Utilizing Ethanol Containing an Antioxidant or Chelator to Produce Stable Brown Rice Products

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ABSTRACT

Cereal Chem. 70(5):562-567

Liquid ethanol (EtOH) extraction of brown rice stabilized the kernels and the flours prepared from them to lipolytic hydrolysis. Oxidation in extracted kernels and their flours was inhibited by using EtOH as a carrier for an antioxidant or iron chelator. The order of effectiveness for the three antioxidants evaluated in this study was: propyl gallate ≈ butylated hydroxytoluene > tocopherols. Increased stability to oxidation was achieved by using these antioxidants in combination with citric acid. Citric acid also lowered residual lipase activity in extracted kernels and their flours, thereby further increasing their stability to lipolytic hydrolysis.

Brown rice lipids are susceptible to hydrolytic and oxidative deterioration. Off-odors and off-flavors are imparted to the rice as the lipids deteriorate, leading to a short shelf life (about three to six months).

A process for stabilizing brown rice to lipolytic hydrolysis by ethanol (EtOH) extraction was previously reported (Champagne et al 1990, 1991). The stabilizing action of EtOH was attributed to: 1) ethanolic denaturation of bran lipases with concomitant deactivation and 2) killing of lipase-producing bacteria and mold located on kernel surfaces. The stabilities of EtOH-extracted kernels and the flours prepared from them to lipolytic hydrolysis increased with higher extraction temperatures and longer extraction times (Champagne and Hron 1992). However, the higher the extraction temperature, the more susceptible the kernels and their flours were to oxidative deterioration during storage (Champagne and Hron 1992). In the case of extracted kernels, this was attributed, in part, to EtOH disrupting and increasing the porosity of the caryopsis coat, leaving lipids exposed to oxygen and susceptible to oxidation. The higher the EtOH temperature, the greater its penetration into the kernel, and the more disruptive it was to the caryopsis coat. EtOH treatments also caused both ethanolic- and heat-denaturation of the hemoproteins, catalase and peroxidase. Unfolding of these enzymes causes greater exposure of the heme groups to lipids, allowing heme iron to initiate oxidation.

The primary purpose of this investigation was to evaluate whether oxidation in EtOH-stabilized brown rice and its flour can be slowed by using EtOH as a carrier for an antioxidant or iron chelator. The effectiveness of butylated hydroxytoluene (BHT), propyl gallate, and tocopherols (alone and in combination with citric acid) was evaluated. These antioxidants are widely used in the food industry (Dziezak 1986). Brown rice and its flour are usually not treated with antioxidants or chelators. Because calcium and magnesium ions augment rice bran lipase activity (Shastry and Raghavendra Rao 1971, Aizono et al 1973, Sidhom et al 1975), a secondary purpose of this investigation was to evaluate whether citric acid, through its ability to chelate divalent cations, would lower residual lipase activity in EtOH-extracted kernels and their flours.

MATERIALS AND METHODS

Rice

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

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Antioxidants

Food-grade propyl gallate (Tenox PG) and tocopherols (Tenox GT-2, containing 70% mixed tocopherols) were obtained courtesy of Eastman Chemical Co. (Kingsport, TN). Food-grade butylated hydroxytoluene (Sustane PB, 15% BHT by weight in EtOH) was obtained courtsey of UOP (Des Moines, IA). Anhydrous citric acid (certified ACS) was purchased from Fisher Scientific.

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. Hot water (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 antioxidants: 0.019–1.90% citric acid, 0.019–1.25% propyl gallate, 0.013–0.28% BHT, and 0.018–1.05% tocopherols (w/w). The solvent was circulated at a flow rate of 1 L/min for 20 min and then drained. Two batches of rice were subjected to each EtOH-extraction treatment. Extracted kernels were placed in shallow stainless steel pans and desolventized at room temperature overnight. Brown rice flours were prepared by grinding extracted kernels to a powder in a Udy cyclone mill (Udy Corp., Fort Collins, CO) with a 20-mesh screen. Unextracted brown rice kernels and the flours prepared from them were the 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.

