

Determination of Phytic Acid in Soybeans and Black Sesame Seeds

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Key Words

- Reagent-Free Ion Chromatography (RFIC) System
- Dionex IonPac AS11-HC
- Food
- Nutrition

Introduction

Phytic acid, also known as inositol hexakisphosphate or phytate when in the salt form, is the storage form of phosphate in many plant tissues, especially seeds and grains. Phytic acid is not digested by humans, and is therefore not a dietary source of inositol or phosphate. In fact, because phytic acid is a good metal chelator, it is believed to have a negative nutritional impact on strongly chelating metals necessary for good health (e.g., iron and calcium) and could prevent their absorption by the intestine. For this reason and because phytic acid is thought to have a positive dietary impact as an antioxidant to prevent carcinogenesis, determining the phytic acid content of foods is of interest.

As a multivalent anion, phytate is ideal for determination by ion chromatography (IC), and there have been a number of studies in which IC was used for determining phytic acid in plants, foods, and animal feeds.¹⁻⁶ Talamond published a series of three papers using an IC method to determine phytate in foods.¹⁻³ These papers compared IC to a spectrophotometric (colorimetric) assay and reported the sample preparation methods needed to prepare food samples for IC analysis for phytate. It was separated with a Thermo Scientific Dionex OmniPac PAX-100 column using a sodium hydroxide eluent (with 1% isopropyl alcohol) and detected by suppressed conductivity. These papers reported the determination of phytate in flours prepared from cowpea (*Vigna unguiculata*) and millet^{1,2} as well as cereals (e.g., maize), oilseeds (e.g., peanuts), and legume seeds (e.g., kidney bean).³

The cereal and flour samples were acid-hydrolyzed in boiling water with 0.5 M HCl. After centrifugation, the sample was augmented with additional HCl to ensure that phytate was decomplexed from metals. For samples containing greater than 15% fat, fat was extracted with light petroleum ether.³ These studies found that the numbers determined by IC were less than those determined by the colorimetric assay, but noted that the colorimetric assay has known nonspecific detections (i.e., compounds other than phytate that are detected); therefore, an accurate determination should have values less than the colorimetric assay. It is possible that the fat extraction step could be replaced with a solid-phase extraction cartridge to remove hydrophobic compounds.⁴

Phillippy et al. used a Thermo Scientific Dionex IonPac AS7 column to determine phytate in roots and tubers, and postcolumn reaction with ferric nitrate and visible absorbance detection or evaporative light scattering detection (ELSD). They preferred absorbance detection due to its superior linearity and saw corrosion problems with the ELSD.⁴ Their study found phytate in 11 of the 15 root and tuber samples analyzed. They also observed similar results with the Dionex OmniPac™ PAX-100 column. Chen and Li, and Pontoppidan et al. reported the determination of phytate along with several inositol phosphates by IC with postcolumn reaction detection.^{5,6} Pontoppidan et al. monitored change in phytate concentrations in feed samples after different heat treatments.⁶

The method reported here uses the hydroxide-selective Dionex IonPac™ AS11 column to separate phytate from other anions (including the remaining chloride from the sample preparation) with an isocratic method. This column is paired with an eluent generator that produces the eluent automatically by just adding deionized water to the system, eliminating the time required and possible error associated with manual eluent preparation. Phytate is detected by suppressed conductivity detection. There is no preparation of acidic regenerant required for detection. Altogether, the system is a Reagent-Free™ IC (RFIC™) system. Therefore, the analyst just prepares the samples, puts them in the autosampler, adds DI water to the eluent bottle, and programs the system with the Thermo Scientific Dionex Chromeleon Chromatography Data System (CDS) software to determine phytate in samples. The IC method requires less than 10 min per injection. This application note describes the determination of phytate in soybeans and sesame seeds using an RFIC system.

Equipment

- Thermo Scientific Dionex ICS-2000* Ion Chromatography System including:
 - Dionex AS Autosampler
 - Chromeleon™ CDS software version 6.80 SR9 or higher
- Thermo Scientific Dionex OnGuard II Ag/H cartridge (P/N 057410)

*This application can be run on any ICS system capable of eluent generation.

