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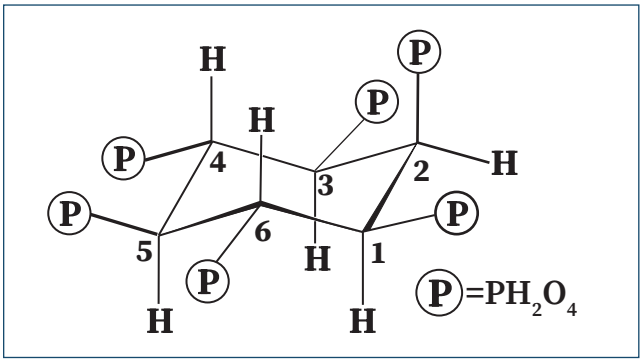
IZiNCG TECHNICAL BRIEF

A Field-Friendly Method for Measuring Dietary Phytic Acid Species in Plant-based Foods

Introduction

Dietary intake of phytic acid (myo-inositol hexaphosphate, IP6; **Figure 1**) or its salt-bound form called phytate, is an important cause of zinc deficiency, especially for populations in low-income countries (LICs) whose diets often rely on phytate-rich unrefined cereals, legumes and oleaginous seeds (1). In contrast, roots, tubers and most leafy vegetables and fruit have low amounts of phytic acid, and animal-source foods have none.

Figure 1. Phytic Acid, myo-inositol hexaphosphate.



Once consumed, phytic acid binds to zinc or other minerals in the intestinal tract, forming insoluble salts that cannot be digested or absorbed and instead are excreted. Hence, phytic acid reduces zinc absorption and may also limit reabsorption of endogenous zinc secreted into the digestive tract. Therefore, to estimate the risk of zinc deficiency or to establish dietary zinc recommendations in a population, measuring the phytate content of food is required (2).

IZiNCG Technical Brief no. 3 outlines how to calculate the phytate-to-zinc molar ratio of individual foods or whole diets, which are used to provide an estimate of the proportion of zinc absorbed (3). Diets with phytate-to-zinc molar rations >15 generally have poor zinc bioavailability, those with ratios between 5 and 15 are said to have medium bioavailability, and those with ratios <5 have good bioavailability (4).

The most effective measurement of phytate levels in food requires extraction and analysis using sophisticated High Performance Liquid Chromatography (HPLC) equipment, but this is not feasible in all research settings. Commercial kits based on enzymatic assays and colorimetric reagents are available, but may have accuracy and reproducibility concerns. A field-friendly method that is high-throughput, widely-available, low-tech and low-cost but that still can measure food phytate with adequate sensitivity, accuracy and precision would therefore be of value to laboratories in LICs and elsewhere. Here “low-tech” means the method would require only widely available reagents and equipment and not require highly specialized skills.

Considerations for Measurements of Phytic Acid

Sensitivity and accuracy

What level of sensitivity is required for a field-friendly method? While many foods have little or no phytic acid, seed-based foods typical of staple diets in LICs contain phytic acid levels ranging from a low of 50 mg/100 g to a high of 500 mg/100 g or more. Thus the limit of detection for a successful method must be adequate to detect phytic acid levels at around 50 mg/100 g.

What level of accuracy is required? Four levels of dietary phytic acid intake (300, 600, 900 and 1,200 mg/d) were used by the European Food Safety

Authority (EFSA) to set the recommendations for adults (5). This provides a useful target for an analytical method. A field-friendly method should be able to detect phytic acid levels typical of staple foods (50 to 500 mg/100 gm) at accuracy of approximately 90% of the actual value, or better.

Ability to separate out IP6 and IP5

A second important consideration is that phytic acid (IP6) can undergo significant breakdown during storage or food preparation to less-phosphorylated inositol phosphates, such as penta- and tetra- phosphates (IP5 and IP4) (6). Food preparation methods that result in phytic acid breakdown include soaking, germination, malting and fermentation. IP5, like IP6, can negatively impact zinc absorption. However IP4 and less-phosphorylated inositol phosphates may have a much lower effect (7, 8).

Several analytical methods for phytic acid measurement do not distinguish IP6 from IP5, IP4 or other inositol phosphates. These include methods that measure total inositol phosphates in an extract using a simple colorimetric assay (9, 10), precipitation of total inositol phosphates such as the "ferric precipitation method" (11), or fractionation using anion-exchange column chromatography (12). The ideal method should be able to separate IP6 and IP5 from IP4 and other less-phosphorylated inositol phosphates. This would typically require some form of chromatography that separates IP6 and IP5 from other breakdown products prior to quantification. The current methods that separate different inositol phosphates best are HPLC and Polyacrylamide Gel Electrophoresis (PAGE).

HPLC versus PAGE

HPLC methods are available that are highly sensitive, accurate and precise, and that chromatographically separate phytic acid from less-phosphorylated species (13). However, HPLC is not high-throughput and requires the use of expensive and dedicated equipment that require specialized skills and training (**Table 1**). Thus, HPLC may not be an ideal method for use in many laboratory settings, whether they are in high-income or low-income countries.

Table 1. Comparison of merits of HPLC (High Performance Liquid Chromatography) versus PAGE (Polyacrylamide Gel Electrophoresis) as a "field-friendly" method for food phytic acid and inositol phosphate assay.

