EPL BIO ANALYTICAL SERVICES

Determination of Molybdenum (Mo) in Canola Seed Using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

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Abstract

Molybdenum is a transition element needed in extremely low amounts for plant development. The molybdenum found in plant tissue is utilized by selected enzymes to carry out redox reactions, in particular for those processes involving nitrogen metabolism. Deficiencies in molybdenum could affect plant development as well as lower crop yield[1]. A method to determine the amount of molybdenum in canola seed with high sensitivity and selectivity was developed and validated. In the reported method, molybdenum is determined by ICP-MS after microwave assisted digestion with a reporting limit of 0.1 ppm. The relative standard deviation (RSD) as a measurement of the assay precision was less than 5% (n=8), and the mean of the spiking recoveries was 99.2%.

Objective

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

Chemicals and Reagents

- 10,000 ppm molybdenum reference substance from High Purity Standards (Charleston, SC, USA) was used to prepare calibration standards ranging from 0.00125 to 0.100 ppm.
- 10 ppm Multi-Component standard from Inorganic Ventures (Christiansburg, VA, USA) containing Li6, Sc, Y, In, Tb, Bi was used to prepare a 0.02 ppm internal standard.

Test Matrices

The method was validated using a non GM commercial variety canola seed, and Standard Reference Material1849a, powdered infant formula obtained from NIST.

Equipment

- MARS 5 microwave system, MARSXpress vessels, CEM (Matthews, NC, USA)
- Inductively Coupled Plasma, Mass Spectrometer, Bruker 820 with Quantum software, Bruker (Billerica, MA, USA)
- ELGA Purelab Flex water purification system, ELGA (Woodridge, IL, USA)
- Retsch grinder, Retsch (Haan, Germany)

Sample Preparation

- Frozen samples were ground to pass through 0.75 mm sieve.
- 0.5 g sample was weighed.
- 6 mL of trace metal grade nitric acid and 2 mL hydrogen peroxide was added.
- Sample dissolution was carried out following the microwave program in Table 1.
- Digested samples were diluted to 40 mL and filtered through regenerated cellulose syringe filters.

Table 1. Microwave program

Stage	Power (W)	Power (%)	Ramp (min)	Temperature (°C)	S (Stir)	Hold (min)
1	1600	100	5	50	0	0
2	1600	100	10	180	0	0
3	1600	100	2	200	0	20

Microwave: MARS 5.

ICP-MS Analysis

- Analytes: Molybdenum 98
- Calibration standards: 0.000125, 0.00500, 0.0500, 0.100 ppm
- Internal standards: Scandium 45, Yttrium 89, Indium 115, Terbium 159, Bismuth 209
- Calibration Parameters: weighted fit, max error 25%, Minimum Correlation Coefficient 0.99

Table 2. ICP-MS parameters

	Parameters	Settings
Gas flow (L/min)	Plasma Flow	17.5
	Auxiliary Flow	1.8
	Nebulizer Flow	1.00
	Sheath Flow	0.19
CRI Gases (mL/min)	Skimmer (with H2)	OFF
RF	RF Power (kW)	1.4
Sample Introduction	Sampling Depth (mm)	6.5
	Pump Rate (rpm)	5
	Stabilization Time (s)	35
	Spray Chamber (°C)	3
Ion Optics (volts)	First Extraction Lens	-1.00
	Second Extraction Lens	-189
	Third Extraction Lens	-242
	Corner Lens	-179
	Mirror Lens Left	45
	Mirror Lens Right	38
	Mirror Lens Bottom	39
	Entrance Lens	1.00
	Entrance Plate	-41
	Fringe Bias	-2.8
	Pole Bias	0.00
Quadrupole Scan	Scan Mode	Peak Hopping
	Dwell Time (ms)	20
	Points Per Peak	1
	Scans/Replicate	30
	Replicates/Sample	3

Results

Validation with Canola Seed and Certified Reference Material (CRM), 1849a

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

Matrices	Test Content (g/kg)	Certified Content (g/kg)	Test RSD	Recovery (%)
1849a	1.667	1.707	4.04 %	97.7
Canola Seed	0.480	N/A	2.50 %	99.2

The calculations for 1849a were based off 5 samples and the canola seed calculation were based on eight samples and three spikes.

Results

Precision of Molybdenum Determination From Two Analysts

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

	Mean (% DB)	Standard Deviation	RSD %
Set 1	0.486	0.0133	2.73
Set 2	0.432	0.0123	2.86
Set 1 & 2	0.459	0.0299	6.51

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

Results

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

	Molybdenum Content (% DB)	Mean (% DB)	Standard Deviation	RSD %
Set 1	0.534	0.494	0.0367	7.43
Set 2	0.526			
Set 3	0.504			
Set 4	0.475			
Set 5	0.436			
Set 6	0.426			
Set 7	0.505			
Set 8	0.514			
Set 9	0.508			
Set 10	0.512			

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

Discussion

Confirmation was achieved in the EPL test result of the CRM (Table 3).

RSD and recovery results of commercial canola seed and CRM showed good accuracy and precision in molybdenum determination (Table 3).

Excellent precision was demonstrated by the QC data in 12 sample sets (Table 4 and 5).

Results

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

Minerals	Low Erucic Acid Rapeseed Meal*		
	Samples	Mean	Range
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.

Conclusions

The EPL analytical method was successfully validated with satisfactory accuracy and precision for commercial canola seeds.

References

(1) Mengel, K., &Kirkby, E.A. (2001) Principles of Plant Nutrition (5th ed.) (p. 480). Dordrecht: Kluwer Academic Publishers.

(2) OECD. (2011). Revised consensus document on compositional considerations for new varieties of low erucic acid rapeseed (canola): key food and feed nutrients, anti-nutrients and toxicants. Series on the Safety of Novel Foods and Feeds No. 24.OECD Publishing.