Antioxidant Properties of Wild Rice†

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Wild rice was extracted with methanol, ethanol, and ethyl acetate. The yields of extracts were 3.9%, 1.9%, and 1.0%, respectively. The antioxidant activities of the extracts were measured by thiobarbituric acid reactive substance values in ground beef and by peroxide values in lard. The methanol and ethanol extracts showed a significant antioxidant activity when added to ground beef and lard. Wild rice hull extract also showed appreciable antioxidant activity in ground beef. Pulverized cooked and uncooked wild rice substantially reduced rancidity in ground beef, and therefore can be used as an "antioxidant ingredient" for commercial applications in food systems such as meat products. By using both ^{31}P and ^{13}C nuclear magnetic resonance, it was established with certainty that the isolate from the wild rice extract is phytic acid.

INTRODUCTION

There has been a growing demand for natural antioxidants due to reports that butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have toxic and carcinogenic effects in animals (Johnson, 1971; Branen, 1975; Ito *et al.*, 1985). Previous studies in this laboratory showed that the incorporation of cooked wild rice (*Zizania aquatica* L.) into beef patties could retard the development of rancidity during frozen storage and improve sensory scores (Minerich *et al.*, 1991). It was postulated that wild rice may contain some natural antioxidant components. Phytic acid is a strong chelating agent and thus possesses antioxidant activity (Graf, 1983; Graf and Eaton, 1990). Becker and Lorenz (1981) reported that wild rice contains 2.1–2.4% phytic acid by weight and so far it is the only documented antioxidant component in wild rice.

In this paper, we present data on the antioxidant properties of different forms of wild rice and its extracts and hulls of wild rice and confirm phytate identification by NMR.

MATERIALS AND METHODS

Materials. Grade A wild rice (*Z. aquatica* L.) and hulls were obtained from New Frontier Foods, Inc., Aitken, MN. Foodgrade antioxidants BHA, BHT, and TBHQ were obtained from Eastman Chemical Products, Inc., Kingsport, TN. δ-Tocopherol, containing 67% δ-tocopherol and 0.5–2% non-tocopherols, was obtained from Sigma Chemical Co., St. Louis, MO. Natural rosemary antioxidant, under the trade name of Herbalox seasoning (type O), was a gift from Kalsec Inc., Kalamazoo, MI.

Preparation of Cooked Wild Rice, Pulverized Cooked Wild Rice, Pulverized Wild Rice, and Wild Rice Hulls. Cooked wild rice (CWR) was obtained by boiling 30 g of wild rice with 300 mL of water for 1 h. The unabsorbed water was drained after cooking. The moisture content of the cooked wild rice was 75%. The pulverized cooked wild rice (PCWR) was prepared by grinding CWR using a Regal LaMachine II food processor Model LM2 (Regal Ware Inc., Kewaskum, WI). The wild rice and wild rice hulls were pulverized by using an All-Grain flour mill Model B-Sox (All-Grain Co., Tremonton, UT).

Solvent Extraction. Five hundred grams of pulverized wild rice (PWR) or pulverized wild rice hulls was extracted with 3 L of solvent (methanol, ethanol, or ethyl acetate) in a 5-L round-bottom flask at 60–65 °C for 2 h under vigorous agitation and reflux conditions. The reflux mixture was filtered, and the residue was extracted again with 2 L of the same solvent at the same conditions. The filtrates from the two extractions were combined, and the solvent was subsequently removed using a Wheaton Heidolph rotary evaporator Type VV 60 (Germany) at 50–60 °C.

Evaluation of Antioxidant Activity. CWR, PCWR, and PWR were directly added to the beef and thoroughly mixed using a household food processor, except for CWR, which remained as whole grains in ground beef. All of the extracts and the commercial antioxidants were dissolved in about 2 mL of absolute ethanol to ensure their uniform distribution in the test food source. Extra lean ground beef (approximately 15% fat), purchased from a local supermarket, was thoroughly mixed with the ethanol/antioxidant solution with a household food processor. The meat was cooked at 78 ● 2 °C for 2 h with the pouch left open to permit the removal of the solvent. The cooked beef was stored at 4 °C, and thiobarbituric acid reactive substances (TBARS) were determined according to the method described by Rethwill *et al.* (1981). For lard, the solvent was removed by a rotary evaporator after mixing with the solutions of additives. The lard was stored at 60 ± 2 °C in an oven for 1 week, and the peroxide values were determined after storage by the Official Method cd 8-53 of the American Oil Chemist's Society (AOCS, 1989).

