

# Stabilizing Brown Rice Products by Aqueous Ethanol Extraction

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## ABSTRACT

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This article describes extraction processes using aqueous ethanol (EtOH) (95%, v/v) for producing stabilized brown rice and brown rice flours. The effects of EtOH extraction on brown rice composition, appearance of kernel surface, bacterial and mold populations, and storage stabilities of brown rice kernels and flours (prepared from extracted kernels) were examined. Less than 3% of the oil was removed from brown rice kernels extracted with EtOH at room temperature (RT) (approximately 24°C), whereas extraction at 70°C removed 15% of the oil. Thiamin retention

was 91 and 37% in kernels extracted with EtOH at RT and 70°C, respectively. Little or no loss of protein, dietary fiber, carbohydrates, or minerals occurred in the extracted kernels. EtOH extraction decreased the bacterial population of the rice to very low levels and, at RT or 70°C, stabilized brown rice kernels to lipolysis. Only flour prepared from kernels extracted with EtOH at 70°C was stable to lipolysis. Brown rice kernels extracted with EtOH at 70°C were more susceptible to oxidative rancidity, probably because the hot alcohol disturbed the caryopsis coat.

Brown rice is a nutritionally valuable food source. The bran layers of the brown rice kernel are rich in dietary fiber, minerals, oil, and vitamins, particularly the B vitamins. The results of recent studies indicate that these bran layers may also have cholesterol-reducing properties (Kahlon et al 1989, Hegsted et al 1990). However, the use of brown rice has been limited because the oil in the bran is susceptible to readily becoming rancid, leading to a shortened shelf life due to off-flavors.

Brown rice lipids are subject to both oxidative and hydrolytic deterioration. Oxidative deterioration can be slowed using optimum packaging materials, temperatures, and atmospheres for storage (Mitsuda et al 1972, Sowbhagya and Bhattacharya 1976, Ory et al 1980, Sharp and Timme 1986). Two approaches have been taken to stabilize brown rice to enzymatic hydrolysis by lipase. The first involves inactivating lipase by subjecting raw or brown rice to moist or dry heat (Van Atta et al 1952, Bardet and Glease 1961, Hirokawa et al 1986) or to parboiling or pre-cooking processes (Miller 1963, McCabe 1976, Sowbhagya and Bhattacharya 1976). The second approach involves extraction with an organic solvent to remove kernel lipids that serve as a substrate for lipase. Kester (1951) demonstrated this approach by stabilizing brown rice using room temperature (RT) petroleum ether or boiling hexane as extractive solvents.

This article describes ethanol (EtOH) extraction processes for producing stabilized brown rice and brown rice flours. The effects of EtOH extraction on brown rice composition, kernel surface appearances, bacterial and mold populations, and on the storage stability of brown rice kernels and the flours prepared from extracted kernels are reported.

## MATERIALS AND METHODS

### Rice Sample

Rough rice samples of Tebonnet (1988 crop) were obtained from the Louisiana State University Rice Experiment Station (Crowley, LA). The samples were dehulled in a McGill sheller (H. T. McGill, Houston, TX).

### 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. Extractions were performed at either room temperature (RT) (approximately 24°C) or at 70°C.

For extractions performed at 70°C, hot water (78°C) was circulated through the extractor jacket. The first extraction was done with 800 g of aqueous EtOH (95%, v/v). The solvent was circulated at a flow rate of 1 L/min. for 20 min, at which time the solvent was drained. Two additional 20-min extractions, each using 600 g of solvent, were performed. The solvent-extracted rice kernels were placed in shallow stainless steel pans and desolventized in RT air overnight or in RT air followed by 1 hr in a vacuum (10 mm, 35°C). 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 sieve screen.

Unextracted brown rice kernels and flours prepared from them were the controls. Brown rice kernels were placed in a 70°C oven for 1 hr to evaluate the effect of heat on kernel stability. To compare the action of aqueous EtOH with that of hexane (a common, commercial vegetable oil extractive solvent), brown rice kernels were extracted with Getty-B hexane at 68°C for 4 hr using a Soxhlet apparatus. The kernels were desolventized overnight in RT air. Flours were prepared by grinding the hexane-extracted kernels.

