JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Ion Chromatography of Phytate in Roots and Tubers

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The ion chromatographic method for the quantification of phytate ($InsP_6$) in foods was adapted for the analysis of roots and tubers. To maximize sensitivity, ultraviolet (UV) detection following postcolumn derivatization was compared with evaporative light-scattering detection (ELSD). Detection limits for phytate were 0.5 and 1 μg for UV and ELSD, respectively. Unidentified peaks eluting close to and after $InsP_6$ were removed by solid-phase extraction. Phytate was detected in 11 of 15 roots or tubers. The highest phytate levels were 0.169 and 0.133% of the fresh weight of taro (*Colocasia esculenta)* and yuca (*Manihot esculenta*), respectively. Potatoes (*Solanum tuberosum*) contained 0.035−0.073% phytate, whereas no phytate at a detection limit of 0.003% of fresh weight was observed in sweet potatoes (*Ipomoea batatas*).

KEYWORDS: Phytic acid; phytate; inositol hexakisphosphate; potato; *Solanum tuberosum*

INTRODUCTION

The amount of phytate (inositol hexakisphosphate, $InsP_6$) in foods has been of interest to nutritionists for many years. Originally perceived as an antinutrient for its ability to precipitate and decrease the bioavailability of minerals such as calcium, iron, and zinc, phytate is now also credited with positive attributes related to the prevention of oxidation, cancer, atherosclerosis, and kidney stones (*1*, *2*). Foods with the highest levels of phytate are seeds of grains, legumes, and nuts, which usually contain from 0.4% to >1% of this compound (*3*). Because phytate is considered to be a storage form of phosphorus and inositol, intermediate levels are present in roots and tubers, but only trace amounts are found in vegetative tissues and animal meats.

In recent years there has been a trend for scientists to analyze phytate in foods by various high-performance liquid chromatography (HPLC) methods rather than the nonspecific precipitation methods of the past (*4*, *5*). This has resulted in an abundance of data for phytate in foods derived from seeds, but not from other plant tissues. Because the underground storage tissues of certain plants are a major part of the diet in many countries, we undertook the measurement of the phytate content of various roots and tubers using ion chromatography. To maximize sensitivity, we first compared the responses of a traditional ultraviolet (UV) detector and an innovative evaporative light-scattering detector (ELSD).

MATERIALS AND METHODS

Materials. All vegetables were obtained from local supermarkets. Stems and leaves were still attached to the carrot, red beet, and radish roots at the time of purchase and were removed and discarded at the time of analysis. Dodecasodium phytate from rice was from Sigma. All other chemicals were of reagent grade.

Preparation of Extracts. Roots and tubers with skin intact were shredded with the thin (2 mm) shredding disk in a KitchenAid model RRKFP600 (11 cup) food processor (http://www.kitchenaid.com). Twenty grams of shedded vegetable was homogenized with 100 mL of 0.75 N HCl for 60 s at low speed in a stainless steel minisample container in a Waring commercial laboratory blender. After the homogenate had been decanted, the container was washed immediately to prevent corrosion and minimize the potential for iron extracted from the stainless steel to interfere with the analysis. Homogenized aliquots of 1.4 mL were centrifuged for 5 min at 10000*g* in an Eppendorf 5415 C microcentrifuge. One milliliter of the supernatant solution was injected with a plastic syringe through a tandem combination of a 225 mg Oasis HLB Plus extraction cartridge (Waters Corp., Milford, MA) connected to a 25 mm Millex-HV 0.45 μm pore size filter unit (Millipore Corp., Bedford, MA) at a flow rate of ~5 mL/min. The Oasis cartridges were used dry without any preconditioning. The several hundred microliters of solution that passed through both the cartridge and the filter was collected, and subsequent calculations were made on the basis of the concentration of $InsP_6$ in this eluate.