Reagents and Standards

Deionized water (DI), Type I reagent grade,
18 MΩ-cm resistivity or better

Phytic acid sodium salt hydrate
($C_6H_{18}O_{24}P_6 \cdot xNa \cdot yH_2O$, SIGMA)

Hydrochloric acid 37% (HCl, Merck)

Conditions

Column:	Dionex IonPac AS11 Analytical (4 × 250 mm) (P/N 044076)
Guard:	Dionex IonPac AG11 (4 × 50 mm) (P/N 044078)
Eluent Source:	Thermo Scientific Dionex EluGen EGC II KOH Potassium Hydroxide Cartridge (P/N 058900) with Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column (P/N 060477)
Eluent:	65 mM KOH
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL
Temperature:	35 °C
Pressure:	~2200 psi
Detection:	Suppressed conductivity, Thermo Scientific Dionex ASRS 300 Anion Self-Regenerating Suppressor 4 mm (P/N 064554), recycle mode, current 200 mA

Preparation of Solutions and Reagents

Eluent Solution

Produce the eluent using the Dionex EluGen EGC II KOH cartridge and DI water supplied by the pump. Control the eluent concentration by Chromeleon CDS software. The RFIC degasser requires at least 14 MPa (2000 psi) of system backpressure to ensure optimal removal of electrolysis gas from the eluent produced by the eluent generator. Refer to the Dionex ICS-2000 Ion Chromatography System Operator's Manual, Document No. 031857, for instructions on adding backpressure.

Standard Solutions

1000 mg/L Phytic Acid Sodium Salt Hydrate Stock Standard Solution

Add 0.1 g phytic acid sodium salt hydrate ($C_6H_{18}O_{24}P_6 \cdot xNa \cdot yH_2O$) to a 100 mL volumetric flask, add DI water to dissolve the salt, bring to volume, and mix.

Working Standard Solutions

Transfer appropriate volumes of stock standard solution into 100 mL volumetric flasks and bring to volume using DI water. Table 1 shows a list of the working standard concentrations used and the volumes of stock standard needed to prepare them.

Table 1: Working standard concentrations and volumes of stock standard solution used for preparing 100 mL of working standard.

	Working Standard Concentration (mg/L)					Volume of Stock Standard Solution (mL)				
Analyte	L1	L2	L3	L4	L5	L1	L2	L3	L4	L5
Phytate	0.1	0.5	1.0	5.0	10	0.01	0.05	0.1	0.5	1.0

3.0 M Hydrochloric Acid

This preparation should be done in a fume hood. Add approximately 250 mL DI water into a 500 mL volumetric flask. Transfer 124 mL of 37% HCl into the same volumetric flask, bring to volume using DI water, and mix well.

Sample Preparation

Purchase soybean and black sesame seed samples from a local supermarket and grind to a fine powder. Weigh 0.2 g sample into a 50 mL glass bottle, add 10 mL 3 M HCl, and shake. Place the sample bottle in boiling water for 10 min. Let the sample cool to room temperature. Transfer the sample into a 50 mL volumetric flask and bring to volume using DI water. Filter the sample using filter paper (Whatman, P/N 1002 110). Transfer 2.5 mL of the sample into a 50 mL volumetric flask and bring to volume with DI water. Pass 6 mL sample through a 2.5 cc Dionex OnGuard™ II Ag/H cartridge prior to sample injection. For more information about how to prepare and use the Dionex OnGuard II Ag/H cartridge, please refer to the product manual (Document No. 031688).

To prepare the spiked sample, add 1 mL of stock standard solution into the sample before extraction to yield 0.1 mg/L phytic acid sodium salt hydrate after sample preparation.

Results and Discussion

Separation

Phytate is a multivalent anion that is strongly retained on an anion-exchange column. Generally either a high eluent concentration or a long run time is required for the determination of multivalent ions. The application described here uses a Dionex IonPac AS11 column designed for rapid elution of strongly retained anions.

