Inhibition of Lipase Activity and Oxidation in Brown Rice Products by Extraction with Ethanol Containing Chelators/Acidulants

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ABSTRACT

Liquid ethanol (EtOH) extraction of brown rice stabilized the kernels and flours prepared from them against lipolytic hydrolysis. The inclusion of a chelator/acidulant (citric acid, phosphoric acid, phytic acid, hydrochloric acid) in the extraction solvent yielded products with lower residual lipase activities. The mechanism of action for the inhibition of lipase activity by these chelators/acidulants was attributed to lipase retaining in the kernels and their flours the ionization states and the corresponding activity that were acquired during the acidic EtOH extraction. Citric acid (reported in Champagne and Hron 1993) and phosphoric acid also inhibited oxidation in extracted kernels and their flours.

Processes have been developed for stabilizing brown rice kernels and their flours against lipolytic hydrolysis by liquid ethanol (EtOH) extraction (Champagne et al 1990, 1991) and by EtOH vapor treatment (Champagne and Hron 1992, Champagne et al 1993) of the kernels. Inclusion of an antioxidant or iron chelator in the liquid extraction process yielded products that were also stable to oxidative deterioration (Champagne and Hron 1993). Citric acid, a chelator and acidulant, inhibited oxidation and lipase activity.

The inhibition of lipase activity by EtOH in these processes was attributed to ethanolic denaturation of bran lipases with concomitant deactivation and destruction of lipase-producing bacteria and mold found on kernel surfaces. The mechanism of action for the inhibition of lipase activity by citric acid was underestimated in our initial investigation (Champagne and Hron 1993). Three possible modes of action were suggested. The first possibility was that citric acid chelates divalent cations (Shastry and Raghavendra Rao 1971, Aizono et al 1973, Sidhom et al 1975) that augments lipase activity, thus preventing the enzyme from having access to them. The second was decreased lipase activity due to lowering of the in situ pHe of the extracted kernels and their flours by citric acid. The third was that the lipase retains in the kernels and flours the ionization states and the corresponding activity that were acquired during the acidic EtOH extraction.

The objectives of this investigation were to: 1) elucidate the mechanism of citric acid inhibition of lipase activity in EtOH-extracted brown rice by determining whether chelators/acidulants besides citric acid inhibit lipase, and 2) determine the effectiveness of these chelators/acidulants in inhibiting oxidation.

MATERIALS AND METHODS

Rice Sample

Rough rice samples of Lemont variety (1990 crop) were obtained from Supreme Rice Mill (Crowley, LA). The samples were dehulled in a McGill sheller (H. T. McGill, Houston, TX).

Chelators/Acidulants

Phytic acid was prepared as described by Evans and Pierce (1982). Reagent grade anhydrous citric acid, phosphoric acid (85.4%), and hydrochloric acid were used.

Extraction Method

A 500-g sample of freshly dehulled brown rice was placed in a jacketed, stainless steel, cylindrical extractor (6 in. in diameter and 6 in. deep) that was fitted at the bottom with a 12-mesh stainless steel sample retaining screen. Water heated to 70°C was circulated through the extractor jacket for temperature control. Extractions were performed at 66°C with 800 g of aqueous EtOH (95%, v/v) containing various amounts of chelators (0.019–1.90% citric acid, 0.021–2.11% phosphoric acid, and 0.031–0.061% phytic acid, w/w) or hydrochloric acid (0.003–0.45%, w/w). The solvent was circulated from top to bottom at a flow rate of 1 L/min for 20 min, at which time it was drained. Two batches of rice were subjected to each EtOH extraction treatment. Extracted kernels were placed in shallow stainless steel pans and desolventized in room temperature air overnight. Brown rice flours were prepared by grinding extracted kernels to a powder in a Udy cyclone mill (Udy Corp, Fort Collins, CO) through a 20-mesh screen.

Unextracted brown rice kernels and flours prepared from them were controls. Control and extracted brown rice kernels and their flours were stored at 36°C in half-pint-sized capped glass jars with air headspace.