Samples of brown rice kernels and their flours were stored in pint-sized capped glass jars at either RT or 36°C, with no humidity control. Analytical tests were performed on two batches of EtOH-extracted, hexane-extracted, and control kernels and flour samples to determine compositions, bacterial and mold populations, and storage stabilities.

### Composition Analysis

Total dietary fiber, nitrogen, moisture, ash, fat, carbohydrate, and thiamin contents of the extracted and control rice samples were determined in duplicate by an outside laboratory using official methods of the AACC (1983) and the AOAC (1990). Elemental compositions were determined in duplicate on HNO<sub>3</sub>-HClO<sub>4</sub> (3:1) digests of ground rice samples using inductively coupled plasma spectroscopy.

### Microbiological Assays

For microbiological assays, 10-g samples of rice were weighed, transferred aseptically into sterile blender jars, and blended with 90 ml of sterile distilled water buffered with phosphate, pH 7.2. Serial dilutions of 10<sup>-1</sup>, 10<sup>-2</sup>, and 10<sup>-3</sup> were prepared, also using sterile distilled water, pH 7.2, buffered with phosphate. Duplicate nutrient agar pour plates for total plate counts and triplicate potato dextrose agar plates for total molds were inoculated with the appropriate dilutions and incubated as described by DeLucca et al (1978).

### Free Fatty Acid Content

As a measure the extent of lipolytic hydrolysis of brown rice kernels and flours during storage, the free fatty acids (FFA) of samples of extracted and control rice samples were determined the day after extraction and then periodically by a micromethod (Hoffpaup et al 1947). Metacresol purple was substituted for the

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phenolphthalein indicator. FFA content was calculated as oleic acid and expressed as percent of oil.

### Conjugated Diene Hydroperoxides

As a measure of oxidative deterioration of unsaturated lipids in brown rice kernels and flours during storage, conjugated diene hydroperoxides (CDHP) were determined by the method of St. Angelo et al (1972). Samples were ground in a Udy cyclone mill to pass through a 20-mesh screen. Duplicate 1-g samples were shaken with hexane (25 ml, high-performance liquid chromatography grade) for 30 min and then filtered through 0.45- $\mu$ m Millex-HV Millipore filters. Absorbancies of the filtrates at 234.0 nm were determined, using hexane as a reference. An  $A_{234}$  of 24,500  $\text{mol}\cdot\text{L}^{-1}\cdot\text{cm}^{-1}$  was used to calculate the concentration of CDHP in micromoles per gram of brown rice (dry basis).

### Scanning Electron Microscopy

A Hitachi S-510 scanning electron microscope was used to examine the surfaces of the extracted and control kernels. The rice grains were attached to aluminum sample stubs with double adhesive tabs. No fixation and dehydration processes were necessary. Prepared stubs were sputter-coated with gold-palladium to prevent charging in the electron beam. The stubs were observed at 10 kV of accelerating voltage.

**TABLE I**  
Compositions of Extracted and Nonextracted (Control) Tebonnet (Long Grain) Brown Rice Kernels<sup>a</sup>

Component	Control	Ethanol		Hexane 68°C
		Room Temperature	70°C	
Moisture, %	12.6	10.4	9.2	10.7
Nitrogen, %	1.5	1.7	1.5	1.4
Fat, %	3.3	3.2	2.8	2.9
Total dietary fiber, %	5.2	5.7	5.1	5.7
Ash, %	1.6	1.6	1.4	1.9
Carbohydrates, %	80.9	79.2	80.3	78.3
Free fatty acids, %	4.1	3.6	1.9	3.5
Magnesium, mg/g	1.3	1.1	1.1	1.3
Phosphorus, mg/g	3.3	3.0	3.0	3.3
Potassium, mg/g	2.8	2.7	2.7	2.9
Calcium, $\mu$ g/g	109	109	117	113
Iron, $\mu$ g/g	25	23	23	16
Zinc, $\mu$ g/g	26	25	26	26
Manganese, $\mu$ g/g	52	48	48	52
Thiamin, $\mu$ g/g	4.6	4.2	1.7	3.7

<sup>a</sup>Values are on a dry basis (except those for moisture) and are means for duplicate analyses. Values for fat analyses are means of 10 determinations. Variations from mean values were less than  $\pm 3\%$ , except for the variations for total dietary fiber and thiamin, which were  $< \pm 6\%$  and  $\pm 5\%$ , respectively. Free fatty acids are expressed as a percent of total oil content.