Antioxidant and Citric Acid Contents

Tocopherol content was determined by separating tocol isomers using high-performance liquid chromatography (HPLC) on a Supelcosil LC-Si, 5-μm, 25-cm × 4.6-mm i.d. column (Supelco, Bellefonte, PA). The mobile phase was 1.8% ethyl acetate and 0.9% acetic acid in isooctane with a flow rate of 1.6 ml/min. The tocol isomers were detected using a Waters 470 scanning fluorescence detector (Milford, MA). The excitation and emission wavelength settings were 290 and 330 nm, respectively. Concentrations of α, β, γ, and δ isomers of tocopherol were summed and reported as total tocopherol. Cargill Analytical Services (Cedar Rapids, IA) used an internal, gas chromatography method for determining BHT content. Propyl gallate content was determined by HPLC using a modification of method 983.15 of the AOAC (1990). Citric acid content was determined by HPLC (Silliker Laboratories, Chicago Heights, IL) according to a method developed by Blake et al (1987). It was cost-prohibitive to determine the antioxidant or citric acid content of all 400 samples monitored in this study; therefore, analyses were performed on duplicate samples treated with each level of antioxidant or citric acid used. Least squares linear regression analyses (GraphPAD InPlot, GraphPAD Software, San Diego, CA) were performed to determine the relationship between the amount of antioxidant or citric acid added to the sample and the amount retained. The antioxidant or citric acid contents given in the figures are predicted

values calculated from the regression lines and are representative of the amounts of each additive retained in the rice.

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 periodically afterwards by a micromethod (Hoffpauir et al 1947). This micromethod uses *m*-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.

Dynamic Headspace Analysis of *n*-Hexanal

A Tekmar LSC 2000 concentrator (Tekmar, Cincinnati, OH) equipped with a 25-ml straight-neck glass sample vessel was used for purge and trap analysis of brown rice volatiles in kernel and flour samples after six months of storage. Each kernel sample was ground to a flour using a Udy cyclone mill immediately before analysis. Samples (2 g) were purged at 50°C for 4 min with helium gas (grade 5) flowing at 40 ml/min. The sparging needle was positioned with the tip centered ~4 mm above the flour bed. Rice volatiles were trapped on Tenax, desorbed at 180°C for 4 min, and cryogenically focused at −130°C at the head of a Hewlett Packard Ultra 2 column (cross-linked 5% phenyl, 94% methyl, 1% vinylsilicon; 50-m × 0.32-mm i.d. with 0.52-μm film thickness) using a Tekmar capillary interface. An HP5890 Series II gas chromatograph (Hewlett Packard Co., Palo Alto, CA) equipped with a flame ionization detector was used with the following operating parameters: injector 230°C; detector 250°C; initial temperature 35°C; initial time 2.0 min; rate 4°C/min; final temperature 230°C; final time 10.0 min.

As a measure of lipid oxidation, *n*-hexanal contents of extracted and control brown rice kernels and their flours were determined. The amount of *n*-hexanal in stored brown rice is linearly proportional to the amount of oxidized linoleic acid, with a correlation coefficient of 0.99 (Shin et al 1986). It is considered a good indicator of oxidation. The *n*-hexanal concentrations in the samples were calculated from a linear regression line (peak area versus *n*-hexanal concentration) established by adding aliquots of standard solution containing 0.0244–0.814 μg of *n*-hexanal to an empty glass sample vessel immediately before purging. Calculated *n*-hexanal concentrations were corrected for recovery using a factor determined by exhaustively purging (Westendorf 1985) representative samples.

Each sample was spiked with 0.800 μg of methyl-3-hexenoate (K&K Laboratories, Plainview, NY) immediately before purging. The amounts recovered were determined from linear regression lines (peak area versus methyl-3-hexenoate concentration) generated for control and EtOH-extracted samples. These curves were established by adding aliquots of standard solution to the surface of the flour bed immediately before purging. The amount of methyl-3-hexenoate recovered in each sample was used to correct the calculated *n*-hexanal concentration in that sample for variations in purging efficiency and instrumental response.

TABLE I
Linear Regression Equations[a] and Correlation Coefficients for Antioxidant and Citric Acid Contents of Brown Rice Extracted with Ethanol Containing Additives

Additive	Linear Equation Coefficient *a*	Linear Equation Coefficient *b*	Correlation Coefficient
Citric acid	0.0794	0.0277	0.9998
Propyl gallate	0.0204	5.4496	0.9939
Butylated hydroxytoluene	0.0468	−4.2154	0.9949
Total tocopherol	0.0241	34.9833	0.9947

[a] $Y = aX + b$ where Y = amount of antioxidant (ppm) or citric acid (%, w/w) remaining in rice; X = amount of antioxidant (ppm) or citric acid (%, w/w) added to rice.

Statistical Analyses

Log-log regression curves were fit to FFA versus time data at each citric acid level for kernel and flour samples to determine whether FFA levels were significantly affected by citric acid content. The curves were compared among levels of citric acid content using analysis of covariance (Steel and Torrie 1980). Statistical analysis software (version 6, SAS Institute, Cary, NC) was used for the statistical analyses.