Ion Chromatography. Fifty-microliter aliquots of the sample solutions were separated on a Dionex AG7/AS7 (guard/analytical) column combination with 0.25 N HNO_3 eluant at a flow rate of 1 mL/min. The eluate was combined with 0.1% $Fe(NO_3)_3$ in 2% $HClO_4$ at a flow rate of 0.5 mL/min in a plastic tee, and the UV absorbance was monitored at 290 nm in a Waters Lambda Max model 480 LC spectrophotometer (*6*). Ten microgram external standards of dodecasodium phytate were analyzed before and after every two sample solutions. For ELSD, the eluate was monitored in an Alltech ELSD 2000 with the impactor off, a drift tube temperature of 118 °C, and a nitrogen gas flow of 0.8 L/min.

Calculations. Three roots or tubers were each extracted, aliquots were analyzed in duplicate, and the means and standard deviations were calculated from the three averages of the duplicates ($n = 3$). The total aqueous extract volumes were calculated using the percent H_2O listed

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10.1021/jf025827m
Published on Web 12/07/2002

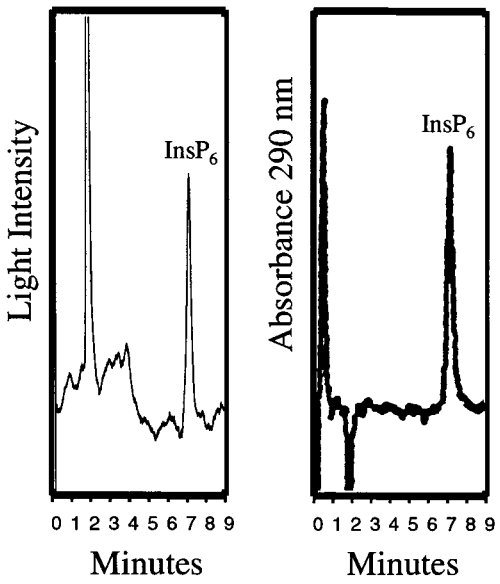

Figure 1. Chromatograms of 10 μg of dodecasodium phytate: (left) ELSD; (right) UV detector.

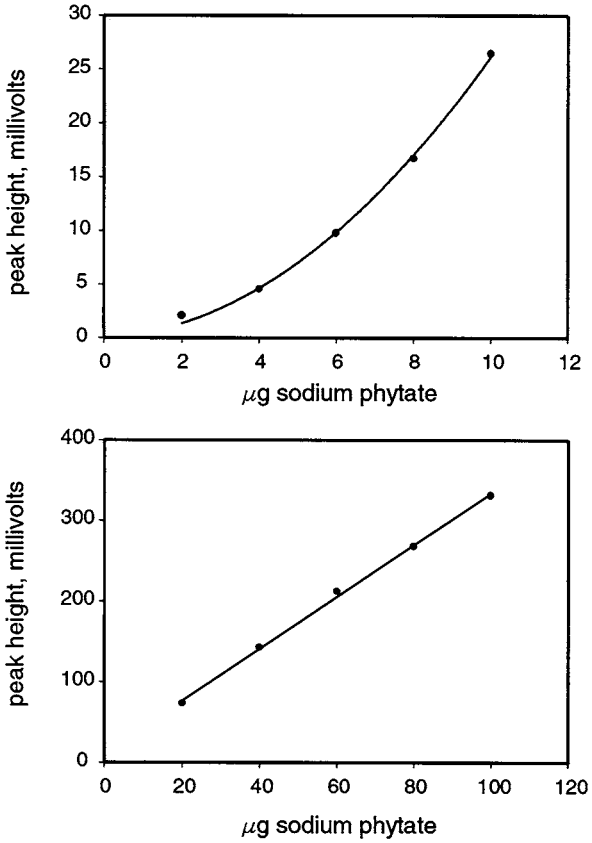

Figure 2. Standard curves with ELSD: (top) 2–10 μg; (bottom) 20–100 μg.

for each vegetable in the USDA Nutrient Database for Standard Reference (*7*).