CHARACTERISTIC	HPLC	PAGE
Ability to separate and detect phytic acid (IP6), IP5 and IP4	Excellent	Adequate
Sensitivity	High	Adequate
Accuracy and Reproducibility	High	Adequate
Cost	High	Low
Specialty Skills and Training Requirements	High	Low
Availability of Equipment and Reagents	High	High

An adequate alternative is a variant of traditional PAGE methods common in many laboratories (14, 15). This PAGE method is not as sensitive as HPLC (**Table 1**). For example, for the trial run data given in **Table 2**, the lower limit of detection for PAGE was deemed to be 25 mg/100 g, as compared with 6 mg/100 g for HPLC. However, this limit of detection for PAGE is still quite adequate for the typical levels of phytic acid in foods. PAGE can also provide adequate separation and quantification of phytic acid, IP5 and IP4. PAGE is also high-throughput, low-cost, widely available, and does not require substantial expertise or training. Therefore, PAGE may represent the best option for a "field-friendly" method.

Besides being easier to use, PAGE also is more cost-conscious than most HPLC approaches. The PAGE equipment used in this example cost approximately $1,700, with reagents and standards costing approximately $1,600. In comparison, a new HPLC system can cost from $25,000 to $100,000, not including reagents and standards. A refurbished HPLC system can currently be obtained for approximately $15,000, but often carries a higher burden of equipment upkeep. Therefore, PAGE costs are approximately just

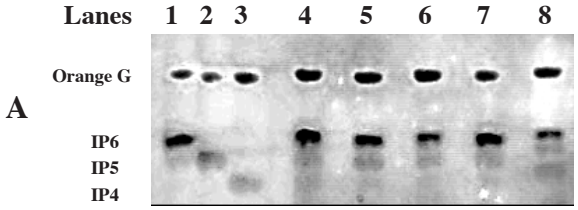
10% of those for HPLC. Moreover, PAGE does not require highly trained technicians common in labs that depend on HPLC, so there are other less quantifiable cost savings too.

An Example of PAGE Analysis of Phytic Acid in Food Samples

First, we will illustrate the utility of PAGE for analysis of phytic acid and less-phosphorylated inositol phosphates in food and flour samples. Extracts of five seed and food samples were prepared. Following PAGE fractionation of extract supernatants and the IP6, IP5 and IP4 standards, the gel was then stained to reveal the inositol phosphate species and the bands quantitated using open-access gel analysis software (**Figure 2**). For a detailed description of this method, refer to the document A Polyacrylamide Gel Electrophoresis (PAGE) Method for Assay of Phytic Acid Species.

These results illustrate the capability of PAGE to separate IP6, IP5 and IP4 species. The relative differences of IP6, IP5 and IP4 between samples were visually apparent, where IP5 and IP4 levels represent about 10% of the total inositol phosphate in a sample. When the bands in **Figure 2** were quantitated, the values were comparable to those obtained via the HPLC method of Lehrfeld (13; **Table 2**). Individual samples from PAGE analysis demonstrated values between 75% to 121% of values obtained via HPLC; averaging across all samples there was only a 1% difference between PAGE and HPLC.

Figure 2. An example of PAGE (Polyacrylamide Gel Electrophoresis) chromatography of phytic acid and inositol phosphates in food and flour samples. Following gel fractionation and staining with Toluidine Blue, the gel image was first converted to 32-bit grayscale, and the amount of phytic acid or inositol phosphate in a given band were quantitated using open-access gel analysis software. Panel A: Lanes 1, 2, and 3 are 20 µL of 1 mM standards (IP6, IP5 and IP4, respectively). Lanes 4 through 8 are 20 µL of extracts of whole mung bean (Lane 4), red kidney bean (Lane 5), raw tofu (Lane 6), barley cv. Harrington (Lane 7) and barley low phytic acid 2 (Lane 8).



These results demonstrate that with the presented PAGE method, a laboratory in a LIC can obtain data that are sufficiently precise to estimate a daily phytic acid intake in the diet, at the four levels defined by EFSA (5). Thus, this method could be used to estimate the impact of phytic acid and other inositol phosphates on zinc bioavailability.

Table 2. Comparison of the phytic acid (IP6), inositol pentaphosphate (IP5) and inositol tetraphosphate (IP4) content of selected plant-based foods and flours as obtained with HPLC and the Polyacrylamide Gel Electrophoresis (PAGE) assay.

	HPLC[a]			PAGE[b]		
Food sample or Flour	**IP6**	**IP5**	**IP4[c]**	**IP6**	**IP5[c]**	**IP4[c]**
	mg/100 g			**mg/100 g**		
White mung beans	614	66	ND	741	ND	ND
Red kidney beans	604	51	ND	612	138	ND
Tofu, raw	547	33	ND	411	89	ND
Harrington barley	724	141	16	666	ND	ND
Low phytic acid 2 barley	307	166	174	325	38	253

[a] The HPLC method used was a modification of that described in (13).
[b] The PAGE method used was a modification of that described in (14).
[c] ND=not detected. The lower limit of detection for inositol phosphates was 6 mg/100g for the HPLC assay and 25mg/100 g for the PAGE assay.

More about phytic acid:

The FAO/INFOODS/IZiNCG Global Food Composition Database for Phytate – Version 1.0 available at http://www.fao.org/infoods/infoods/tables-and-databases/faoinfoods-databases/en/

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This Technical Brief was prepared by Dr. Victor Raboy and was reviewed by members of the IZiNCG Steering Committee.

About IZiNCG

IZiNCG is the International Zinc Nutrition Consultative Group whose primary objectives are to promote and assist efforts to reduce global zinc deficiency through interpretation of nutrition science, dissemination of information, and provision of technical assistance to national governments and international agencies. IZiNCG focuses on identification, prevention and treatment of zinc deficiency in the most vulnerable populations of low-income countries.

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