Chelator/Acidulant Content

Citric acid content was determined by high-performance liquid chromatography (HPLC) (Silliker Laboratories, Chicago Heights, IL), according to a method developed by Blake et al (1987). Phytate and phosphate contents were determined by a modification of the ion-exchange method of Ellis and Morris (1983). Five gram samples of flour from EtOH-extracted and control kernels were stirred with 100 ml of 1.2% HCl for 1 h and then centrifuged for 20 min at 10,000 × g. Ten milliliters of supernatant was applied to 0.8 × 4 cm plastic columns that contained 0.35 g of Dowex 1–×8, 200–400 mesh (Biorad, Richmond, CA). Inorganic phosphate was eluted from the columns with 10 ml of 0.1M HCl and collected in a 25-ml volumetric flask. Phytic acid was eluted from the columns with 10 ml of 2.0M HCl. Recovery was 97%. The phosphate and phytate contents of the eluants were determined by measuring their phosphorus contents using inductively coupled plasma (ICP) spectroscopy.

Free Fatty Acids Content

As a measure of the extent of lipolytic hydrolysis in brown rice kernels and their flours during storage, free fatty acids (FFA) contents were determined the day after extraction and then periodically by a micro method (Hoffpaur et al 1947). This micro method uses meta-cresol purple instead of phenolphthalein as an indicator. FFA were measured in oil extracted by petroleum ether from 5 g of ground rice using a Soxhlet extraction apparatus. FFA content was calculated as oleic acid and expressed as percent of oil.

1Southern Regional Research Center, USDA, ARS, New Orleans, LA. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

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Fluorometric Determination of Lipase Activity

EtOH-extracted and control kernels were freshly ground to flours in a Udy Cyclone mill through a 20-mesh screen before assaying by a fluorometric method. Samples (100 mg) of flour were mixed with 20 ml of 0.2M Tris-HCl buffer (pH 7.6) for 1 min using a vortex mixer. While the sample was still suspended, a 3-ml aliquot was transferred to a cuvette in the cell compartment of a fluorimeter (Giford Fluoro IV, Oberlin, OH). Five microliters of 0.1M fluorescein dibutyrate (Research Organics, Inc., Cleveland, OH) in 2-methoxyethanol (PHOTREX Reagent, J. T. Baker Chemical Co., Phillipsburg, NJ) was added to the cell with stirring. Fluorescence values for emission at 520 nm, resulting from excitation at 490 nm, were recorded at 10-s intervals for 4-10 min, depending on the hydrolysis rate, and continuously plotted against time on a chart recorder. The slope of the line of fluorescence intensity versus time corresponds to the rate of appearance of fluorescein resulting from the hydrolysis of nonfluorescent fluorescein dibutyrate to butyrate and fluorescent fluorescein. This reaction is catalyzed by hydrolases, preferably by lipase (Guilbault and Kramer 1964). No autoysis of fluorescein dibutyrate was observed in the Tris-HCl buffer.

Dynamic Headspace Analysis of n-Hexanal

n-Hexanal contents of EtOH-extracted and control kernels and their flours were determined as a measure of lipid oxidation. The amount of n-hexanal in stored brown rice has been found to be linearly proportional to the amount of oxidized linoleic acid with a correlation coefficient of 0.99 (Shin et al. 1986). A Tekmar LSC 2000 concentrator (Tekmar, Cincinnati, OH) equipped with a 25-ml straight-neck glass sample vessel was used for the purge and trap analysis of n-hexanal in samples after six months of storage. n-Hexanal was separated from other volatiles on a Hewlett Packard Ultra 2 column (cross-linked 5% phenyl, 94% methyl, 1% vinylsilicone; 50-m × 0.32-mm i.d. with 0.52-μm film thickness) using a Hewlett Packard 5890 Series II gas chromatograph (Hewlett Packard Co., Palo Alto, CA) equipped with an FID detector. Details of the method used and operating parameters for the concentrator and gas chromatograph are given elsewhere (Champagne and Hron 1993).