When using a Dionex IonPac AS11 column and isocratic elution with 65 mM KOH, phytate is eluted in seven min (Figure 1). Under these conditions, the common inorganic ions elute early and do not interfere with phytate determinations. As HCl is used for extracting phytate from samples, the samples were passed through a layered Dionex OnGuard II Ag/H cartridge to ensure that there were no peaks (i.e., chloride) that interfered with phytate determination. The bottom trace in Figure 1 shows that there are no peaks that interfere with phytate determinations, and the Dionex OnGuard II Ag/H cartridge removes the majority of the chloride.

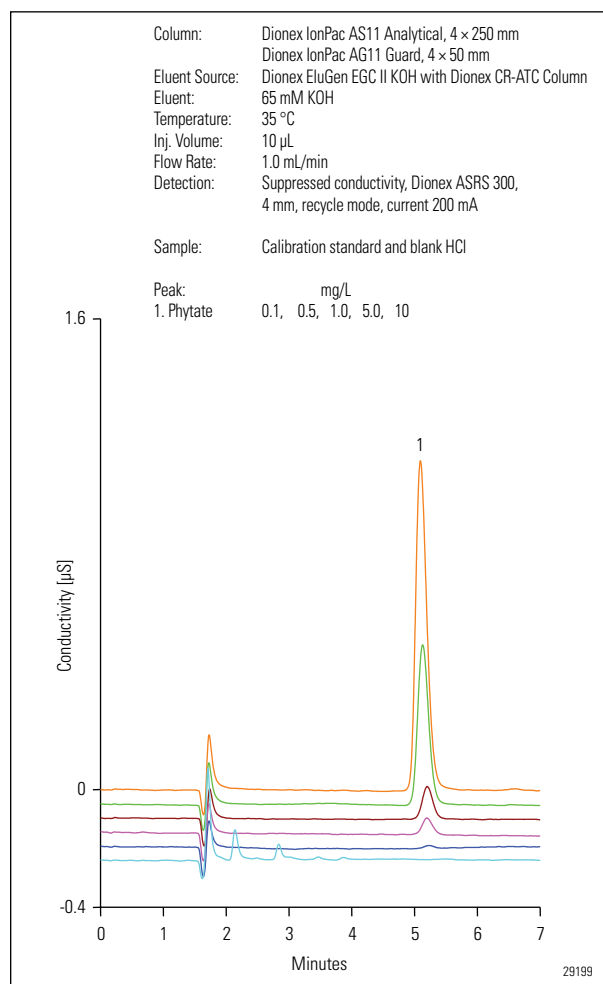


Figure 1: Overlay of the calibration standards and an HCl blank (treated with the Dionex OnGuard II Ag/H cartridge) chromatograms.

Method Detection Limited (MDL)

To estimate method sensitivity, 0.1 mg/L phytic acid sodium salt hydrate was injected seven times.

The MDL was then calculated using the equation $\text{MDL} = t(n-1, 0.99) (S)$, where $\text{MDL} = t(n-1, 0.99)$ = the Student's t value appropriate for a 99% confidence level and a standard deviation estimate with $n-1$ degrees of freedom, S = standard deviation of the replicate analyses. This yielded a value of 0.03 mg/L. During this experiment the conductivity background was $\sim 1.5 \mu\text{S}$ and background noise was $\sim 2 \text{ nS}$. Note that this MDL for phytate is more of an estimate due to the nature of the standard. The phytic acid sodium salt hydrate used to prepare the standard does not specify the molar amount of sodium or water, so the exact amount of phytate in the standard is not known. When converting the MDL concentration to mass (10 μL injection), the value is $3 \times 10^{-4} \mu\text{g}$. This is lower than the 0.5 and 1 μg determined using postcolumn reaction UV and ELSD,⁴ and compares favorably to the $1.32 \times 10^{-4} \mu\text{g}$ previously reported using IC with suppressed conductivity detection.² Talamond et al. also reported an MDL of $1 \times 10^{-4} \mu\text{M}$ with an injection of 200 μL and using a MW of 660.²

Method Calibration

The method was calibrated before sample analysis using five concentrations of phytic acid sodium salt hydrate standard ranging from 0.1–10 mg/L. Again, the concentration of standards were calculated based on the amount of the salt used to prepare the standard and not the amount of phytate, as that is unknown. Figure 2 shows the calibration plot with the calibration results (regression equation and coefficient of determination) displayed on the plot. The figure shows that the method is linear in the concentration range tested.

