good shape, and the mill is assembled correctly (see owners manual). Line the aluminum canister with a plastic sample bag before clamping it in place on the mill.

2. Weigh 40 ± 2 grams of sample and transfer to a plastic sample bottle.

3. Add an equal weight (± 0.1 gram) of celite to the sample bottle and mix by shaking. The celite must have been dried at 130 °C for at least two hours within the past two days.

4. Slowly pour the sample/celite mixture into the hopper being careful to avoid dispersing the celite into the air, then turn the mill on.

5. Feed the mixture into the mill by turning the feed screw in a clockwise direction.

6. Tap the hopper to loosen the remnants of celite and use a brush to get as much of the mixture as possible to go through the mill.

7. Turn the mill off and disassemble it, being careful to catch the ground sample in a clean, dry pan as the mill is opened and parts are removed.

8. Brush the ground sample from all parts into the pan.

9. Remove the aluminum canister from the mill and transfer the ground sample from it to a plastic sample bottle.

10. Add the ground sample from the pan to the plastic sample bottle and mix

thoroughly by shaking vigorously by hand.

11. Reassemble the mill.

12. Preferably, ground sunflower samples should be allowed to set overnight before analyzing them to permit the oil to become more evenly distributed throughout the mixture. However, time constraints may require the analysis to be performed without this delay.

8.1.b Soybeans

1. Turn on the Udy grinder and allow it to reach maximum RPM.

2. Place a clean, empty Udy sample jar under the cyclone body and slowly pour the sample into the feed hopper a few beans at a time and allow gravity and vibration to move the sample through the feed gate, or pour the entire sample into an automatic feed hopper if one is being used. A 30-gram portion should be completely ground in approximately 90 seconds.

3. Press the plunger two to three times after the sample is ground and tap firmly above the clear plastic cyclone body top clear the grinder of all loose meal.

4. Hold the plunger down, remove the sample jar, and cap it. Turn off grinder. Technicians should remove all jars from the grinder in a similar manner to obtain uniform drying of ground samples. Shake and rotate the sample jar to loosen caked meal.

5. Disassemble and clean the grinder according to information in section 7.1.e after grinding each sample.

8.1.c Corn

1. Place the sample into a Stein Mill cup, raise the cup to the upper cup support and lock in place with the retaining knob.

2. Turn the mill on and crack the corn by grinding for five to ten seconds.

3. Lower the cup and brush the corn from the blade, blade arbor, and lower surface of the upper cup support into the cup.

4. Transfer the sample from the cup to a glass sample bottle.

5. Grind the cracked corn on the Udy following the procedure under 8.1.b.

8.1.d Processed Commodities

Most are already in ground condition. Check with CTL supervisor for any necessary prep work.

8.2 Sample Analysis

1. Processed commodity samples are analyzed in single cuts. All other samples are analyzed in duplicate.

2. Turn the service unit(s) and the recirculating chiller on.

3. Open Excel and enter the sample ID into a spreadsheet with the barcode scanner, or manually enter the ID if the sample has no barcode.

4. If the sample is a processed commodity, enter the commodity code into the spreadsheet.

5. Place a dry, empty cellulose thimble on the balance. Make sure the thimble has no tears or holes, and that it has been cleaned if previously used.

6. Tare the balance by pressing the tare button.

7. Weigh 4 ± 0.10 grams of the sample into each of two thimbles. **Note: For processed commodity samples, weigh 3 to 5 grams into only one thimble.**

8. Transfer the sample weight to the spreadsheet by pressing the send button on the balance.

9. Remove the thimble from the balance and plug the opening with a piece of cotton.

10. Press a thimble adapter onto the thimble and place the thimble into a thimble stand.

11. Enter the ID number of a clean, dry extraction cup into the spreadsheet.

12. Tare the balance and place the extraction cup on the balance.

13. Enter the weight of the cup into the spreadsheet by pressing the send button on the balance.

14. Remove the extraction cup from the balance and place it in a cup holder.

15. Repeat steps 2-13 for the remainder of the samples.

16. Check the temperature display of the recirculating chiller and the service unit(s). The water should be 7-15 °C and the silicone oil should be 83-87 °C. Make adjustments if necessary.

17. Insert the thimbles into the base of the condensers and move the extraction mode levers to the boil position to allow the magnets to fasten to the thimble adapters.

18. Move the extraction mode levers to the rinse positions.

19. Dispense 50 ± 1 ml of petroleum ether into each extraction cup.

20. Place the extraction cups into position and clamp in place against the condensers by lowering and locking the handle.

21. Move the extraction mode levers to the boil position. Record the time in the Excel spreadsheet. Also record the time for the rinse and evaporation cycles.