**TABLE II**  
Microbial Populations of Solvent-Extracted and Nonextracted (Control) Brown Rice Kernels and Their Flours, Initially and After Five Months of Storage at Room Temperature

Treatment	Total Plate Count (No./g of rice)		Mold Count (No./g of rice)	
	Initially	After 5 Months	Initially	After 5 Months
No extraction				
Control	20,000	5,300 (k) <sup>a</sup> 2,500 (f)	10	10 (k) 20 (f)
Extraction				
Ethanol, 70°C	180	20 (k) 20 (f)	<10	<10 (k) <10 (f)
Ethanol, RT	120	70 (k) 120 (f)	10	10 (k) 20 (f)
Hexane, 68°C	180	80 (k) 240 (f)	10	10 (k) 20 (f)

<sup>a</sup>k = kernels, f = flour, RT = room temperature.

## RESULTS

### Effect of EtOH Extraction on the Composition of Brown Rice Kernels

Table I compares the composition of brown rice kernels extracted with EtOH at RT and 70°C and with hexane at 68°C and the unextracted control. Less than 3% of the oil was removed from the kernels extracted with EtOH at RT. Extraction with EtOH at 70°C or with hexane at 68°C removed approximately 15% of the kernel oil. Kernels extracted with EtOH at RT retained 91% of thiamin, whereas at 70°C thiamin content was reduced 63%; extraction with hexane at 68°C resulted in 20% loss of thiamin. No loss of protein (nitrogen), total dietary fiber, or carbohydrate occurred as a result of extraction with EtOH or hexane, and loss of minerals was minimal.

EtOH extraction of freshly dehulled brown rice kernels at 70°C yielded a product with an FFA content approximately 50% that of the control kernels. The FFA content of the brown rice products extracted with EtOH at RT or with hexane at 68°C was approximately 85% that of the control. Likewise, FFA content was reduced when kernels with high FFA content were extracted. EtOH-extraction at 70°C of kernels with 27% FFA content led to a 77% decrease in FFA. Extraction with EtOH at RT and hexane at 68°C led to 33 and 57% reductions in FFA, respectively (data not shown).

The residual EtOH contents of brown rice extracted with EtOH at RT and 70°C with air-desolventization were approximately 830 and 115 ppm, respectively. After desolventizing by vacuum, residual EtOH contents were approximately 260 and 50 ppm, respectively, for kernels extracted with EtOH at 70°C and RT.

### Effect of EtOH Extraction on Surface Appearance of Brown Rice Kernels

Visually no differences could be seen between the kernels extracted with EtOH at RT and control kernels. Kernels extracted with hexane at 68°C appeared slightly dull; those extracted with EtOH at 70°C appeared duller and drier. Scanning electron micrographs revealed the extent of disruption of the caryopsis coat (pericarp, testa, nucellus) caused by extraction. The undulating caryopsis coat of the control kernels appeared more wavy at  $\times 20$  magnification following solvent extraction. Magnification at  $\times 2,000$  revealed narrow crease marks on the surfaces of the kernels extracted with EtOH at RT and those extracted with hexane at 68°C. The surfaces of the control kernels did not have these marks. The marks were wider and more pronounced on the kernels extracted with EtOH at 70°C, giving their surfaces a hill-and-valley appearance.

### Effect of EtOH Extraction on Microorganisms in Brown Rice Kernels and Flour

Table II shows the bacterial and mold populations of extracted and control kernels and their flours, initially and after five months of storage at RT. Total plate counts decreased during storage and were very low in the extracted kernels and their flours. The mold counts of the kernels and flours were low. No countable mold was observed in the kernels extracted with EtOH at 70°C or their flours.