Statistical Analyses

Regression analysis was used to produce a FFA versus time curve (Figs. 1–3) at each chelator/acidulant level for kernel and flour samples to determine whether FFA levels were significantly affected by chelator/acidulant contents. The curves were compared among levels of chelator/acidulant using analysis of covariance (Steel and Torrie 1980). SAS (Statistical Analysis Software, Version 6, SAS Institute Inc., Cary, NC) was used for the statistical analyses.

RESULTS AND DISCUSSION

Retention of Phosphoric Acid and Phytic Acid by EtOH-Extracted Brown Rice

The inorganic phosphorus content of brown rice was lowered ~18% by EtOH extraction. Extracting the rice with EtOH containing 0.34–33.70 mg of phosphoric acid per gram of rice yielded products with phosphate contents ranging from 1.33 to 5.12 mg/g of rice. The inorganic phosphorus content of control kernels expressed as phosphate was 1.47 mg/g of rice; that of kernels extracted with EtOH alone was 1.21 mg/g of rice. The retention of phosphate by brown rice extracted with EtOH containing various amounts of phosphoric acid is described by the following linear regression equation:

\[ Y = (0.1152 \times X) + 1.4055 \]

where \( Y \) = amount of phosphate (mg/g of rice) remaining in the rice; \( X \) = amount of phosphate (mg/g of rice) added to the rice; correlation coefficient \( r = 0.9923 \), \( P < 0.0001 \).

EtOH extraction lowered the phytate content of brown rice by ~10%. There was no significant increase \( (P = 0.1464) \) in phytic acid content (7.38 mg/g of rice) with the addition of 0.50–0.98 mg of phytic acid/g of rice.

Fig. 1. Effect of extraction with liquid ethanol containing various amounts of phosphoric acid on free fatty acid levels in brown rice kernels and their flours during storage at 36°C. Each curve was generated by log-log regression and fits 14 data points from analyses on two batches of rice. Free fatty acid is expressed as percent of kernel oil.

Fig. 2. Effect of extraction with liquid ethanol containing various amounts of phytic acid on free fatty acid levels in brown rice kernels and their flours during storage at 36°C. Each curve was generated by log-log regression and fits 14 data points from analyses on two batches of rice. Free fatty acid is expressed as percent of kernel oil.
Effects of Phosphoric Acid and Phytic Acid on FFA Levels

The effects of extracting brown rice with EtOH containing various amounts of phosphoric acid or phytic acid on FFA levels in the kernels and their flours after six months storage at 36°C are depicted in Figures 1 and 2. These curves were generated by log-log regression and are of the form:

\[ \log(\text{FFA} + 1) = m \times \log(\text{time} + 1) + b \]

where \( m \) = slope and \( b \) = y-intercept at time 0. This function also described the effect of citric acid content on FFA levels in brown rice kernels and their flours (Champagne and Hron 1993). Residual lipase activity in EtOH extracted kernels and their flours was lowered by the addition of phosphoric acid or phytic acid to the EtOH extractant, as indicated by significantly lower (\( P < 0.0001 \)) FFA levels after six months of storage. For example, at the highest level of phosphoric acid added (33.70 mg/g of rice), FFA levels in the extracted kernels and their flours were 38 and 85% lower, respectively, than those in kernels extracted with EtOH alone and their flours after storage. The FFA level in kernels extracted with EtOH containing 0.98 mg of phytic acid/g of rice was 69% lower than that in kernels extracted with EtOH alone; the level in the flour of the former was 85% lower than that in the flour of the latter.