RESULTS AND DISCUSSION

Retention of Antioxidants and Citric Acid by EtOH-Extracted Brown Rice

Brown rice kernels, extracted with EtOH containing various amounts of antioxidants or citric acid, retained only small percentages of the additives: ~2% propyl gallate, ~4% BHT, ~3% total tocopherols, and ~8% citric acid. Extraction with EtOH did not lower endogenous citric acid (0.028%) or tocopherol (26 ppm of total tocopherols) contents. The linear regression equations describing the relationships between the amounts of antioxidant or citric acid added and the amounts retained are shown in Table I.

Effects of EtOH Extraction on FFA Levels

The effects of extraction with EtOH at 66°C for 20 min on the accumulation of FFAs (as a percent of oil) in brown rice kernels and flours prepared from them during storage at 36°C are shown in Fig. 1. During six months of storage, FFA levels in EtOH-extracted kernels and their flours increased from 1.2% to 4.4 and 18.7%, respectively. In contrast, FFA levels in control kernels and flours increased from 1.9% to 9.6 and 56.0%, respectively, during this storage time. Grinding kernels to flours mingled lipases and oil, allowing lipolytic hydrolysis to proceed more readily. The increases in FFA levels in EtOH-extracted kernels and their flours during storage indicate residual lipase activity. Thus, extraction with EtOH at 66°C for 20 min partially stabilized the kernels and their flours to lipolytic hydrolysis.

Effects of Citric Acid on FFA Levels

The effects of citric acid content on FFA levels in EtOH-extracted brown rice kernels and their flours during six months

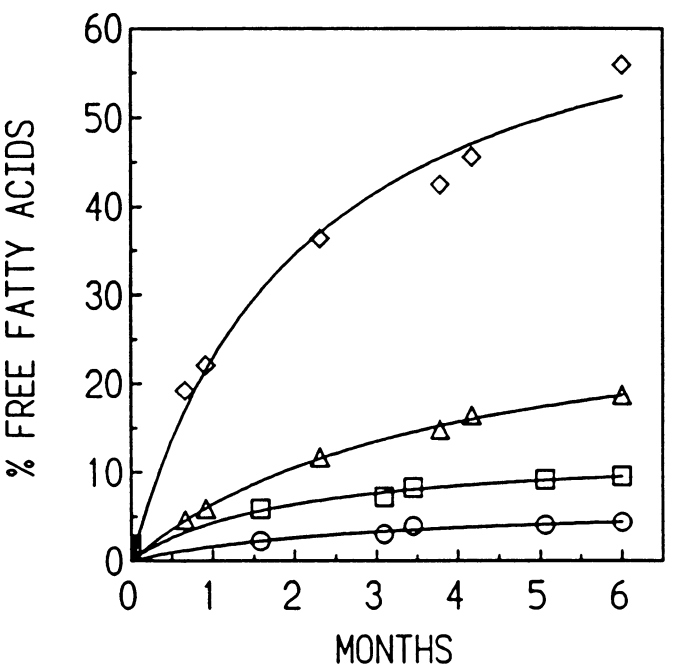

Fig. 1. Effect of ethanol extraction at 66°C for 20 min on the accumulation of free fatty acids in brown rice kernels (○) and flours prepared from them (△). Compared with control (unextracted) kernels (□) and their flours (◇). Samples were stored at 36°C. Means of analyses on 10–20 batches of rice. Average deviation from the mean was 3.5%. Free fatty acids expressed as percent of kernel oil.

of storage at 36° C are depicted in Fig. 2. The curves were generated by log-log regression in the form:

$$\text{Log}(\text{FFA} + 1) = m \times \log(\text{time} + 1) + b$$

where m = slope and b = y intercept at time 0. Increasing the citric acid level of brown rice from 0.028% (endogenous) to 0.030, 0.052, and 0.266% reduced the increase in FFA levels in kernels after six months of storage by 24, 32, and 40%, respectively. It reduced the increase in FFA levels in their flours by 43, 70, and 87%, respectively. Increased citric acid content significantly decreased ($P < 0.01$) FFA levels in kernels and their flours during storage, as indicated by analysis of covariance. Knowledge of time and citric acid content accounted for 90.4 and 94.3% of all the variability observed in the kernel and flour data, respectively. The trends in FFA levels in flour samples occurring across time at each citric acid concentration were all statistically distinct ($P < 0.0001$). With two exceptions, the trends in FFA levels with time in kernel samples were also statistically distinct ($P < 0.01$). The curves fitted to kernel data at citric acid concentrations of 0.030 and 0.052% could not be statistically distinguished; their slopes were marginally different ($P = 0.0466$). Conversely, the curves fitted to kernel data at citric acid concentrations 0.030 and 0.266% showed no statistical differences in slopes, but the overall curves were statistically distinct ($P < 0.01$). Hence, the FFA levels in the kernels containing 0.266% citric acid were significantly lower than those containing 0.030% citric acid.

