EPL BIO ANALYTICAL SERVICES

Crude Fat Determination in Maize Grain and Canola Seed Using an Automated Hydrolysis System and an Automated Extraction System

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Abstract

Manual methods for the determination of fat in grain are labor intensive, use concentrated hydrochloric acid, and are subject to analyst variability. New technology, using an automated hydrolysis system and an automated extraction system, reduces the actual hands-on time of the analyst, utilizes more dilute (safer) 3N HCl in a closed system, and eliminates analyst inconsistency regarding pipetting technique and transferring of solutions. After automated hydrolysis, the samples were extracted in the automated unit containing a mixture of 45% ethyl ether, 45% petroleum ether and 10% ethyl alcohol. The results of the automated analysis fell within the published data ranges for both matrices. The relative standard deviation (RSD) was less than 15% (2.74% for maize and 0.534% for canola). The automated method allows for increased sample throughput, reduced solvent consumption, enhanced safety and the elimination of technician variability.

Objective

To validate an analytical method for the determination of crude fat in maize grain and canola seed.

Chemicals and Reagents

- Petroleum Ether, Chromatography Grade, EMD, Billerica, MA
- Ethyl Ether, Diethyl Ether, HPLC Grade, Honeywell Burdick & Jackson, Muskegon, MI
- Ethyl Alcohol, Anhydrous, Denatured, EMD, Billerica, MA
- 6N Hydrochloric Acid (HCl), Volumetric Solution, J.T. Baker, Center Valley, PA
- Deionized (DI) water, as delivered by the Barnstead NANOpure water system
- Celite, Diatomaceous Earth, Not Acid Washed, 545 filter aid, Fisher Scientific, Fair Lawn, NJ

3N HCl Solution
Per liter of 3N HCl solution to be prepared, add 500 mL of 6N HCl to 500 mL of DI water, mix thoroughly, and let cool.

45:45:10 Petroleum Ether: Ethyl Ether: Ethyl Alcohol (extraction solution)
To make 4 L, combine and mix 1800 mL of petroleum ether, 1800 mL of ethyl ether and 400 mL of ethyl alcohol into a suitable container.

Test Matrices: The method was validated using commercial lines of dent corn (*Zea mays*) and canola seed (*Brassica napus*).

Equipment

- Retsch centrifugal grinder with 0.75 mm screen, Hahn, Germany
- Balances, 0.1 mg accuracy, Mettler-Toledo, Columbus, OH
- Oven – capable of maintaining a temperature of 110 ± 10°C (AnkomRD Dryer), Ankom Technology, Macedon, NY
- Filter bags – Ankom Technology XT4, Ankom Technology, Macedon, NY
- Heat Sealer, TEW Electric Heating Equipment CO, LTD., Reseda, CA
- Solvent resistant marking pen
- Desiccator pouch – MoistureStop weigh pouch or equivalent, Ankom Technology, Macedon, NY
- Extraction instrument – Ankom^{XT15}, Ankom Technology, Madecon, NY
- Hydrolysis instrument – Ankom^{HCL} Hydrolysis System, Ankom Technology, Macedon, NY
- Ankom blotter, Ankom Technology, Macedon, NY
- Weigh paper
- Paper Towel, Wipe-All or equivalent, Kimberly-Clark, Surrey, UK

Procedures

The extraction process described herein is similar to AOCS Method Am 5-04 *(1)*. The starting points for this method were taken from the ANKOM^{HCl} manual *(2)* and the ANKOM^{XT15} manual *(3)*. Maize grain and canola seed samples were ground with dry ice using a Retsch centrifugal grinder equipped with a 0.75 mm screen. Samples were stored frozen at -20°C.

- Sub-samples (0.75 g) of grain and seed were weighed into tared filter bags. In addition, celite was weighed (0.5g) onto weigh paper and transferred into the filter bags. Two separate filter bags of only celite were weighed to serve as blanks. The bags were heat sealed within approximately 4 mm from the top of the bag.
- Samples were analyzed separately per matrix. Samples were placed into the Ankom^{HCl} Hydrolysis System with the addition of 500 mL of 3N HCl solution. Samples were hydrolyzed at 90°C for 60 minutes with a rinse cycle of 40 minutes.
- After hydrolysis the filter bags were spread out between paper towels and blotted using the Ankom blotter. The blotting was repeated at least once with fresh paper towels to remove excess moisture.

Procedures

- The sample bags were then placed into a drying oven set at 110°C for 3 hours.
- Samples were removed from the oven then were placed into a desiccant pouch to cool. The desiccant pouch contained a pH test strip to show the acidity of the samples. If they were still acidic they were placed back into the drying oven at 30 minute increments until no longer acidic.
- Once cooled, samples were weighed and returned to the desiccant pouch until extraction.
- Samples were then placed into the Ankom^{XT15} extraction unit. The extraction solution was added and the instrument was set at 90°C for 60 minutes.
- When the extraction was complete, the sample bags were removed and placed again into the drying oven for 30 minutes at 110°C.
- Samples were removed from the oven then placed into a desiccant pouch to cool. Once cooled, a final weight was taken.

Results/Discussion

Seven replicate sub-samples of maize grain and canola seed were analyzed to determine the precision of the developed method via calculation of the relative standard deviation (RSD). Crude Fat concentrations were calculated on a dry weight basis %DB. Crude fat results ranged from 5.10 to 5.47% DB for maize grain and 43.7 to 44.4% DB for canola seed. The RSD was 2.74% for maize grain and 0.534% for canola seed (Table 1).

Table 1. Summary of Sample Analysis Results (Precision Assessment)

Matrix	Mean (n=7) (%DB)	Std. Dev.	RSD (%)
Maize Grain	5.24	0.144	2.74
Canola Seed	44.1	0.235	0.534

Conclusions

An analytical method for the determination of crude fat in maize grain and canola seed was successfully validated. The method demonstrated acceptable accuracy and precision. The precision for both analyses was less than the 15% RSD maximum required for successful validation of the method. The results of the maize grain were comparable to the published range of 3.1 to 5.8% DB *(5)*. The results of the canola seed replicates were also comparable to the published range of 42.7 to 43.1% DB *(4)*.

References

(1) AOAC. Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction. Method Am 5-04. In *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 6th ed.; Firestone, D., Ed.; American Oil Chemists' Society: Champaign, IL, 2009

(2) Ankom Technology. In ANKOM^{HCl} *Hydrolysis System Operator's Manual*; Ankom Technology: Macedon, NY, 2008; pp12-18

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(4) Fereidoon Shahidi, *Canola and Rapeseed Production, Chemistry, Nutrition and Processing Technology* (New York: Van Nostrand Reinhold, 1990), p. 278.

(5) OECD. Consensus Document on Compositional Considerations for New Varieties of Maize (Zea Mays): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites. Aug. 20, 2002. *Environment Directorate Joint meeting of the chemicals committee and the working party on chemicals, pesticides and biotechnology. Series on the Safety of Novel Foods and Feeds, No.6.* p. 20