Determination Of Sulfur in Canola Seed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

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Abstract

Canola seed has been developed from rapeseed for production of edible oil with low erucic acid and feed meal with low glucosinolates. In the canola plant sulfur is an essential component of both structural molecules (amino acids) and functional species (such as glutathione and thiamine). Glucosinolates also contain sulfur. The canola plant has high demand for sulfur and develops high sulfur content in seed. However, when canola seed is processed to produce canola oil, sulfur poisons the nickel catalyst commonly used for hydrogenation. In addition, the aglycone derivatives of glucosinolates including isothiocyanates and thiocyanates are toxic to poultry and swine. Since the 1920s gravimetric methods (AOAC 920.10 and 923.01) have been used for sulfur determination in plants. Although ICP-OES methods have been published since the 1990s, as the sulfur emission signals are in the ultraviolet region and are susceptible to interferences, rigorous sample digestion using $HClO_4$, aqua regia or HF was necessary[1], which poses safety concerns. In addition, the interference of minerals could not be removed by any digestion. Here we present an improved method which is safe and efficient for canola seed. Sulfur was quantified by ICP-OES using indium as an internal standard after microwave assisted digestion with dilute nitric acid. The method was validated for accuracy and precision on commercially available canola seed in which excessive amounts of interfering elements were present, followed by 100% confirmation with a rapeseed Certified Reference Material. With a limit of quantitation (LOQ) of 0.025%, this method has a 3.7% RSD and average recovery of 99.2%. Copper, iron, manganese, and zinc can be determined simultaneously.

Objective

Develop and validate an analytical method for the determination of total sulfur in canola seed.

Materials and Methods

Chemicals and Reagents

- Sulfur as methane sulfonic acid (MSA) standards were prepared.
- Indium of 2 µg/mL concentration was used as internal standard.

Test Matrices

- The method was validated using rapeseed (colza) as European Reference Material ERM®-BC367, and commercially available canola seed.

Equipments

- MARS 5 microwave system, MARS Xpress vessels, CEM (Matthews, NC, USA)
- Inductively coupled plasma, optical emission spectrometer, Optima 2000, or Optima 7300 with WinLab32 software, Perkin Elmer (Waltham, MA, USA)
- Purelab Flex water purification system, ELGA (Woodridge, IL, USA)
- Grinder, Retsch (Haan, Germany)

Sample Preparation

- Frozen samples were ground to pass through 0.75 mm sieve.
- 1.0 g sample was weighed.
- Added 10 mL of trace metal grade nitric acid and 10 mL de-ionized water.
- Sample dissolution was carried out by microwave (Table 1).
- Digested samples were diluted to 25 mL and filtered through syringe filters.

Table 1. Microwave program.

Power (watts)	Max Power %	Ramp (min)	T (°C)	Hold (min)
1600	100	15	200	15

Microwave: MARS 5.

ICP-OES Analysis

- Analyte: Sulfur (S) at 181.980 nm
- Calibration standards: 10, 50, 100, 300 and 600 µg/mL
- Internal standard: Indium (In) at 325.609 nm
- Calibration parameters: Linear, Minimum Correlation Coefficient 0.99

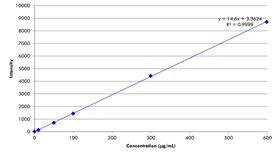
Table 2. ICP-OES parameters.

	Parameters	Settings
Gas flow (L/min)	Plasma Flow	13
	Auxiliary Flow	0.2
	Nebulizer Flow	0.8
RF	RF Power (KW)	1.5
Plasma	View	Radial (S), Axial (In)
	View Distance (mm)	15.0
Sample Introduction	Flow Rate (mL/min)	1.5
	Rinse (sec)	90
	Delay Time (s)	100

Results: Linearity

Sulfur Linear Range

Figure 1. Calibration curve of sulfur.



Results: Validation

Validation with Canola Seed and Certified Reference Material (CRM)

Table 3. Test results of CRM and commercially available canola seed.

Matrices	Test Content (g/kg)	Certified Content (g/kg)	Test RSD	Certified Uncertainty Values		Recovery (%)
				(g/kg)	RSD	
ERM® – BC367	10.3	10.3	3.01 %	0.5	4.85%	100
Canola Seed*	6.01	N/A	3.76 %	N/A		99.2

*The calculations were based on seven samples and three spikes.

Results: Reproducibility

Precision of Sulfur Determination from Two Analysts

Table 4. Precision assessment of sulfur determination by two analysts on two separate days.

Sample Set	Mean (% DB)	Standard Deviation	RSD %
Set 1	0.619	0.0184	2.97
Set 2	0.612	0.0281	4.58
Set 1 & 2	0.615	0.0236	3.84

Calculations were based on 20 samples in each sample set. DB = Dry Basis.