### Effect of EtOH Extraction on Extent of Lipolytic Hydrolysis of Lipids in Brown Rice Kernels and Flours During Storage

FFA levels in extracted kernels that were air- or vacuum-desolventized did not change during six months of storage at RT, whereas levels of control kernels increased from 4 to 6%. Figure 1 shows the effect of storage time at 36°C on FFA levels in extracted and control kernels. At a storage temperature of 36°C, the FFA levels in kernels extracted with EtOH at RT and hexane at 68°C increased from 3.3 to 4.3%; the level in the kernels extracted with EtOH at 70°C remained at 1.8%. When stored at 36°C for six months, the FFA level of the control kernels increased from 4 to 9.8%. No differences were seen in the FFA levels during storage of control kernels and those heated at 70°C for 1 hr before storage.

Figure 2 shows the effect of storage time at RT and 36°C, respectively, on FFA levels in flours prepared from control and extracted kernels that were air-desolventized. FFA levels increased rapidly in control flour samples that were stored at RT or 36°C. The rate of increase in FFA levels was greater for flours stored at the higher temperature. FFA levels also increased rapidly in flour samples prepared from kernels extracted with EtOH at RT and hexane at 68°C that were stored at RT or 36°C, but the rates of increase were lower than those of the control flours. Only slight increases occurred in FFA levels in flour prepared from kernels extracted with EtOH at 70°C that were stored at RT or 36°C. Decreasing the residual EtOH content of this flour by vacuum-desolventizing to the amount found in the flour prepared from kernels extracted with EtOH at RT (approximately 100 ppm) did not change the rate of FFA formation. During storage, the FFA content of flour prepared from kernels heated in an oven at 70°C for 1 hr was less than 10% lower than that of the control flour (data not shown).

#### Effect of EtOH Extraction on the Extent of Oxidative Deterioration of Unsaturated Lipids in Brown Rice Kernels and Flours During Storage

Figure 3 depicts the development of CDHP in extracted and control kernels during storage at RT and 36°C. Initial CDHP levels in the kernels extracted with EtOH at 70°C and hexane at 68°C were lower than those in kernels extracted with EtOH at RT and control kernels. The hot solvents apparently extracted CDHP. CDHP levels in the kernels extracted with EtOH at 70°C increased rapidly during storage at RT or 36°C. In contrast, CDHP levels in control kernels and kernels extracted with EtOH at RT and hexane at 68°C increased only gradually during storage at RT or 36°C. In flours prepared from extracted and control kernels, CDHP levels increased rapidly during the first three months of storage and then leveled off (Fig. 4). After three months of storage at RT, CDHP levels were approximately the same in the flours prepared from control and EtOH-extracted kernels; the level in flour from hexane-extracted kernels was slightly lower. After three months of storage at 36°C, CDHP levels in flours prepared from control kernels and kernels extracted with EtOH at RT and hexane at 68°C were approximately the same; the level in flour from kernels extracted with EtOH at 70°C was higher.