Analysis of covariance indicated that the trends in FFA levels occurring across time in kernels at each level of added phosphoric or phytic acid were statistically distinct (\( P < 0.0001 \)) from those of kernels extracted with EtOH containing no additive. Likewise, these trends in FFA levels in their flours were statistically distinct (\( P < 0.0001 \)). Trends in FFA levels with time could not be distinguished (\( P > 0.05 \)) among kernels extracted with EtOH containing 0.34 mg of phosphoric acid or 0.73 mg of phytic acid per gram of rice and larger amounts of these additives. Similarly, trends in FFA levels with time could not be statistically distinguished (\( P > 0.05 \)) among flours prepared from kernels extracted with EtOH containing 0.84 mg of phosphoric acid or 0.73 mg of phytic acid per gram of rice and larger amounts of these additives.

Effect of Hydrochloric Acid on FFA Levels

Brown rice kernels were extracted with EtOH containing various amounts of hydrochloric acid to determine whether lipase activity would be reduced in EtOH-extracted kernels and their flours by an acidulant that is not a chelator. In contrast to hydrochloric acid, citric acid, phosphoric acid, and phytic acid are acidulants and chelators of divalent cations. Lipase activity in EtOH-extracted kernels and their flours was reduced by the addition of hydrochloric acid to the EtOH extractant, as depicted in Figure 3. Decreasing the pH of EtOH extractant to 3.1 and lower values significantly decreased (\( P < 0.0001 \)) FFA levels in kernels and their flours during storage, as indicated by analysis of covariance. Decreasing the pH of the EtOH extractant from 7.5 (approximate) to 3.1, 2.5, 2.2, 1.7, and 1.2 reduced the increase in FFA levels after six months of storage by 61, 71, 74, 83, and 82%, respectively, in kernels and by 33, 56, 82, 92, and 96%, respectively, in their flours. The trends in FFA levels occurring across time in kernels extracted with EtOH adjusted to pH 3.1 or lower were statistically distinct (\( P < 0.0001 \)) from those in kernels extracted with EtOH not containing hydrochloric acid. Likewise, these trends in FFA levels in their flours were statistically distinct (\( P < 0.01 \)). Trends in FFA levels with time could not be statistically distinguished (\( P > 0.05 \)) in kernels extracted with EtOH adjusted to pH values lower than 3.1 from that in kernels extracted at pH 3.1. In flours prepared from kernels extracted at pH values lower than 2.2, trends in FFA levels were not statistically distinct (\( P > 0.05 \)) from that in flour prepared from kernels extracted at pH 2.2.

Effect of pH on Lipase Activity in EtOH-extracted Kernels and Their Flours

The pH values of EtOH treatment solutions, extracted kernels, and flours prepared from extracted kernels are listed in Table I. The pH values of flours prepared from kernels treated with EtOH containing 0.019–0.19% citric acid, 0.021–0.105% phosphoric acid, 0.031–0.061% phytic acid, or 0.003–0.018% hydrochloric acid (w/w) were 0.1–0.2 pH units lower than that of flour from kernels extracted with EtOH having no added acidulant. A drop in in situ pH from 7.0 to 6.8–6.9 should decrease lipase activity in the flours only 3–5%, based on the observation of Aizono et al (1973) that rice bran lipase activity is 95 and 70% of its maximum at pH 7.0 and 6.9, respectively. However, the FFA data in Figures 1–3 (see also Figure 2 in Champagne and Hron 1993) indicate markedly lower lipase activities in the flours than would be predicted from these small decreases in in situ pH. Thus, the mode of action of these acidulants/chelators in inhibiting lipase activity does not appear to have resulted from lowering of the in situ pH. The acidity of the EtOH treatment solutions were in the pH 1–4 range, as shown in Table I. Exposing the kernels to these low pH values should have had little effect on lipase stability (Aizono et al 1973) and its resultant activity at the higher in situ pH of the extracted kernels and their flours. However, least squares linear regression analysis indicated a fairly high correlation (\( r = 0.8481 \)) between the pH of the treatment solutions and the change in FFA during six months of storage in flours prepared from kernels extracted with EtOH containing various amounts of citric acid, phosphoric acid, phytic acid, or hydrochloric acid (Fig. 4). The correlation is higher (\( r = 0.9463 \)) when the two data points furthest from the line (pH 3.1 and 2.5 from HCl addition) are not included. These high correlations suggest that the pH of the treatment solution may have been a factor in the extracted rice having reduced lipase activity.