The effectiveness of citric acid in stabilizing kernels and their flours to lipolytic hydrolysis using a higher (70° C) and lower (24° C) extraction temperature is shown in Table II. Although the same amount of citric acid was added to the kernels treated at the different temperatures, the amount of citric acid retained increased with higher extraction temperatures. FFA levels after storage were dependent on the amount of citric acid retained and the extraction temperature. For example, the FFA level at six months in flour containing 0.112% citric acid prepared from kernels extracted at 24° C (Table II) was approximately that of flour containing less than a third as much citric acid (0.030%) prepared from kernels extracted at 66° C (Fig. 2). Thus, the same degree of stability to lipolytic hydrolysis was obtained with a higher citric acid content and a lower extraction temperature as with a lower citric acid content and a higher extraction temperature.

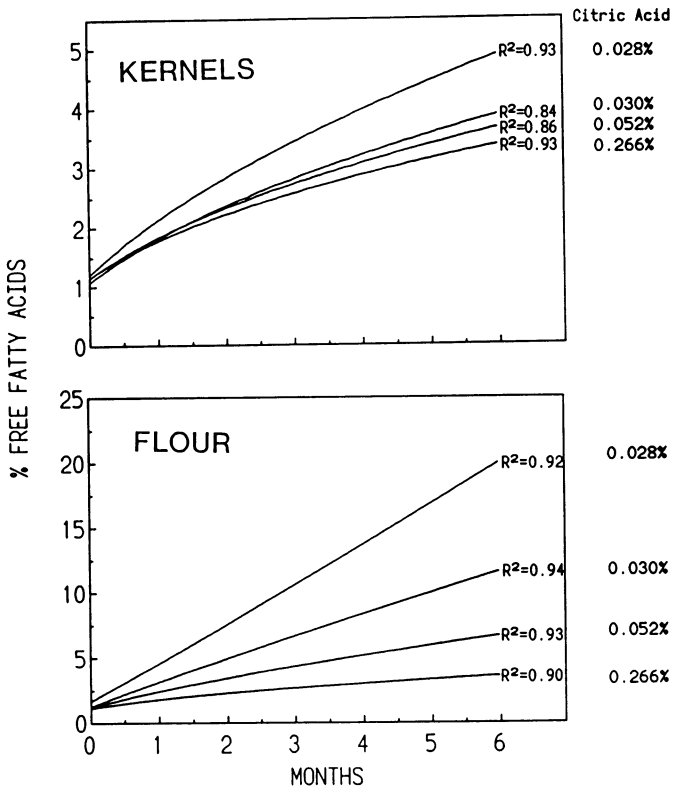

Fig. 2. Effect of citric acid content on free fatty acid levels in ethanol-extracted brown rice kernels and their flours during storage at 36° C. Curves were generated by log-log regression and fit functions of the form: $\text{Log}(\text{FFA} + 1) = m \times \log(\text{time} + 1) + b$ where m = slope and b = y intercept at time 0. Each curve fits 100–200 data points from analyses on 20–40 batches of rice. Citric acid contents are predicted values from linear regression line of amount added to sample versus amount retained. Free fatty acids expressed as percent of kernel oil.

TABLE II
FFA Levels in Kernels Extracted with EtOH Containing 0.19% Citric Acid (Weight Citric Acid/Weight EtOH) and in Their Flours Following 6 Months Storage at 36° C.

Extraction Temperature (° C)	Citric Acid Content, %	% Free Fatty Acids	
		Kernels	Flour
24	0.112	5.9 ± 0.2	12.9 ± 0.4
66	0.266	3.4 ± 0.1	3.6 ± 0.1
70	0.350	2.9 ± 0.1	2.8 ± 0.1

TABLE III
pH Values[a] of Ethanol-Citric Acid Treatment Solutions, Extracted Kernels,[b] and Flours Prepared from Extracted Kernels[c]

Citric Acid Content, %	Kernel	Flour, pH	Treatment Solution
0.028 (endogeneous)	7.00 ± 0.01	7.05 ± 0.02	7.5[d]
0.030	6.26 ± 0.02	6.99 ± 0.01	4.22 ± 0.02
0.052	5.23 ± 0.03	6.80 ± 0.02	3.71 ± 0.02
0.266	4.30 ± 0.01	5.55 ± 0.01	3.30 ± 0.02

[a] Means of duplicate determinations.
[b] pH determined on slurry (10 g of kernels/100 ml of deionized water) stirred for 5 min.
[c] pH determined on slurry (10 g of flour/100 ml of deionized water) stirred for 30 min.
[d] Unstable pH readings.