Identification of Antioxidant. On the basis of the results obtained (Tables I–III), it was suspected the antioxidant is phytate, and another method of extraction and purification was implemented. Pulverized wild rice was extracted with 0.5 N HCl.

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† Published as Paper 20158 of the contribution series of the Minnesota Agricultural Experiment Station on research conducted under Minnesota Agricultural Experiment Station Project 18-23, supported by Hatch funds. This project was funded by the Greater Minnesota Corp. (Minnesota Technology Corp.), New Frontier Foods, Inc., the Minnesota Paddy Wild Rice Research and Promotion Council, and the Minnesota Association of Meat Processors.

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0021-8561/94/1442-0034$04.50/0

Table I. Effect of Cooked Wild Rice on the TBARS[a] Values of Cooked Ground Beef Stored for 10 Days at 4 °C

treatment	TBARS, ppm[b]
beef (control)	3.5
beef + BHA[a] (200 ppm)	0.3**
beef + BHT[a] (200 ppm)	0.5**
beef (85%) + CWR[a] (15%)	1.8[c]**
beef (85%) + water (15%)	3.5[c]

[a] Abbreviations: TBARS, thiobarbituric reactive substances; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; CWR, cooked wild rice. **Values significantly different ($p < 0.01$) from control. [b] SD = ±0.1 ppm. [c] TBARS values are based on the weight of the beef to eliminate the dilution effect of the rice.

The sample (0.5 g) was added to a 50-mL centrifuge tube (28.5 × 104 mm polyallomer) containing 2 mL of 0.5 M HCl and stirred to ensure removal of air pockets. The tip of the ultrasonic microprobe (ultrasonic liquid processor, Model W-385, equipped with a $^1/_8$-in. standard tapered microtip probe; Heat Systems-Ultrasonics, Inc., Farmingdale, NY) was inserted halfway into the liquid, and the sample was sonicated for 1–1.5 min. (1-s cycle, 50% duty at energy level 5.0). The alternative to sonication is vigorous mechanical agitation for 2 h at room temperature. The suspension was centrifuged at 1500 rpm for 15 min. An aliquot (1–5 mL) of supernatant was removed, diluted with 20 mL of distilled water, and poured onto an Analytichem silica-based, anion-exchange (SAX) column (quaternary amine Bond Elut column, Analytichem International, Harbor City, CA). The loaded SAX column was washed with 10 mL of 0.05 M HCl, and the resin-bound inositol polyphosphates were then eluted with 2 mL of 2 M HCl. The eluted sample was evaporated to dryness. The residue was resuspended with 1 mL of water and analyzed by HPLC.

A PRP-1 5-m (150 × 4.1 mm) reversed-phase analytical column (Hamilton Co., Reno, NV) was used. The mobile phase was prepared by mixing 500 mL of 52% methanol in water, 0.015 M formic acid, 5 mL of tetrabutylammonium hydroxide (40% w/w in water), and 2 mg of phytic acid hydrolysate and adjusting the pH to 4.3 with 10 N sulfuric acid. A refractive index (RI) detector was used. Sodium phytate was used as a standard.

The fractions in the wild rice extract that had the same elution time as the standard were examined by NMR for confirmation. The NMR spectra of both ^{31}P and ^{13}C were used. The ^{31}P spectra were run on a Bruker MSL 300 operating at 121.5 MHz using 85% phosphoric acid as an external reference. The ^{13}C (75.5 MHz) spectra were run on a Bruker WM-300 WB. The samples were dissolved in D_2O and placed in a 5-mm probe.