RESULTS

Phytic acid eluted from the AG7 plus AS7 columns at ~7 min. A similar result was obtained with Dionex PA-100 guard and analytical columns (*4*) using 0.20 N HNO_3 as the eluant, but no direct comparisons using new columns were made. It can be seen in **Figure 1** that the signal-to-noise ratio was lower for the ELSD than for the UV detector. UV-detected noise was

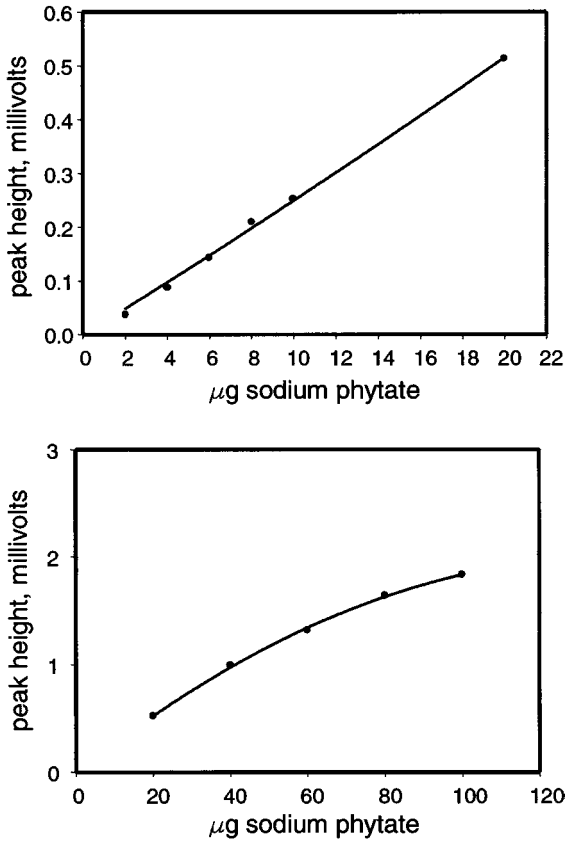

Figure 3. Standard curves with UV detector: (top) 2–20 μg; (bottom) 20–100 μg.

consistent and short-term with a period of several seconds, whereas ELSD noise was inconsistent and long-term over the course of minutes. Detection limits for phytic acid (molecular weight = 660) were 0.5 μg for UV and 1.0 μg for ELSD. Standard curves prepared with 2−100 μg of sodium phytate gave an exponential response at low concentrations and a linear response at high concentrations with the ELSD (**Figure 2**). In contrast, a linear response at low concentrations followed by a logarithmic response at high concentrations was obtained with the UV detector (**Figure 3**). After initial experiments were performed with the ELSD, the optical system became coated with white material, presumably from the roots and tubers, and corroded, possibly as a result of condensation from the nitric acid eluant. Due to the cost of repairing the damage, the manufacturer recommended that it not be used with this procedure. Therefore, subsequent experiments and analyses were performed with the UV detector.

When centrifuged root and tuber extracts were analyzed, peaks eluting near and after phytic acid were observed. These peaks, which were not previously observed upon analysis of extracts from seeds, could be removed by rapidly passing the extracts through an Oasis HLB Plus extraction cartridge (**Figure 4**). The Oasis HLB Plus cartridges bind hydrophobic molecules and do not retain the hydrophilic inositol phosphates. No attempt was made to identify the unknown compounds. As shown in **Table 1**, complete recovery of phytate added to a russet potato extract was obtained after injection through the cartridge.

Russet and red-skinned potatoes contained 0.073 and 0.051% phytate, respectively, on a fresh weight basis (**Table 2**). The highest levels were 0.169 and 0.133% phytate in the starchy tropical roots taro and yuca, respectively. The presence of phytate was detected in 11 of the 15 vegetables analyzed. Some

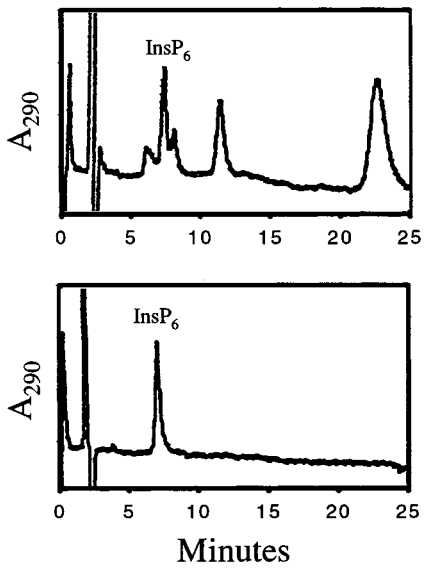

Figure 4. Chromatograms of russet potato extract without (top) and with (bottom) Oasis HLB Plus extraction.