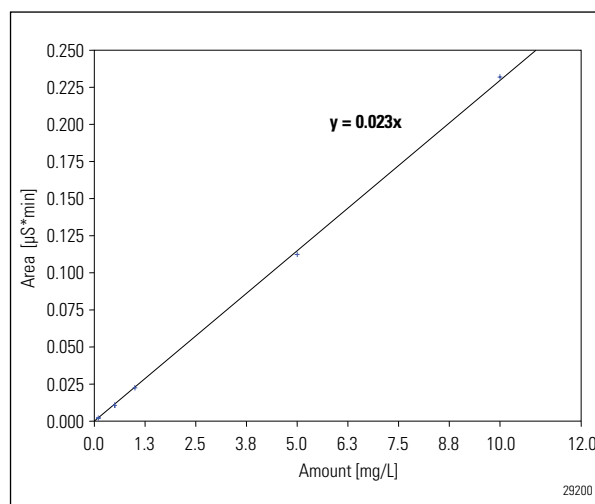


Figure 2: Calibration plot for phytate (forced through the origin).

Sample Analysis

Soybeans and black sesame seeds were chosen to be analyzed for phytate as seeds are known to have elevated phytic acid concentrations compared to other parts of a plant. The sample was extracted using 3 M hydrochloric acid and then treated with a layered Dionex OnGuard II Ag/H cartridge. The Dionex OnGuard II Ag/H cartridge removes chloride from the sample. This prevents overloading of the column with the chloride in the sample, which would prevent an accurate determination of phytate. Figure 3 shows a chromatogram of a prepared soybean sample with and without sample pretreatment with a Dionex OnGuard II Ag/H cartridge. There is a peak at the retention time of phytate in the untreated sample that affects the measurement, necessitating pretreatment of the sample. Figure 4 shows chromatograms of prepared soybean and black sesame seed samples. Note that there are no interfering peaks in either chromatogram. Each sample was prepared three times and three injections were made of each preparation. Reproducibility of sample preparation and injection are shown in Tables 2 (soybean) and 3 (sesame seed) along with a quantification of the amount of phytate in each sample. Both tables show that the method has good reproducibility. Quantifying the amount of phytic acid in each sample based on the phytic acid sodium salt hydrate working standard yielded values of 1.59 g/100g and 1.90g/100g for soybean and black sesame seed, respectively.

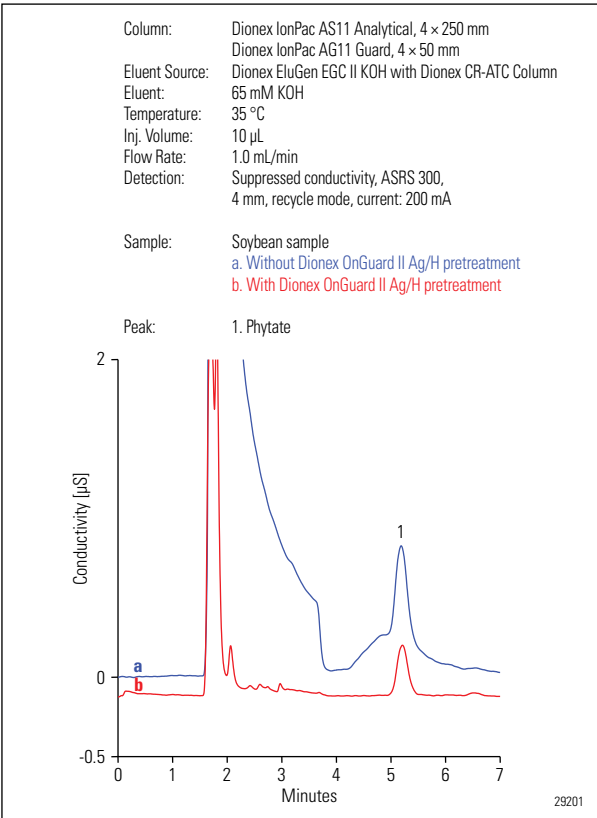


Figure 3: Overlay of chromatograms of the soybean sample with and without Dionex OnGuard Ag/H cartridge pretreatment.