RESULTS AND DISCUSSION

Confirmation of Antioxidant Activity. Minerich *et al.* (1991) reported that the addition of CWR at levels of 15% and 30% by weight decreased the TBARS of beef patties during frozen storage. These data suggest that some natural antioxidants may be present in wild rice. The possibility that a portion of the reduction of TBARS as a result of the dilution of beef by the added substances cannot be excluded since high proportions of wild rice were used. Therefore, an experiment was conducted in which water was compared with the CWR. That is, the calculation of TBARS values was based on the weight of the beef only to eliminate the possible dilution effect by the wild rice. As shown in Table I, the addition of 15% cooked wild rice decreased TBARS values by almost 50%, while the addition of plain water had no effect. These data support the findings by Minerich *et al.* (1991) that the reduction of TBARS values was not due to a dilution of beef with inert substances but more likely attributable to antioxidant components in the wild rice. It was not surprising that CWR, which contains only low concentrations of antioxidant components, was much less effective than the highly pure synthetic antioxidants BHA and BHT.

Table II. Antioxidant Activity of Wild Rice Extracts in Cooked Ground Beef and in Lard[a]

treatment (%)	TBARS, beef,[b] ppm	PV[a] lard,[c] mg/kg
control (no additive)	3.8[d]	40.3[e]
BHA (0.02)	0.3**	4.3**
TBHQ (0.02)	0.3**	1.6**
MeOH[a] extract (0.02)	3.5	34.0**
MeOH extract (0.05)	2.7**	28.2**
EtOH[a] extract (0.02)	3.3*	43.4
EtOH extract (0.05)	3.0**	29.9**
EtOAC[a] extract (0.02)	3.6	45.9
EtOAc extract (0.05)	3.5	57.6
hull extract (0.10)	2.4**	
hull extract (0.20)	0.9**	

[a] Abbreviations: PV, peroxide value; MeOH, methanol; EtOH, ethanol; EtOAc, ethyl acetate; see Table I for other abbreviations used. *Values significantly different ($p < 0.05$) from control in the same column. **Values significantly different ($p < 0.01$) from control in the same column. [b] Stored for 6 days at 4 °C. [c] Stored for 7 days at 60 °C. [d] SD: TBARS = ±0.1 ppm. [e] SD: PBV = ±0.2 mg/kg.

Isolation of Antioxidant Components by Solvent Extraction. Phenolic compounds are widely distributed in plants (Salunkhe *et al.*, 1989), and some of them have been identified as natural antioxidants (Pratt and Birac, 1979; Wu *et al.*, 1982). Ramarathnam *et al.* (1988, 1989) identified isovitexin as a natural component in white rice hulls. Although wild rice belongs to a different botanical family, it is probable that the antioxidant activity of wild rice may be due to certain phenolic compounds and the presence of phytic acid as mentioned earlier. Therefore, solvent extractions were conducted in an attempt to isolate the natural antioxidant components. Organic solvents of different polarities, i.e., methanol, ethanol, and ethyl acetate, were used, and the yields of extraction were 3.1%, 1.9%, and 1.0%, respectively, which is in order of decreasing solvent polarity. All extracts were semisolids with a distinct wild rice flavor and were relatively insoluble in oil. Both the methanol and the ethanol extracts had a brown color, while the ethyl acetate extract had a dark green color, probably because chlorophylls are more soluble in ethyl acetate than in methanol or ethanol. Wild rice hulls were extracted with methanol, and the yield of the extraction was 3.2%. The physical appearance of the extract was similar to that of the methanol extract of the rice.

Antioxidant Efficacy of Wild Rice and Hull Extracts. The antioxidant efficacy of the extracts was evaluated in cooked ground beef by the TBARS value test (Rethwill *et al.*, 1981) and also evaluated in lard by the peroxide value test (AOCS, 1989). The results are shown in Table II. The methanol and ethanol extracts showed similar activities in both beef and lard. On the other hand, the ethyl acetate extract had little effect in beef and had a prooxidant effect in lard, which may be caused by the presence of chlorophyll, a known prooxidant. No prooxidant effect was observed in beef, probably because beef contains high levels of the prooxidant iron. Compared to BHA and TBHQ, the antioxidant activity of the extracts was rather low, partly due to the low solubility in oil. It is interesting that the hull extract also showed appreciable activity. The fact that wild rice hulls currently are a waste product of no use to wild rice processors makes them potentially a more economically attractive source of natural antioxidants than the wild rice *per se*.