22. Boil with the thimbles immersed for one hour. Make sure the condenser valves are open.

23. Move the extraction mode levers to the rinsing position.

24. Rinse for 3 ½ hours if sunflower seeds, 2 ½ hours for everything else.

25. Close the condenser valves, press the air switch on the service units, and open the evaporation valve on the extraction units. This will collect the solvent in the condensers.

26. Close the evaporation valve and turn off the air and power for the service units.

27. Remove the extraction cups and thimbles from the extraction units.

28. Put the used thimbles in the fume hood and reclaim the ether from the extraction units. Turn off the recirculating chiller.

29. Used thimbles can be emptied, scraped and/or vacuumed clean, and reused. The cotton plugs may also be reused.

30. Dry the extraction cups in an oven at 100 ºC for 30-35 minutes. Record the time in and out of the oven on the oven log, Form Oil-1.

31. Place the extraction cups in a desiccator and allow them to cool to room temperature.

32. Tare the balance and place the extraction cup on the balance.

33. Enter the weight of the extraction cup plus oil into the spreadsheet by pressing the send button on the balance.

9. Determination

The calculation for the percentage of oil in the sample (on an "as is" basis) is done using the following equation:

$$\%\ Oil = \frac{(Wt_1 - Wt_2)}{sample\ \ weight} * Factor$$

Where Wt_1 = weight of extraction cup + oil,
Wt_2 = weight of extraction cup.
Factor = 200 for sunflower samples, 100 for all other samples

Oil results are normally corrected to a particular moisture basis using the following equation:

$$\%\ Oil_{MB} = \frac{Oil_{AI}\ x\ (100 - MB)}{100 - M}$$

Where Oil_{MB} is the oil content of the sample on the desired moisture basis,

Oil_{AI} is the "as is" oil content of the sample,
MB is the desired moisture basis, and
M is the moisture content of the sample.

10. Data Validation

10.1 Data Entry

Oil results are either sent directly to a LIMS via an Excel spreadsheet interface, or are hand-entered into the spreadsheet on an "as is" basis. The oil is then moisture corrected by the spreadsheet using the calculation above.

10.2 Acceptance Criteria

10.2.a Precision can be verified by comparing the differences between replicate analyses. Differences between replicates should be ≤ 0.20% oil for soybean and corn samples, and ≤ 0.60% oil for sunflower samples.

1. If the difference between the LCS's replicates falls within the cut tolerance for that oilseed, the average of the two replicates is checked for accuracy as under 10.2.b below.

2. If the difference between the LCS's replicates exceeds the tolerance, the data is stored in the LIMS with the designation of "Fail" and the sample must be repeated (unless an assignable cause is known to have caused one of the two replicates to be in error, in which case the unaffected replicate may be considered "Valid" and used in the accuracy check below).

10.2.b Accuracy can be verified by comparing the LCS's replicate analyses to the target value.

1. Determining the Target Value

   a. In order to obtain a good estimate of the method's variability and have a control chart in place for a new LCS by the time the old LCS is expended, an action date should be estimated, and twenty portions of the new LCS should be analyzed in duplicate (one portion every couple of days over a

period of eight-to-ten weeks). If for any reason this cannot be accomplished before the new LCS is needed, a less robust estimate can be developed by analyzing ten portions of the new LCS in duplicate on the same day.

b. Calculate the average value and standard deviation of the results.

c. The average is the target value for all future analyses of the LCS.

d. The standard deviation value is used to set the acceptable tolerance of all future analyses of the LCS.

2. Comparing the LCS to the Target Value

a. The LCS result is acceptable and data for all like samples within the batch will be validated and reported to the customer (pending agreement of the other samples' replicates with the tolerance specified under 10.2.a) if none of the following is true:

i. The result is outside the 3 standard deviation lines
ii. The result is the 8th point in a row on one side of the average line
iii. The result is the 6th point in a row all going up or down
iv. The result is outside the 2 standard deviation lines, along with more than 5% of the others

b. If any of the above scenarios are true, the data for all like samples within the batch are stored in the LIMS with the designation of "Fail" and the process, equipment, materials, etc. are investigated for an assignable cause.

i. If an assignable cause is found, document and correct the problem.

ii. Whether or not an assignable cause was found, grind and analyze another portion of the affected LCS, along with all of the samples from the batch.

1. If the LCS does not violate any of the four rules above the entire batch will be validated and reported to the customer.

2. If the LCS again violates one of the four rules above, the Project Leader (or, in his/her absence, the technician) must use discretion

in deciding how to proceed.

10.2.c Commodity samples

Repeat the analysis on any sample for which a Quality Control (QC) request is issued through LIMS.