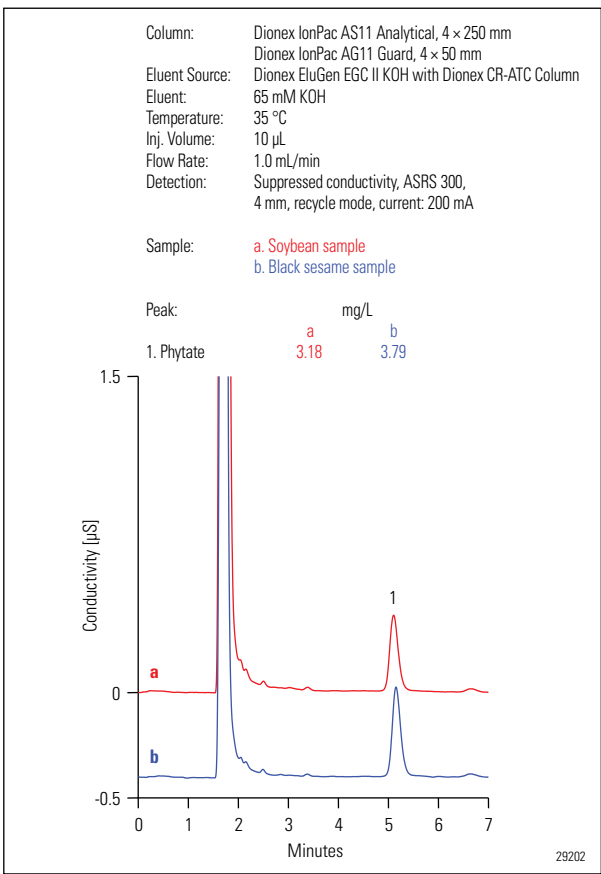


Figure 4: Chromatograms of soybean and black sesame seed samples.

The value for phytate in soybeans was 25–33% higher than that previously determined for two different soybean samples.³ Given the nature of the standard discussed earlier and the understanding that phytate levels will vary with samples from different locations and harvest times, there is reasonable agreement. To determine method accuracy, analyte recovery was measured by adding a known amount of phytic acid sodium salt hydrate standard into both samples before sample extraction. The results show that the recoveries were good, with values ranging from 96.8–115% for both samples (Tables 2 and 3), suggesting method accuracy.

Table 2: Amount of phytic acid in the soybean sample and recovery results.

Injection No.	Amount (mg/L)					
	Sample			Spiked Sample		
	# 1	# 2	# 3	# 1	# 2	# 3
1	3.19	3.19	3.23	4.29	4.12	4.08
2	3.17	3.00	3.23	4.26	4.14	3.97
3	3.16	3.21	3.25	4.25	4.08	4.13
Average	3.17	3.13	3.24	4.27	4.12	4.06
RSD	0.56	3.63	0.31	0.48	0.71	2.06
Average	3.18			4.15		
Spiked Conc (mg/L)	1.0					
Recovery (%)	96.8					
Amount (g/100 g)	1.59					

Table 3: Amount of phytic acid in the black sesame seed sample and recovery results.

Injection No.	Amount (mg/L)					
	Sample			Spiked Sample		
	# 1	# 2	# 3	# 1	# 2	# 3
1	3.75	3.86	3.74	4.93	4.97	4.95
2	3.78	3.85	3.74	4.91	5.00	4.89
3	3.78	3.89	3.74	4.95	4.98	4.91
Average	3.77	3.86	3.74	4.93	4.98	4.92
RSD	0.49	0.52	0.01	0.34	0.31	0.60
Average	3.79			4.94		
Spiked Conc (mg/L)	1.0					
Recovery (%)	115					
Amount (g/100 g)	1.90					

Conclusion

This study demonstrated an IC method for determining phytic acid (as phytate) in soybeans and black sesame seeds. This method was shown to be accurate and reproducible for these two samples and should be applicable to other food samples, although the sample preparation described here must be tested to ensure that it is accurate for any new samples. The method uses an RFIC system which is easy to use, as eluent and regenerant preparation are not required.

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