Lipase Activity

We propose that lipase retains in the kernels and flours the ionization states and the corresponding activity that were acquired during the acidic EtOH extraction. This phenomenon has been observed in lipase-catalyzed processes in nearly anhydrous organic solvents (Zaks and Klibanov 1985). Zaks and Klibanov demonstrated that the lipase enzyme “remembered” the pH of the latest aqueous medium to which it was exposed and its corresponding activity when the enzyme was dried or added to an organic solvent.
TABLE I
pH Values* of Ethanol Treatment Solutions, Extracted Kernels, and Flours Prepared from Extracted Kernels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Solution %, w/w</th>
<th>mg/g of rice</th>
<th>Treatment Solution</th>
<th>Kernel</th>
<th>Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>...</td>
<td>...</td>
<td>7.5*</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Ethanol and citric acid</td>
<td>0.019</td>
<td>0.30</td>
<td>4.2</td>
<td>6.3</td>
<td>7.0</td>
</tr>
<tr>
<td>Ethanol and phosphoric acid</td>
<td>0.053</td>
<td>0.34</td>
<td>3.7</td>
<td>5.2</td>
<td>6.8</td>
</tr>
<tr>
<td>Ethanol and phytic acid</td>
<td>0.021</td>
<td>0.84</td>
<td>3.9</td>
<td>4.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Ethanol and hydrochloric acid</td>
<td>0.053</td>
<td>1.69</td>
<td>6.2</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>Ethanol and phytic acid</td>
<td>1.930</td>
<td>3.37</td>
<td>5.3</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Ethanol and hydrochloric acid</td>
<td>0.015</td>
<td>3.15</td>
<td>5.9</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Ethanol and phytic acid</td>
<td>0.046</td>
<td>3.77</td>
<td>5.0</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Ethanol and hydrochloric acid</td>
<td>0.061</td>
<td>0.98</td>
<td>2.7</td>
<td>3.7</td>
<td>5.3</td>
</tr>
<tr>
<td>Ethanol and phytic acid</td>
<td>0.004</td>
<td>0.98</td>
<td>2.2</td>
<td>4.6</td>
<td>6.9</td>
</tr>
<tr>
<td>Ethanol and hydrochloric acid</td>
<td>0.018</td>
<td>0.98</td>
<td>2.9</td>
<td>4.5</td>
<td>6.8</td>
</tr>
<tr>
<td>Ethanol and phytic acid</td>
<td>0.042</td>
<td>0.98</td>
<td>3.0</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Ethanol and hydrochloric acid</td>
<td>0.133</td>
<td>0.98</td>
<td>2.9</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Ethanol and phytic acid</td>
<td>0.446</td>
<td>0.98</td>
<td>3.0</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Ethanol and hydrochloric acid</td>
<td>0.046</td>
<td>0.98</td>
<td>2.9</td>
<td>4.4</td>
<td></td>
</tr>
</tbody>
</table>

*Means of duplicate determinations; average deviation from mean = 0.02.
| pH determined on slurry (10 g of kernels/100 ml of deionized water) that was stirred for 5 min.
| pH determined on slurry (10 g of flour/100 ml of deionized water) that was stirred for 30 min.
| Unstable pH readings.

Fig. 4. Relationship between the pH of the liquid ethanol extractant containing an acidulant and the change in free fatty acid content (Δ%) during six months of storage. The acidulants added were citric acid (○), phosphoric acid (●), phytic acid (■), and hydrochloric acid (□). ○ = Liquid ethanol alone.