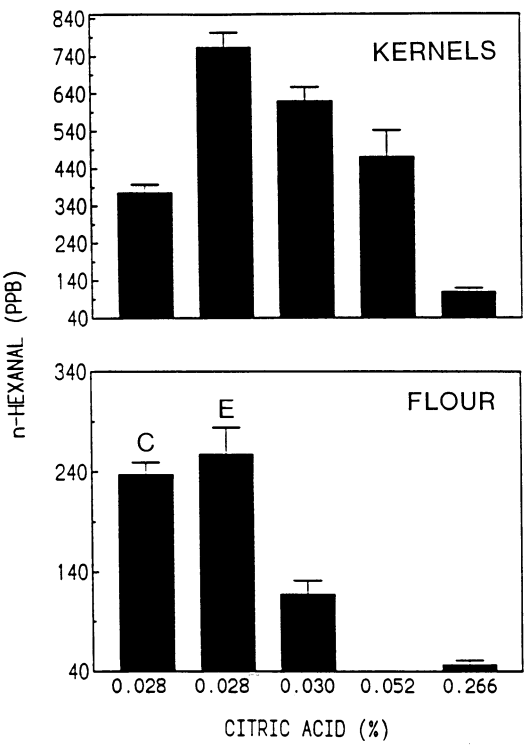

Fig. 3. Effect of citric acid content on n-hexanal levels in ethanol-extracted brown rice kernels and their flours. Samples were stored at 36° C. Citric acid contents are predicted values from linear regression line of amount added to sample versus amount retained. Endogenous citric acid content is 0.028%. C = control (unextracted), E = Ethanol-extracted (no added citric acid). Detection limit 40 ppb of n-hexanal. Error bars indicate standard error of the mean ($p = 0.05$).

Two possibilities may explain the inhibitory effect of citric acid on lipase activity. The first possibility is that citric acid chelates divalent cations that augment lipase activity, thus preventing the enzyme from having access to them. The second is that the decrease in lipase activity is due to a lowering of pH. As shown in Table III, the acidity of the EtOH-citric acid treatment solutions were in the pH 3–4 range. Exposing the kernels to these low pH values should have had little effect on lipase stability for two reasons. First, rice bran lipase is highly stable at acidic pH values (Aizono et al 1973); the enzyme retains approximately 75, 90, and 100% of its activity at pH 2, 3, and 4, respectively. Second, kernel constituents buffer the penetrating acidic EtOH solution, thus preventing the lipase from being exposed to these low pH values. The resultant pH of the in situ environment, however, could influence lipase activity. Maximum rice bran lipase activity occurs between pH 7.5–8.0 (Aizono et al 1973). Activity decreases at lower pH values: pH 7.0 (95%), pH 6.0 (70%), and pH 5.0 (30%). The pH values of flours (suspended in water) with 0.030 and 0.052% citric acid contents were only slightly lower than that of flour prepared from EtOH-extracted kernels with no added citric acid (Table III). The small effect these differences in pH could have on the enzyme's activity does not explain the marked differences in lipase activities observed among these flours (Fig. 2). Thus, inhibition of lipase activity by citric acid apparently was not the result of pH, unless lipase retains in the flour the ionization states that were acquired during the acidic EtOH extraction and the corresponding activity. Studies are currently underway to elucidate the mode of action of citric acid and to evaluate the influence of other divalent cation chelators on lipase activity.

Effects of Antioxidants and Citric Acid on *n*-Hexanal Levels

The effects of citric acid and antioxidants, alone or in combination with citric acid, on *n*-hexanal levels in brown rice kernels and their flours after six months of storage at 36°C are depicted in Figs. 3–6. Except for the *n*-hexanal concentrations of the EtOH-extracted and control samples, the plotted values are means of duplicate analyses on two batches of rice. Mean *n*-hexanal concentrations of EtOH-extracted and control samples were calculated from duplicate analyses on 10 batches of rice.