Results: Reproducibility

Precision of Sulfur Determination from Multiple Analysts

Table 5. Precision assessment of sulfur determination by combined study data.

Sample Set	Sulfur Content (% DB)	Mean (% DB)	Standard Deviation	RSD %
Set 1	0.583	0.607	0.0412	6.78
Set 2	0.619			
Set 3	0.588			
Set 4	0.681			
Set 5	0.594			
Set 6	0.668			
Set 7	0.553			
Set 8	0.585			
Set 9	0.625			
Set 10	0.573			

Calculations were based on the QC data acquired in 10 sets of 40 samples in each set analyzed using two instruments in 3 weeks. DB = Dry Basis.

Discussion

Method Validation

- 100% confirmation was achieved in EPL test result of the CRM (Table 3).
- RSD and recovery results of commercial canola seed and CRM canola seed showed good accuracy and precision in total sulfur determination (Table 3).
- Excellent precision was demonstrated by the QC data from multiple sample sets (Table 4 and 5).

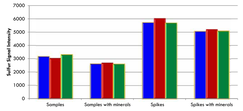
Discussion: Interference

Interference of Minerals to Sulfur Determination

Sulfur signals of the canola samples with and without sulfur spikes were measured to show the signal differentiation by addition of minerals (Fig. 2).

The result demonstrated the minerals in canola seed adversely impact sulfur determination. Sulfur signal suppression of >20% was observed, which resulted in low sulfur spike recoveries (60 - 70%).

Figure 2. Sulfur signal suppression by minerals. Samples, canola seeds; Spikes, canola seeds spiked with 1 × sulfur; minerals, 0.5 – 6 µg/mL Cu, Fe, Mn, Zn, Na, 100 – 300 µg/mL Ca, K, Mg and P.



Discussion: Interference

Canola meal is usually referred to as a good source of essential minerals (Table 6) compared to other vegetable-origin oilseed meals[2, 3].

Since mineral contents are usually expected to be invariable after sample digestion, the interference of the component minerals in canola to sulfur determination is inevitable, and is impossible to be removed or reduced by any sample digestion. Therefore, an internal standard is necessary and critical (Table 7).

Table 6. Range in mineral composition of low erucic acid rapeseed meal (DM basis).[2]

Minerals	Low Erucic Acid Rapeseed Meal*		
	Samples	Mean	Range
Calcium, %	589	0.74	0.49–0.99
Phosphorus, %	597	1.12	0.94–1.39
Magnesium, %	556	0.53	0.39–0.68
Potassium, %	557	1.28	1.11–1.46
Sodium, %	557	0.06	0.00–0.31
Sulfur, %	379	0.71	0.54–0.89
Chloride, %	137	0.12	0–0.27
Iron, ppm	553	243.02	56.85–429.19
Zinc, ppm	553	61.25	10.53–111.96
Copper, ppm	553	5.92	0–24.24
Manganese, ppm	553	64.06	15.25–112.86
Molybdenum, ppm	553	0.93	0.31–1.55

Source: Dairy One Cooperative Inc. *Canola Meal, Dry Accumulated crop years: 05/01/2000 through 04/30/2010.

Discussion: Internal Standard

Comparison of Internal Standards

Indium was identified as the best internal standard for correction against interferences including the mineral interference demonstrated in Fig. 2, to achieve excellence in accuracy (Table 7) and precision (Table 3, 4 and 5).

Table 7. The impact of internal standard on the accuracy of sulfur determination.

Internal Standard	Sulfur Spike Recovery (%)
Indium	99.2
Yttrium	107
Germanium	111

Calculations were based on canola sample set of 7 samples and 4 spikes. The listed sulfur spike recoveries were the average value.

Discussion: Multiple Elements

Simultaneous Analysis of Multiple Elements

Table 8. The accuracy and precision of simultaneous analysis of multiple elements on ICP-OES.

Test Results	Cu	Fe	Mn	Zn	S
Content (%DB)	0.000290	0.00649	0.00374	0.00503	0.601
RSD (%)	3.34	3.33	1.69	1.59	3.76
Recovery (%)	99.8	92.1	94.7	102	99.2

Calculations were based on the average values of canola sample set of 7 samples and at least 3 spikes.

Conclusions

- An analytical method for the determination of sulfur in canola seed was successfully validated with satisfactory accuracy and precision for both commercial and CRM canola seeds.
- The National Research Council recommends that total dietary sulfur not exceed 0.2 percent on a dry-matter basis[4, 5]. EPL sensitivity and LOQ (0.025 %DB) fits the requirement.

References

1. Ackerman, L., Rohovec, J. and Šebek, O. (2012), Determination of total sulfur in fifteen geological materials using inductively coupled plasma-optical emission spectrometry (ICP-OES) and combustion/infrared spectrometry. Geostandards and Geoanalytical Research, 36: 407–414.
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