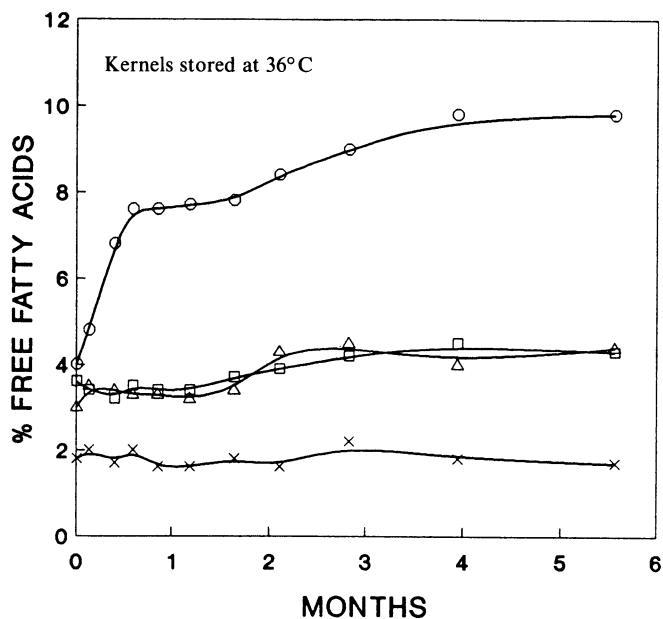


Fig. 1. Effects of storage at 36°C on levels of free fatty acids in solvent-extracted and nonextracted (control) brown rice kernels. RT - room temperature, EtOH = ethanol, ○ = control kernels, □ = kernels extracted with EtOH at RT, × = kernels extracted with EtOH at 70°C, △ = kernels extracted with hexane at 68°C.

## DISCUSSION

Extraction of brown rice with aqueous EtOH (95%, v/v) at RT yielded a full-fat product with an FFA content approximately that of freshly dehulled rice. A product with approximately 15% lower oil content and an FFA content approximately half that of freshly dehulled rice resulted from kernels extracted with EtOH at 70°C.

Thiamin was chosen as an indicator of the degree of retention of B vitamins, which can be extracted from rice bran by EtOH (Talwalkar et al 1965). Thiamin retention was 91 and 37% in the kernels extracted with EtOH at RT and 70°C, respectively. In comparison, thiamin retention was 80% in the kernels extracted with hexane at 68°C. Thiamin is soluble in 95% EtOH (1 g/100 ml) and is practically insoluble in hexane (Windholz 1976). The EtOH apparently penetrated the kernel layers to a greater extent at 70°C than at RT, thus leaching more thiamin. Some thiamin probably was also lost by heat destruction at the higher temperatures. Little or no loss of protein, dietary fiber, carbohydrates, and minerals occurred in the kernels extracted with EtOH or hexane.

EtOH and hexane extractions decreased the bacterial population of brown rice to very low levels. Total plate counts