Note that *n*-hexanal levels were markedly lower in flours prepared from control and extracted kernels (with and without antioxidant or chelator) than they were in the kernels themselves. Within the rough rice kernel, oil is localized in the aleurone and germ of the caryopsis coat (Shastry and Raghavendra Rao 1971). Shelling of the kernel disrupts the bran layers, allowing oil to migrate towards the surface and be oxidized. Grinding the kernels to a flour dilutes the oil by a factor of 12 with the starchy endosperm. When stored in a jar, the flour particles pack tightly and exclude more air than kernels stored in a jar. Thus, in intact kernels, more oil would be exposed to air and be susceptible to oxidation. The effectiveness of antioxidants and citric acid in inhibiting oxidation depends on the extent that the EtOH carrier penetrates the bran layers and makes contact with the oil bodies within. Grinding kernels to flour improves the contact between the oil and antioxidant or citric acid, thus increasing the effectiveness of the additive in inhibiting oxidation. The *n*-hexanal level in brown rice kernels extracted with EtOH without an antioxidant or chelator was approximately twice that for control kernels. The oil in EtOH-extracted kernels was more susceptible to oxidation than the oil in control kernels because EtOH increased kernel porosity, leaving oil exposed to oxygen (Champagne et al 1991). The extent of oxidation in flours prepared

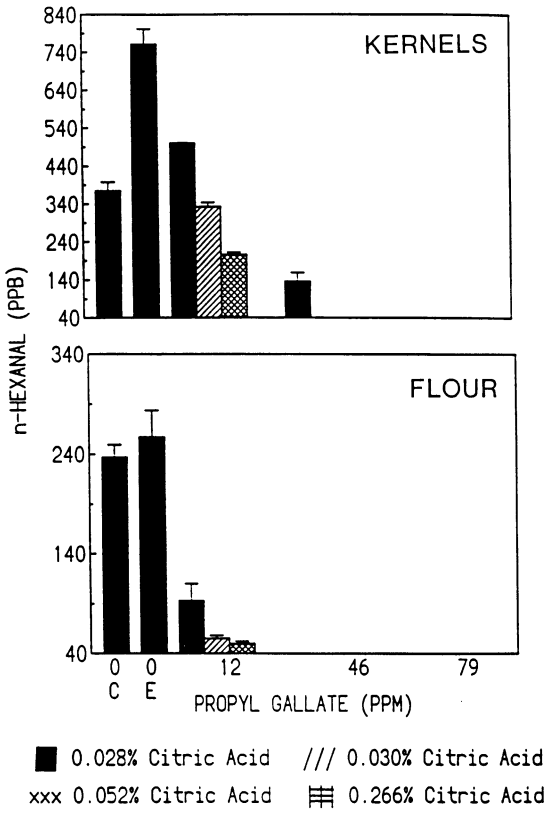

Fig. 4. Effect of propyl gallate, alone and in combination with citric acid, on *n*-hexanal levels in ethanol-extracted brown rice kernels and their flours. Samples were stored at 36°C. Propyl gallate and citric acid contents are predicted values from linear regression lines of amount added to sample versus amount retained. C = control (unextracted), E = Ethanol-extracted (no additives). Detection limit, 40 ppb of *n*-hexanal. Error bars indicate standard error of the mean ($p = 0.05$).

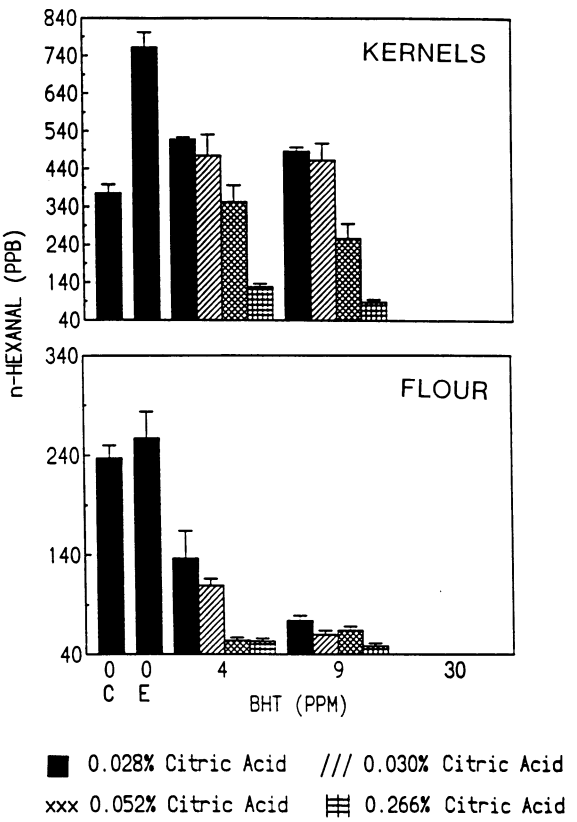

Fig. 5. Effect of butylated hydroxytoluene, alone and in combination with citric acid, on *n*-hexanal levels in ethanol-extracted brown rice kernels and their flours. Samples were stored at 36°C. Butylated hydroxytoluene and citric acid contents are predicted values from linear regression lines of amount added to sample versus amount retained. C = control (unextracted), E = Ethanol-extracted (no additives). Detection limit, 40 ppb of *n*-hexanal. Error bars indicate standard error of the mean ($p = 0.05$).

from EtOH-extracted and control kernels was the same after six months of storage. There was no significant difference ($P = 0.4754$) in the *n*-hexanal levels of these flours.