They hypothesized that the ionogenic groups of the enzyme acquire certain ionization states in an aqueous solution of a given pH and retain these ionization states in both the solid state and in organic solvents. To demonstrate that this phenomenon explains our observations, we dispersed flours from kernels extracted with EtOH containing and not containing an acidulant in pH 7.6 Tris-HCl buffer and measured the lipase activities using fluorescein dibutyrate as a substrate. Lipase activities, as indicated by rates of production of the hydrolysis product fluorescein, in these flours are compared in Figure 5. The time trend slopes of the relative fluorescence intensity of fluorescein produced in flours from kernels extracted with EtOH containing citric acid, phytic acid, or hydrochloric acid were not statistically different (P > 0.05) from that corresponding to flour from kernels extracted with EtOH not containing an acidulant, as determined by analysis of covariance. In flour from kernels extracted with EtOH containing phosphoric acid, the time trend slope of the relative fluorescence intensity of fluorescein was significantly steeper (P < 0.0001) than that corresponding to flour from kernels extracted with EtOH not containing an acidulant. These results concur with our hypothesis. Upon dispersing the flours in pH 7.6 Tris-HCl buffer, their residual active lipases "forgot" the ionization states they acquired in the acidic extractants and the corresponding activities and obtained the same ionization states and activities.

The mechanism of action for the inhibition of lipase activity by citric acid, phosphoric acid, and phytic acid does not appear to result from chelation of divalent cations that augment lipase activity. If chelation played a significant role, lipase activities measured fluorimetrically would have followed the same trends as those measured titrimetrically.

Dispersing flours from EtOH-extracted kernels in pH 7.6 Tris-HCl buffer did not cause them to regain the lipase activity observed in control (untreated) flour. Thus, inactivation of the lipase by EtOH was irreversible. In our earlier work, we contended that the higher the temperature of the EtOH extractant and the longer the extraction time, the greater the penetration of the EtOH into the kernel, and the greater the amount of lipase exposed to the EtOH and inactivated. The results presented here, however, suggest that some of the lipase not inactivated by the extraction was exposed to EtOH. For the residual lipase to have been influenced by the pH of the EtOH extractant, it had to have been exposed to the EtOH. We propose that some of the lipase activity...
taining 0.52 mg of citric acid/g of rice (Champagne and Hron 1993). On a molar basis, phosphate does not appear to be as effective an inhibitor of oxidation in EtOH-extracted brown rice and its flour as citric acid.

It is interesting to note that the flour prepared from kernels extracted with EtOH containing 0.34 mg of phosphoric acid/g of rice (yielding a product with 1.33 mg of phosphate/g of rice) had an n-hexanal level 30% that of control flour with a higher phosphate level (1.47 mg/g of rice). Also, flour with an n-hexanal level 50% of that of control flour was obtained by slightly increasing the citric acid content from the endogenous level of 0.28 to 0.30 mg/g of rice (Champagne and Hron 1993). Apparently, the EtOH extractant penetrates the kernel surfaces allowing the phosphate or citrate to bind with free iron and copper that serve as catalysts during oxidation. Retention of the phosphate or citrate by the rice does not appear to be necessary.

The extent of oxidation in kernels extracted with EtOH containing phytic acid or hydrochloric acid was not significantly different \((P > 0.05)\) from that in kernels extracted with EtOH alone after six months of storage at 36°C, as indicated by n-hexanal contents. Likewise, the extent of oxidation in flours prepared from these kernels did not significantly differ \((P > 0.05)\). As noted earlier, phytic acid, a strong chelator of divalent cations, was not retained by the kernels. Acidified EtOH is used commercially to extract phytate from bran. No losses of iron, copper, zinc, calcium, or magnesium occurred when kernels were extracted with EtOH containing phytic acid.

In conclusion, the inclusion of an antioxidant in the EtOH extraction process yields brown rice products with very low residual lipase activities. Inclusion of citric acid or phosphoric acid, two antioxidants/chelators widely used in the food industry, yields brown rice flours that are also highly stable to oxidative deterioration.