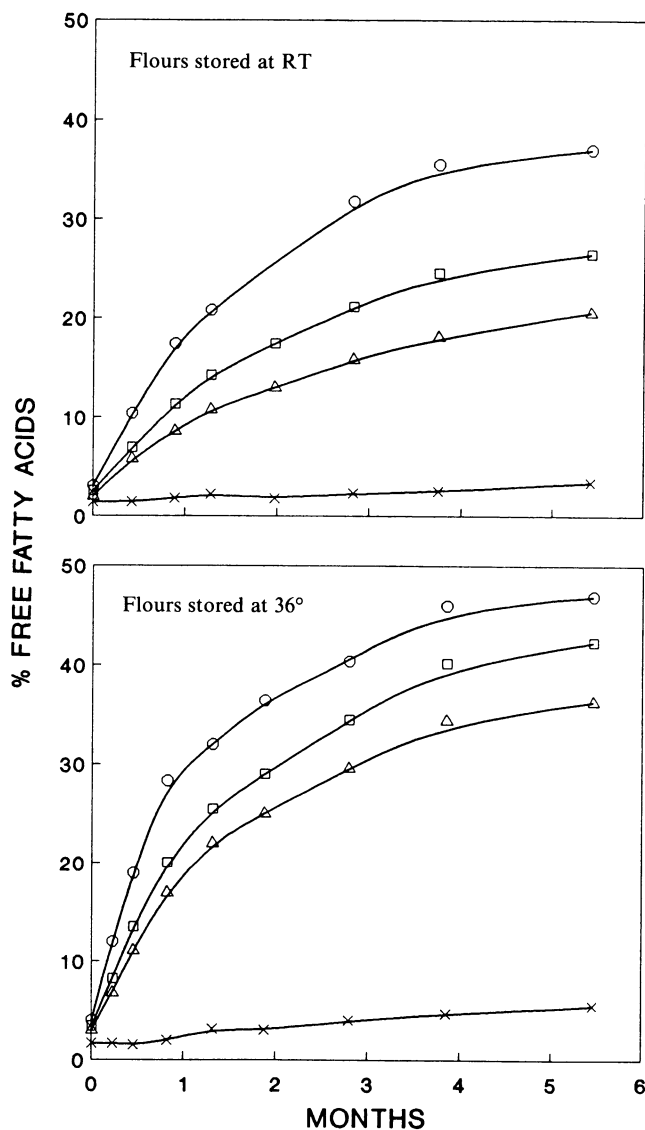


Fig. 2. Effects of storage at room temperature (RT) and 36°C on free fatty acids in flours prepared from solvent-extracted and nonextracted (control) brown rice kernels. EtOH = ethanol, ○ = control kernels, □ = kernels extracted with EtOH at RT, × = kernels extracted with EtOH at 70°C, △ = kernels extracted with hexane at 68°C.

decreased during storage, as previously observed (Ory et al 1980).

Brown rice has a short shelf life (approximately 3–6 months) because of enzymatic and oxidative deterioration of bran lipids. Brown rice oil is readily hydrolyzed by the action of lipases (both natural to the bran and of microbial origin) that release FFA (DeLuca et al 1978). Accumulation of FFA imparts to the rice unacceptable off-flavors and sour odors. Hydroperoxides are the primary products of the reaction of oxygen with unsaturated lipids (Frankel 1961). These degrade to form volatile carbonyl compounds, which impart off-flavors and rancid odors to brown rice (Frankel 1961, Sharp and Timme 1986).

EtOH and hexane extractions stabilized brown rice kernels to lipolytic hydrolysis, as indicated by the little or no increase in FFA in the extracted kernels. EtOH acts in several ways in stabilizing brown rice. First, EtOH denatures bran lipases and thus deactivates them. Second, EtOH reduces bacterial and mold populations by killing the organisms. DeLuca et al (1978) determined that approximately 10% of the total bacterial population on rough rice and all of the isolated molds showed lipolytic action. Third, which also explains the action of hexane, EtOH removes kernel oil that serves as a substrate for lipase. Within the intact rice kernel, lipases are localized in the testa-cross layer

region of the caryopsis coat, while the oil is localized in the aleurone and germ (Shastry and Raghavendra Rao 1971). Damage to kernels during shelling disrupts these regions, allowing oil and lipase to mingle and lipolysis to proceed. Solvent extraction of this "freed" oil removes the substrate from the lipase. Kester (1951) found that the oil that is removed from whole brown rice kernels with fat solvents was characteristically unstable in the grain (in contact with lipase) and stable when extracted from the grain (enzyme absent).

Flours prepared from kernels extracted with EtOH at 70°C were stable to lipolysis, whereas flours prepared from control kernels and kernels extracted with EtOH at RT and with hexane at 68°C were not stable. The large increases in FFA in flour samples prepared from kernels extracted with EtOH at RT and hexane at 68°C can be explained by these solvents not penetrating the kernel surface sufficiently to deactivate all of the lipase. Grinding these kernels to flours allows the oil and lipase to make contact and lipolysis to occur. In contrast, EtOH at 70°C apparently penetrated farther into the bran layers and deactivated nearly all of the lipase. Scanning electron micrographs revealed that EtOH at 70°C was more disruptive to the caryopsis coat than the EtOH at RT or hexane at 68°C, supporting the supposition