Antioxidant Activity of Different Forms of Wild Rice. As discussed above, the antioxidant activity and the yields of the extract may not be high enough to be of

Table III. Antioxidant Activity of Various Forms of Wild Rice in Beef[a]

treatment (%)	TBARS,[a,c] ppm[b]	treatment (%)	TBARS,[a,c] ppm[b]
control (no additive)	2.7	PCWR (10/2.5)[d]	1.0**
δ-tocopherol (0.05)	0.3**	PCWR (15/3.75)[d]	0.2**
rosemary antioxidant (0.05)	0.5**	PWR[a] (2.5)	0.6**
CRW[a] (15/3.75)[d]	0.8**	PWR (5)	0.2**
PCWR[a] (5/1.25)[d]	1.8**	PWR (10)	0.3**

[a] Abbreviations: PCWR, pulverized cooked wild rice; PWR, pulverized wild rice; see Table I for other abbreviations. **Values significantly different ($p < 0.01$) from control. [b] SD = ±0.1 ppm. [c] Stored for 6 days at 4 °C. The TBARS values are based on weight of beef to eliminate the dilution effect by rice. [d] Figures in parentheses are the percentage on a wet/dry basis based on the average water content of 75%.

commercial significance. Therefore, an effort was made to optimize the antioxidant efficacy of the whole wild rice. Three different forms were tested, i.e., CWR, PCWR, and PWR. As shown in Table III, PCWR was much more active than CWR, apparently due to higher contact surface area between the rice and the beef components. Therefore, a smaller amount of the rice is needed to achieve the same effect. This is especially desirable when the amount of wild rice added to the food product is limited because of cost. PWR also showed substantial reduction on TBARS values (down to 0.6) even at a concentration as low as 2.5%. This compares to the TBARS values of 0.5 for beef treated with 0.05% rosemary antioxidant (Table III). Wild rice has been shown to increase consumer preference for beef (Minerich *et al.*, 1991). Therefore, the fact that comparable TBARS values were noted between rosemary and PWR is a significant finding.

Application of Wild Rice. Though many foodstuffs have been found to contain natural antioxidants, the concentrations are often too low to be of practical significance. They may be categorized as "antioxidant ingredients" and used in a whole or a rather crude form. However, it is essential that the presence of the bulk antioxidant ingredient should not have any undesirable effect on the quality of the food to which it is added. Therefore, the functionality of the antioxidant ingredient from wild rice in particular food systems requires further study.

There has been substantial interest in developing "microwavable" precooked meat products as convenience foods. One of the major problems is the development of rancidity, or warmed-over flavor, resulting from lipid oxidation. Therefore, there is a significant potential to use wild rice in meat products, not only for the antioxidant action but also for the flavor and nutritional benefits (Minerich *et al.*, 1991).

The antioxidant activity of the whole grain CWR is limited by its surface area. Second, it may affect the appearance of the product and could be misidentified by the consumer. Although the extracts are more efficient than the CWR, the cost of the extracts might be prohibitive for commercial applications. PCWR and PWR provide attractive alternatives for practical applications to utilize the antioxidant and other properties of wild rice. It is worth mentioning that since the starch in wild rice has a very high water binding capacity (Lorenz, 1981), it is advantageous to use PWR to absorb water and water-soluble nutrients during the subsequent cooking process. However, the rice must be sufficiently cooked because an otherwise undesirable grainy texture may result.

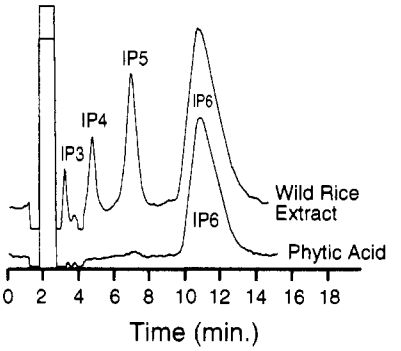

Figure 1. HPLC chromatogram of wild rice extract and phytic acid standard.