Increasing citric acid content from endogenous (0.028%) to 0.030 or 0.052% resulted in small decreases in *n*-hexanal levels in kernel samples (Fig. 3). The *n*-hexanal level in kernels containing 0.266% citric acid was markedly less (~30% that of control kernels). Flour with a citric acid content of 0.030% had an *n*-hexanal level ~50% that of control flour (Fig. 3). A high degree of stability to oxidation was achieved with a citric acid content of 0.052 or 0.266%. The *n*-hexanal levels in flours with citric acid contents of 0.052 or 0.266% were <40 ppb (detection limit) and 46 ppb (~15% that of control), respectively.

The *n*-hexanal level in kernels containing 12 ppm of propyl gallate was approximately 66 and 133% that of EtOH-extracted and control kernels, respectively (Fig. 4). The *n*-hexanal level decreased to about that of the control in kernels containing 12 ppm of propyl gallate and 0.030% citric acid. Increasing the citric acid content to 0.052 or 0.266% in kernels containing 12 ppm of propyl gallate resulted in *n*-hexanal levels ~55% of the control and not detectable (<40 ppb), respectively. In kernels containing 46 ppm of propyl gallate, the *n*-hexanal level was ~37% that of control kernels; in combination with added citric acid, no *n*-hexanal was detected. At 79 ppm and higher levels (128–413 ppm) of propyl gallate (alone or in combination with added citric acid) no *n*-hexanal was detected. In flour containing 12 ppm of propyl gallate, the *n*-hexanal level was ~39% that of the control (Fig. 4). The *n*-hexanal levels were ~20% that of the control in flours containing 0.030 or 0.052% citric acid in conjunction with 12 ppm of propyl gallate. No *n*-hexanal was detected in flours containing 12 ppm of propyl gallate and 0.266% citric acid or higher levels (46–413 ppm) of propyl gallate, alone or in combination with added citric acid.

A drawback of having propyl gallate in the rice was the kernels having a grayish tinge; the antioxidant forms a blue-black complex

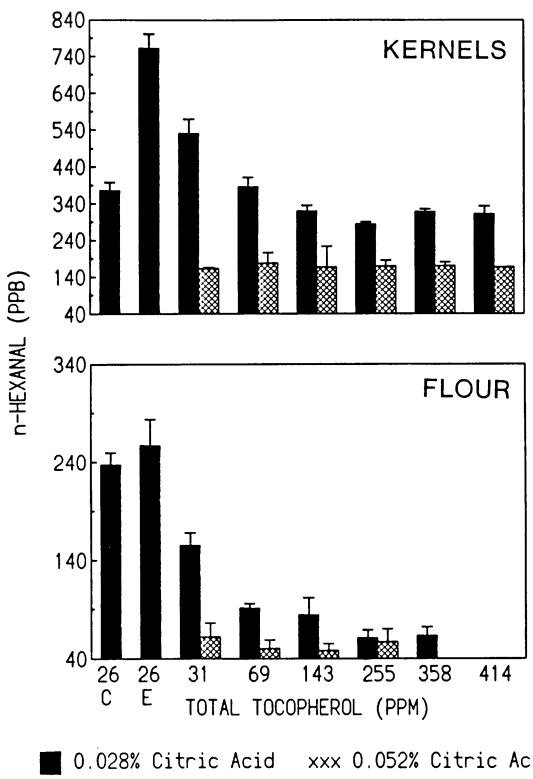

Fig. 6. Effect of total tocopherols, alone and in combination with citric acid, on *n*-hexanal levels in ethanol-extracted brown rice kernels and their flours. Samples were stored at 36°C. Total tocopherols and citric acid contents are predicted values from linear regression lines of amount added to sample versus amount retained. Endogenous total tocopherols content, 26 ppm. C = control (unextracted), E = Ethanol-extracted (no additives). Detection limit, 40 ppb of *n*-hexanal. Error bars indicate standard error of the mean ($p = 0.05$).

with iron in the bran. Adding the stronger chelator citric acid eliminated this color problem by binding the pro-oxidative iron.