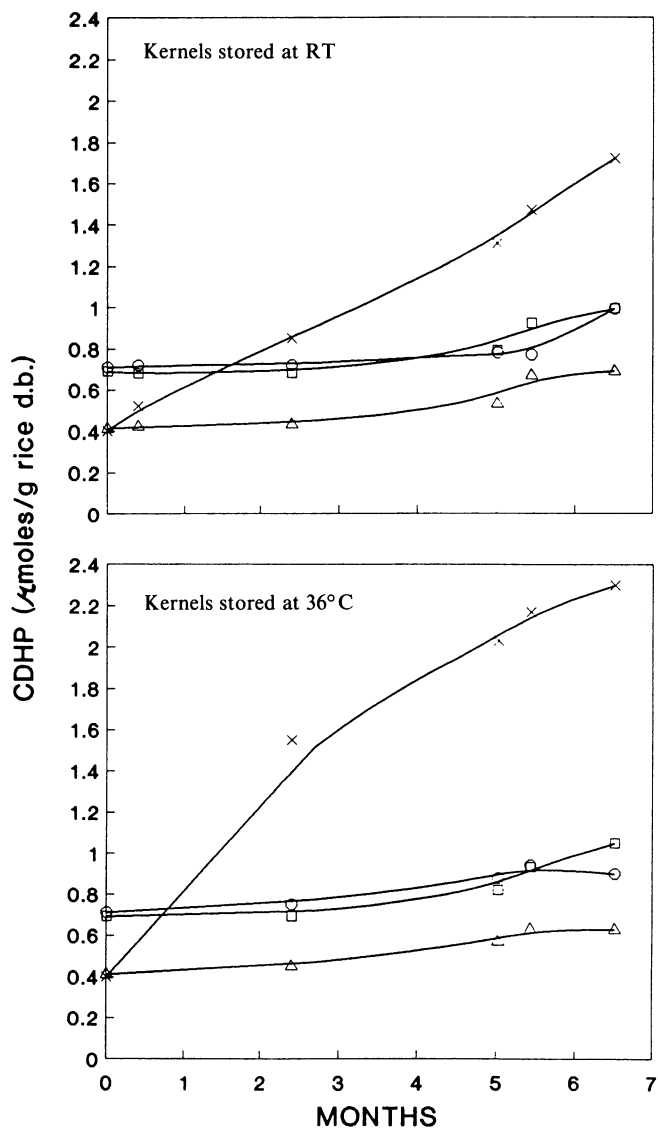


Fig. 3. Development of conjugated diene hydroperoxides (CDHP) in solvent-extracted and nonextracted (control) brown rice kernels during storage at room temperature (RT) and 36°C. EtOH = ethanol, ○ = control kernels, □ = kernels extracted with EtOH at RT, × = kernels extracted with EtOH at 70°C, Δ = kernels extracted with hexane at 68°C.