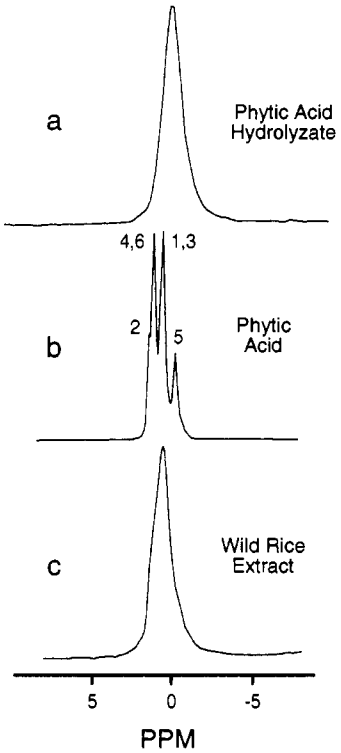

Figure 2. NMR phosphorus spectrum of phytic acid hydrolysate (a), phytic acid standard (b), and wild rice extract isolate (c).

Confirmation of the Antioxidant Identification. Sodium phytate was used as a standard and showed only one peak by HPLC analysis (Figure 1). Four standards were run covering a range from 0.10 to 0.40 mg/mL phytic acid. Results of the HPLC analysis (Figure 1) showed that the pulverized wild rice samples contain 0.42% of inositol hexaphosphate (IP6) "phytic acid" with 60.3% of isomer distribution; 0.185% of inositol pentaphosphate (IP5) with 26.5% of isomer distribution; 0.067% of inositol tetraphosphate (IP4) with 9.61% of isomer distribution; and 0.025% of inositol triphosphate (IP3) with 3.59% of isomer distribution.

The phosphorus spectra of the phytic acid hydrolysate (Figure 2a), phytic acid standard (Figure 2b), and wild rice isolate (Figure 2c) examined by nuclear magnetic resonance (NMR) show similar chemical shifts for phosphates. The loss of definition of the wild rice isolate spectra is due to the averaging of the multiple isomers present in the extract. The ^{13}C spectra of the wild rice isolate of the phytic acid hydrolysate (Figure 3a), phytic acid standard (Figure 3b), and wild rice isolate (Figure 3c) show the carbons of the inositol ring. The chemical shifts and

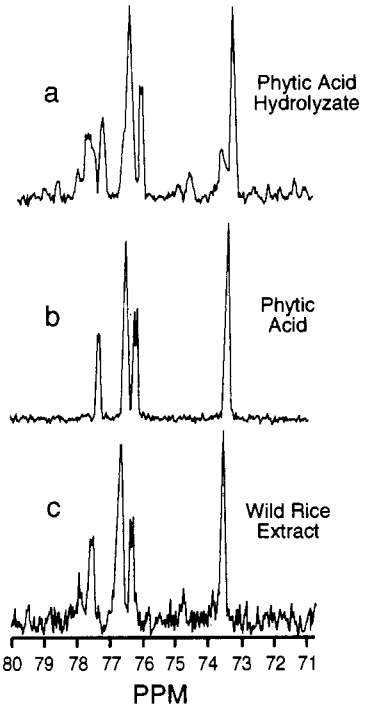

Figure 3. NMR carbon spectrum of phytic acid hydrolysate (a), phytic acid standard (b), and wild rice extract isolate (c).

intensity of patterns clearly match, showing that they correspond to the same compound.

Conclusions. The incorporation of CWR resulted in significant reduction of TBARS values in cooked ground beef during refrigerated storage. Solvent extraction using methanol, ethanol, and ethyl acetate indicated that the higher the polarity of the solvent, the higher the yield of the extract. The methanol and ethanol extracts showed antioxidant activity in both ground beef and lard, while the ethyl acetate extract had little effect in beef and even prooxidant activity in lard. PCWR and PWR showed much higher antioxidant activity than CWR due to increased surface areas and therefore have high potential for commercial applications in food systems, especially in meat products. By using both ^{31}P and ^{13}C NMR, we can conclude that one of the potent antioxidants in wild rice is phytic acid. The antioxidant properties of phytate are well established (Graf, 1983; Graf and Eaton, 1990).

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Received for review May 20, 1993. Revised manuscript received August 23, 1993. Accepted October 4, 1993.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1993.