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**LITERATURE CITED**


Characterization of Phosphorus in Starch by $^{31}$P-Nuclear Magnetic Resonance Spectroscopy

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ABSTRACT

Phosphorus in starches from various botanical sources (cereals, roots, tubers, and legumes) was examined by $^{31}$P-nuclear magnetic resonance spectroscopy. Normal cereal starches contained phosphorus (0.02-0.06% starch dry weight) mainly in the form of phospholipids, whereas waxy starches had much less phosphorus (<0.01%), mainly in the form of starch phosphate monoesters. High-amylose corn starch (70% amylose) contained organic phosphorus (0.02%) as starch phosphate monoesters and phospholipids in a 1:4 ratio. Root and tuber starches were phospholipid-free, and the residual organic phosphorus was exclusively phosphate monoesters. Phosphate monoesters were exceptionally high in potato starch (0.88%). Legume starches contained phosphorus (<0.01%), mainly in the form of starch phosphate monoesters. Rice and lentil pea starches displayed signals absent in the other starches at 1.5-2.5 ppm on $^{31}$P-nuclear magnetic resonance spectra. Phosphate monoesters of all the starches were located more on the primary carbon (C-6) than on the secondary carbon (C-3) of the anhydroglucose unit.

Most cereal starches contain phosphorus that is mainly in the form of phospholipids (Schoch 1942a,b; Tabata et al 1975; Meredith et al 1978), whereas root and tuber starches contain phosphorus in the form of starch phosphate monoesters (Posternak 1935, 1951; Hodge et al 1948; Hizukuri et al 1970). Several cereal starches from corn, waxy corn, rice, and waxy rice were reported to contain minor amounts (6-15 ppm) of starch phosphate monoesters located mostly at C-6 of their anhydroglucose units (Tabata et al 1975).

As major phosphoryl sources in cereal starches, lysophospholipids from helical complexes with starch. This reduces water-binding capacity (Tester and Morrison 1990) and increases opaqueness of a starch paste (Schoch 1942a, Swinkels 1985). Phosphate monoesters in root or tuber starches, however, promote its hydrophilic nature by "wedging" apart individual starch chains with negatively charged phosphate groups, increasing water-binding capacity, swelling power, and paste clarity (Swinkels 1985, Lim 1990).

$^{31}$P-nuclear magnetic resonance (NMR) spectroscopy has been used to locate the phosphorylations in modified wheat and corn starches and in native potato and taro starches (Lim 1990, Muhrbeck and Tellier 1991, Jane et al 1992, Lim and Seib 1993) and locations of phosphodiester cross-linkage in corn starch (Kasemsawan and Jane 1994).

The objective of this study was to use $^{31}$P-NMR spectroscopy to characterize naturally existing phosphorus in starches from various botanical sources.

MATERIALS AND METHODS

Corn, rice, and potato starches were purchased from Sigma Chemical Company (St. Louis, MO). Several starches were gifts of respective companies: waxy and du-waxy corn starches (American Maize-Products Co., Hammond, IN); oat starch (ConAgra, Omaha, NE); wheat starch (Midwest Grain Products Co., Atchison, KS); high-amylose corn starch (70% amylose) (National Starch and Chemical Co., Bridgewater, NJ); tapioca starch (A. E. Staley Mfg. Co., Decatur, IL). Arrowroot starch was purchased from Frontier Cooperatives (Norway, IA). Other starches were isolated in our laboratory. α-1-Lysophosphatidyl choline, α-1-lysophosphatidyl ethanolamine, and crystalline α-amylase of Bacillus species were purchased from Sigma. The activity of α-amylase was 2,100 U/mg. One unit is defined as release of 1 mg of maltose from starch in 3 min.

Starch Isolation

Starches were isolated from a number of sources. Cattail millet (Pennisetum americanum) and waxy amaranth (Amaranthus hypochondriacus) starches were isolated by the method of Yanez and Walker (1986). Waxy rice starch was isolated by the method of Tester and Morrison (1990). Sweet potato (Ipomoea batatas), lotus (Nelumbo nucifera), and water chestnut (Trapa natans) starches were isolated by the method of Shiotani et al (1991). Legume starches from mung bean (Vigna radiata), green pea (Pisum sativum), lentil pea (Lens culinaris), and lima bean

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