Table 1. Recovery of Phytate from Oasis HLB Plus Cartridges

μg of phytate added[a]	μg of phytate recovered[b]	% recovery
40	42 ± 9	105
80	77 ± 11	96
120	121 ± 18	101
160	157 ± 4	99

[a] Dodecasodium phytate added to 1 mL of centrifuged russet potato extract containing on average the equivalent of 164 μg of dodecasodium phytate/mL. [b] Mean ± SD (n = 4).

Table 2. Phytate Content of Roots and Tubers

root/tuber	Latin name	phytate (% of fresh wt)[a]
russet potato	*Solanum tuberosum*	0.073 ± 0.024
red-skinned potato	*Solanum tuberosum*	0.051 ± 0.022
new potato (baby red-skinned)	*Solanum tuberosum*	0.035 ± 0.019
sweet potato	*Ipomoea batatas*	ND[b]
boniato (white-fleshed sweet potato)	*Ipomoea batatas*	ND
ñame (yam)	*Discorea trifida*	0.046 ± 0.032
taro	*Colocasia esculenta*	0.169 ± 0.026
yuca (cassava)	*Manihot esculenta*	0.133 ± 0.019
malanga (yautía)	*Xanthosoma sagittifolium*	0.083 ± 0.033
radish	*Raphanus sativus*	ND
beet (red beet)	*Beta vulgarus*	0.005 ± 0.002
carrot	*Daucus carota*	0.015 ± 0.003
parsnip	*Pastinaca sativa*	0.061 ± 0.049
turnip	*Brassica rapa*	ND
sunflower (Jerusalem artichoke)	*Helianthus tuberosus*	0.046 ± 0.020

[a] Mean ± SD (n = 3). [b] Not detected. The detection limit corresponded to 0.003% of fresh weight.

of the standard deviations were high, most likely because of the limited number of samples analyzed.

Surprisingly, sweet potatoes had no detectable phytate at a detection limit corresponding to 0.003% of fresh weight. Two experiments were performed to determine whether the apparent lack of phytate in sweet potatoes might be explained by phytase activity or insufficient extraction time. When 10 mg of dodecasodium phytate was homogenized with 20 g of sweet potato and 90 mL of H_2O and stirred 60 min at room temperature, 83% of the phytate was recovered after the addition of 10 mL of 7.5 N HCl and analysis by ion chromatography (results not shown). When a similar recovery experiment was performed by homogenizing 10 mg of dodecasodium phytate with 20 g of sweet potato and 100 mL of 0.75 N HCl and stirring 60 min at room temperature, 87% of the added phytate was recovered. Therefore, the apparent absence of phytate in sweet potatoes was probably not due to high levels of phytase activity or problems with the extraction procedure. However, it is theoretically possible that the phytase also may have been degraded or inactivated during storage.

DISCUSSION

The traditional UV detector was superior to the ELSD used in this study for several reasons. First, UV detection gave significantly lower detection limits than the ELSD. Second, the response of the UV detector was linear at low phytate concentrations, whereas the response of the ELSD was nonlinear. Third, the UV detector was easier to maintain than the ELSD, which developed corrosion in parts of the optical system due to condensation of acidic vapors. Improvements in ELSD technology have the potential to overcome these problems, and the development of more competitive detectors is anticipated.