BHT contents of 4 and 9 ppm resulted in *n*-hexanal levels in kernels ~66 and 134% that of EtOH-extracted and control kernels, respectively (Fig. 5). Citric acid contents of 0.052 and 0.266%, in conjunction with 4 ppm of BHT, resulted in *n*-hexanal levels about that and 34% that of the control, respectively. The *n*-hexanal levels in kernels containing 9 ppm of BHT, in combination with 0.052 or 0.266% citric acid, were 68 and 24% that of the control, respectively. In flours containing 4 or 9 ppm of BHT, *n*-hexanal levels were ~57 and 31% that of the control, respectively (Fig. 5). Increasing the citric acid content of the flour containing 4 ppm of BHT to 0.052 or 0.266% resulted in a *n*-hexanal level ~23% that of the control. The *n*-hexanal levels were slightly lower in flours containing 9 ppm of BHT in combination with added citric acid than they were in those with no added citric acid. No *n*-hexanal was detected in kernels or flours containing 30 ppm or higher levels (65–202 ppm) of BHT, alone or in combination with citric acid.

Tocopherols inhibited oxidation in EtOH-extracted brown rice kernels and their flours, but not to the extent of propyl gallate and BHT. Increasing total tocopherols content of kernels from endogenous (26 ppm) to 31 ppm resulted in an *n*-hexanal level ~65 and 132% that of EtOH-extracted and control kernels, respectively (Fig. 6). Kernels containing 69 ppm of total tocopherols had a *n*-hexanal level about that of the control. In kernels containing 143–414 ppm of total tocopherols, and in those containing these amounts of total tocopherols in combination with 0.052% citric acid, *n*-hexanal levels were ~82 and 45%, respectively, that of the control. In flours, increasing total tocopherols content to 31, 69, 143, 255, or 358 ppm resulted in *n*-hexanal levels ~65, 38, 35, 26, and 26%, respectively, that of the control (Fig. 6). The *n*-hexanal levels were <32% that of the control in flours containing 31–358 ppm of total tocopherols in combination with 0.052% citric acid. No *n*-hexanal was detected in flours containing 414 ppm of total tocopherols, alone or in combination with citric acid.

Oxidation in EtOH-stabilized brown rice kernels and the flours produced from them can be slowed by using EtOH as a carrier for an antioxidant or chelator (Figs. 3–6). The effectiveness of the three antioxidants evaluated in this study followed the order: propyl gallate ≈ BHT > tocopherols. Increased stability to oxidation was achieved by using these antioxidants in combination with citric acid. Citric acid alone was ineffective in reducing the extent of oxidation in EtOH-extracted kernels to less than that of control kernels, except at the 0.266% level. However, at this level, an acidic odor and sour, fruity flavor was imparted to the rice that was not noticeable at lower levels. In flours prepared from EtOH-extracted kernels, a high degree of stability to oxidation was achieved with a citric acid content of 0.052%.

The inclusion of citric acid in the EtOH-extraction process would be of particular value in producing stable brown rice flour because total inactivation of lipase is difficult to achieve, even when high extraction temperatures and long extraction times are used. Total inactivation of lipases is not required to produce stable kernels (Champagne and Hron 1992), but all, or nearly all, of the lipase activity must be eliminated to produce stable flours. Any residual lipase activity results in lipolytic hydrolysis proceeding in the flours because the lipases and lipids are mingled. Citric acid would reduce or eliminate this residual lipase activity and would allow the use of lower extraction temperatures and shorter times.

The effect of citric acid on the stability of brown rice to lipolytic hydrolysis may have implications for stabilization of rice bran by extrusion. Including citric acid in rice bran before or after extrusion may be valuable in yielding a product with higher stability to lipolytic hydrolysis. The use of citric acid may allow the use of lower extrusion temperatures and shorter times. This may be valuable in minimizing the extent of heat-denaturation of the hemoproteins, catalase and peroxidase. Upon heat-denaturation, these enzymes act as iron catalysts and increase the susceptibility of the bran to oxidative deterioration. Lower

extrusion temperatures and shorter times may also prevent the loss of unsaponifiables (tocotrienols, γ-oryzanol) implicated in lowering low-density lipoprotein cholesterol levels in humans (Yoshino et al 1989, Qureshi et al 1991).

In conclusion, EtOH serves well as an agent for inhibiting lipolytic hydrolysis and as a carrier for an antioxidant or chelator for slowing down oxidative deterioration in extracted brown rice kernels and their flours.

ACKNOWLEDGMENTS

We acknowledge Samuel E. Ellzey, Jr., and Hector Huerta for technical assistance and Bryan T. Vinyard for statistical analyses. Acknowledgment is also given to Tai-Sun Shin (Food Science Department, Louisiana State University) for tocopherol analyses.

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[Received October 21, 1992. Accepted May 24, 1993.]