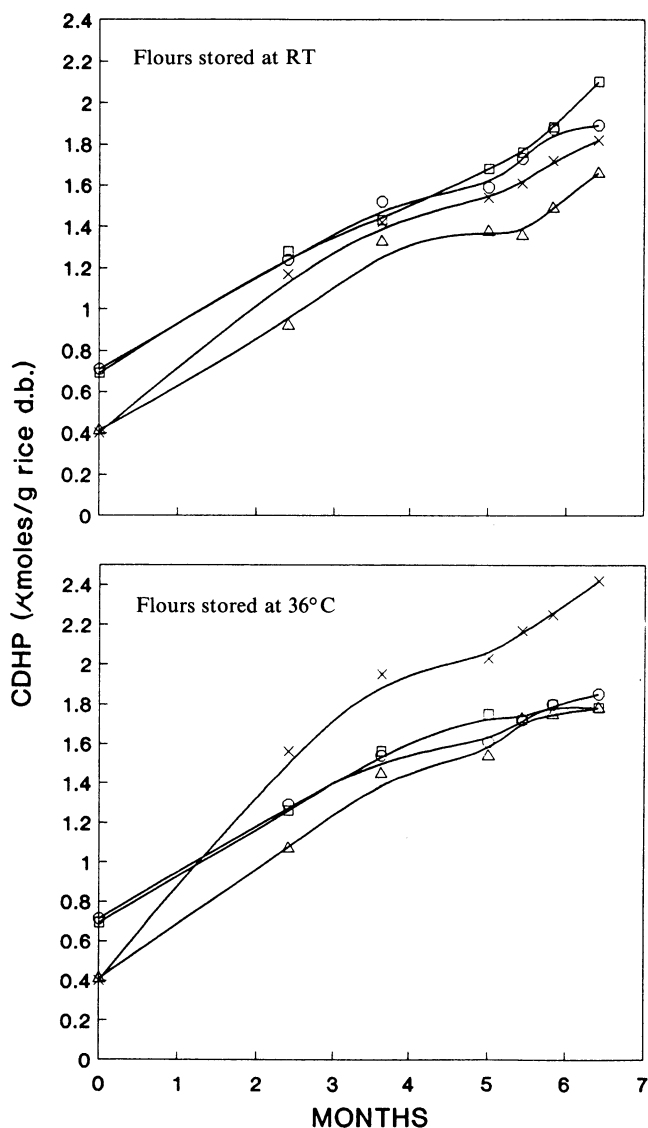


Fig. 4. Development of conjugated diene hydroperoxides (CDHP) in flours prepared from solvent-extracted and nonextracted brown rice kernels during storage at room temperature (RT) and 36°C. ○ = control flour, □ = flour from kernels extracted with ethanol (EtOH) at RT, × = flour from kernels extracted with ethanol (EtOH) at 70°C, Δ = flour from kernels extracted with hexane at 68°C.

that the 70°C EtOH was more effective in penetrating the coat and deactivating the kernel lipase.

The large increases in FFA in the flours prepared from control kernels and kernels extracted with EtOH at RT and hexane at 68°C cannot be attributed to differences in the lipolytic bacterial and mold populations of the flour and kernel samples, since no significant differences in microbial populations were observed between these samples. The stability to lipolysis of the flour prepared from kernels extracted with EtOH at 70°C was not due to the higher residual level of EtOH in the flour or the higher temperature of extraction, as supported by our experimental results.

Levels of CDHP increased rapidly in the kernels extracted with EtOH at 70°C, indicating susceptibility of these kernels to oxidative rancidity. Only slight increases in CDHP levels were observed in the control kernels and kernels extracted with EtOH at RT and with hexane at 68°C. Since EtOH at 70°C disturbed the caryopsis coat to a greater extent than did the other solvents, it left the kernel lipids more susceptible to oxidative deterioration. Also, the moisture content of the kernels extracted with EtOH at 70°C was lower than that of the other kernel samples (Table I). EtOH at 70°C dehydrated the kernel surfaces, which may have led to lower water activity in the kernels. A decrease in water activity accelerates the oxidation of lipids in food systems (Koch 1961).

An economically feasible, stable, full-fat product can be produced by extracting brown rice kernels with aqueous EtOH at RT. Extraction at 70°C produces a partially defatted product that is stable to lipolysis. However, this product would be more susceptible than unstabilized brown rice to oxidative deterioration. With proper packaging, oxidative deterioration can be slowed (Ory et al 1980, Sharp and Timme 1986) and thus should not be a deterrent to extraction with EtOH at 70°C. Thiamin retention in kernels extracted with EtOH at 70°C is poor. This extraction would be the preferred method for obtaining a product suitable for making brown rice flour that is stable to lipolysis or for extracting rice high in FFA and restoring it to a product low in FFA.

A large percentage of rice exported by the United States is shipped as brown rice since other countries find it more economical to mill it themselves. At the elevated temperatures during transport, the oil in unstabilized brown rice is subject to lipolysis. Bran high in FFA loses its value as food and animal feed. The higher the FFA in the oil, the more uneconomical it is to refine. The losses of potentially edible oil during refining are two to three times the FFA content of the oil (Enochian et al 1981). Shipping brown rice stabilized by extraction with EtOH at 70°C would provide importers with a product that can be milled to yield a partially defatted, stabilized bran of food quality. The full-fat bran obtained from extraction with EtOH at RT would require further processing to eliminate residual lipase activity. Both of these ethanol-extracted brown rice products would be suitable for obtaining oil low in FFA content.

Hexane is also a suitable solvent for stabilizing brown rice. However, there are advantages to extraction with EtOH rather than hexane. 1) Flour stable to FFA formation can be produced from brown rice kernels extracted with 70°C EtOH and not with 68°C hexane. 2) EtOH is more effective than hexane in extracting FFA from rancid brown rice. 3) EtOH is generally recognized as safe, and residual amounts remaining in the extracted rice will not affect the suitability of the product for human consumption. 4) EtOH is a safer, less volatile solvent than hexane.

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