A simple solid-phase extraction pretreatment with Oasis HLB Plus cartridges greatly facilitates the determination of phytate in roots and tubers by ion chromatography. **Table 2** contains the first data for phytate obtained by HPLC for some frequently consumed roots and tubers. Most of the data previously available in the literature were obtained by nonspecific methods that may detect other compounds in addition to $InsP_6$ and therefore have the potential to overestimate the amount of phytate present (*8*). The phytate contents of potatoes in **Table 2** were 0.035−0.073% of the fresh weight, which corresponded to 0.18−0.34% of the dry weight. Others have found 0.38% (*9*), 0.18% (*10*), and 0.16% (*11*) of the dry weight of potatoes to be phytate. Potatoes grown organically with cattle manure fertilizer contained 0.024−0.086% of the dry weight as phytate (*12*). In the present study sweet potatoes were found to contain <0.003% phytate according to fresh weight, which agrees with the 0.01% of dry weight found by Oboh et al. (*13*) but is much lower than the 0.32 and 0.18% of dry matter reported by Harland et al. (*14*) and Ravindran et al. (*10*), respectively. Sweet potatoes are cured for about a week under conditions of warm temperature and high humidity to increase storage life and sweetness (*15*). Whether curing destroys some of their phytate is unknown. The 0.015% of the fresh weight of carrots determined to be phytate is similar to the 0.09% of dry weight listed in the review by Frossard et al. (*16*) but considerably lower than the 0.63% of dry weight found by Khokhar et al. (*11*). The highest level of phytate in any root or tuber was 0.169% of fresh weight in taro in this study, whereas 0.28, 0.85, 0.32, and 0% of dry weight in taro were measured by Harland et al. (*14*), Marfo et al. (*17*), Ravindran et al. (*10*), and Adeyeye et al. (*18*), respectively. Others (*10, 14, 16*−*19*) also found considerable amounts of phytate in the starchy tropical roots cassava (yuca) and yams. In contrast, Oladimeji et al. (*20*) reported <0.02% phytate in yams, sweet potatoes, taro, and cassava on a dry weight basis.

Research on the positive and negative nutritional aspects of phytate in foods has centered on foods derived from seeds. In most raw seeds $InsP_6$ is the predominant inositol phosphate and $InsP_6$ and $InsP_5$ account for >95% of the total inositol polyphosphates (*8*). Because only $InsP_6$ was measured in the present study, it is not known for certain whether the inositol phosphate profiles of most fresh roots and tubers are similar to those of seeds. The current results show that many roots and

Table 3. World Food Supply Statistics for 1999[a]

commodity	metric tons	commodity	metric tons
rice	515,851,044	maize	112,969,322
wheat	415,957,860	cassava	97,709,842
potatoes	184,667,944		

[a] FAO (*21*).

tubers contain levels of phytate comparable to those of seeds on a dry weight basis. One large potato may contain up to several hundred milligrams of phytate. In 1999, of the 297,099,503 metric tons of potatoes grown worldwide, 62% (184,667,944 metric tons) was used as food, 12% was used as animal feed, and 12% was used as seed potatoes (*21*). On a worldwide basis, potatoes and cassava rank among the top staple starchy foods (**Table 3**). Thus, in many diets roots and tubers may represent the main source of phytate, in amounts comparable to grain- and legume-based diets. Unlike wheat and rice, which contain the highest levels of phytate in the bran (*3*), which is removed during milling, potatoes contain phytate evenly distributed throughout the tuber (B. Q. Phillippy, unpublished data). Additional research is needed to determine what environmental and genetic factors influence the accumulation of phytate in roots and tubers and the effects of storage conditions and food-processing methods on its retention. Future efforts to sort out the nutritional attributes of phytate should not overlook the insights that may be gleaned from an awareness of the phytate contents of roots and tubers.

ABBREVIATIONS USED

$InsP_6$, *myo*-inositol hexakisphosphate; $InsP_5$, *myo*-inositol pentakisphosphate; HPLC, high-performance liquid chromatography; ELSD, evaporative light-scattering detector.

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Received for review July 16, 2002. Revised manuscript received November 7, 2002. Accepted November 8, 2002.

JF025827M