Utilizing Ethanol to Produce Stabilized Brown Rice Products

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Oil in brown rice is susceptible to hydrolytic and oxidative deterioration, which can lead to off-odors, off-flavors, and shortened shelf life. This paper discusses lipolytic hydrolysis and oxidation of kernel oil and methods for stabilizing the oil. An overview of processes in which ethanol is used in liquid and vapor states to stabilize brown rice to lipolytic hydrolysis is presented.

KEY WORDS: Brown rice, brown rice flour, ethanol, lipase, lipolytic hydrolysis, oxidative deterioration.

Brown rice is a nutritionally valuable food. The bran layers of brown rice kernels are rich in dietary fiber, minerals, oil, protein and vitamins, particularly B vitamins. When added to the diet, the bran layers are effective in reducing cholesterol levels in humans (1,2). Utilization of brown rice and its bran, however, has been limited due to susceptibility of oil in the bran to hydrolytic and oxidative deterioration.

Lipolytic hydrolysis. Lipases, both endogenous to the bran and of microbial origin, initiate hydrolytic deterioration of kernel oil. Within intact rice kernels, endogenous lipases are localized in the testa layer of the caryopsis coat, while oil is localized in the aleurone and germ, as depicted in Figure 1 (3). Surface damage during dehulling disrupts these regions, lipases make contact with oil (the substrate), and hydrolysis of triglycerides to free fatty acids (FFA) proceeds. Following dehulling, lipase-producing mold and bacteria found on kernel surfaces would also interact with bran oil. Approximately 10% of the total bacterial population and all of the molds on rough rice are lipase-producing and have been suggested to be the primary cause of FFA formation in brown rice (4).

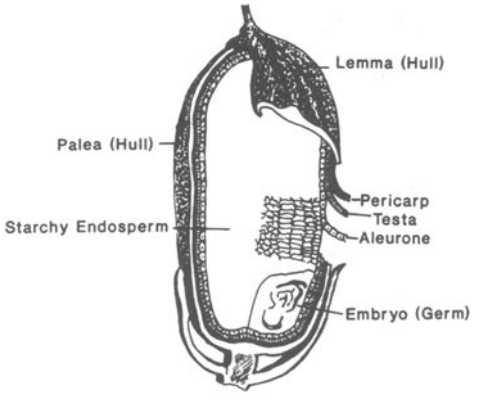

FIG. 1. Cross-section of a rice kernel.

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The level of FFA in brown rice after 6 mon of storage can typically be from 6% to 25%, depending on the extent of surface damage, moisture content of the rice, and temperature of storage. The rate of FFA formation in bran or brown rice flour is high; approximately 30% of the oil can be converted to FFA within a week under high humidity and temperature conditions (5). Bran high in FFA loses its value as animal feed and human food, and its oil is uneconomical to refine. The losses for potentially edible oil during refining are two to three times the FFA content of the oil (5).

Enzymatic oxidation. Free unsaturated fatty acids act as the preferred substrate for lipoxygenase, an enzyme found in the germ to which enzymatic oxidation of bran oil can be primarily attributed (6). Thus, the extent of enzymatic oxidation resulting from lipoxygenase is linked to the extent of lipolytic hydrolysis as determined by lipase activity. Oxidation initiated by lipoxygenase leads to rancid off-flavors and off-odors developing in kernels.

Stabilizing brown rice and rice bran. Lipases are inactivated by stabilizing the bran on or off the kernel. Numerous processes for stabilizing the bran off the kernel have been developed and are classified as "retained-moisture heating," in which the bran is heated under pressure until completion of heating; "added-moisture heating," in which the moisture content of the bran is increased during heating, followed by drying; and "dry heating" at atmospheric pressure (7). In the United States, stabilized bran is produced commercially by extrusion at 125°-135°C for 1-3 sec at 11-15% moisture (8) or from parboiled rough rice, a process that stabilizes the bran on the kernel.

Three approaches have been taken for inactivating lipases in bran on the kernel to produce stabilized brown rice. The first approach inactivates lipases by subjecting brown rice to moist or dry heat (9-11) or to precooking processes (12-14). These processes have been used for producing quick-cooking brown rice products. A second approach involves extraction with an organic solvent to remove "freed" kernel oil that serves as a substrate for lipase (15). A third approach stabilizes brown rice by ethanolic denaturation of bran lipases and lipase-producing bacteria and mold (16-19).

Lipoxygenase is inactivated by heat-denaturation together with lipases during stabilization of bran. However, heat treatments designed to stabilize brown rice or rice bran to lipolytic hydrolysis or lipoxygenase-initiated oxidation may increase the susceptibility of the product to nonenzymatic oxidation. Heat processing leads to redistribution of oil, destruction of endogenous antioxidants and increased surface-area exposure to oxygen (20). Heat and ethanol denature the hemoproteins catalase and peroxidase found in brown rice kernels (18). Unfolding of these enzymes causes greater exposure of the heme groups to substrate oil, allowing heme iron to initiate oxidation (21). Nonenzymatic oxidation initiated by heme groups of denatured hemoproteins has been observed to proceed more rapidly than lipoxygenase-initiated oxidation (22). Enzymatic and nonenzymatic oxidative deterioration in

brown rice and rice bran is slowed by maintaining low levels of oxygen in the product by means of optimum packaging materials and atmospheres for storage (14,23–25).

Ethanol (EtOH) for brown rice stabilization. Processes for stabilizing brown rice to lipolytic hydrolysis by liquid EtOH extraction and by treatment with EtOH vapors were recently reported (16–19). The remainder of this paper will present an overview of these processes and the resulting products.

Stabilization by liquid EtOH extraction. Liquid EtOH (95%, v/v), at temperatures ranging from 24°C (room temperature) to 70°C, stabilized brown rice to lipolytic hydrolysis. The effectiveness of EtOH in stabilizing kernels increased with higher extraction temperatures and longer extraction times. Figure 2 illustrates the effect of EtOH extraction temperature and time on the accumulation of FFA in brown rice kernels stored at 36°C. Brown rice kernels extracted with 70°C EtOH for 60 min were the most stable to lipolytic hydrolysis; the FFA level in these kernels increased from 1.0 to 1.4% during 6 mon storage at 36°C. Kernels extracted for 60 min at 24°C (room temperature) were less stable to lipolytic hydrolysis than those extracted at 70°C, as indicated by an increase in FFA from 2.0 to 3.8%. Reducing extraction time from 60 min to 10 min left kernels extracted at 70°C slightly less stable to lipolytic hydrolysis; FFA increased from 1.9 to 3.6% in 10 min-extracted kernels during 6 mon of storage. Only partial stabilization was achieved by extracting kernels with EtOH for 10 min at 24°C; FFA increased from 2.6 to 7.3%. To produce stabilized kernels, liquid EtOH needs to make contact with lipases that have been activated during dehulling of the kernel and with lipase-producing bacteria and mold found on kernel surfaces. Since these interactions are on or near the kernel surface, "deep" penetration of EtOH into the kernel is not required. Thus, low extraction temperatures and/or short extraction times, although not as effective as higher temperatures for longer times, can be used to produce kernels that show high stability to FFA formation compared to untreated kernels.

To produce stabilized flours from extracted kernels, EtOH must penetrate the testa layer and denature nearly all of the lipase. This requires higher extraction temperatures and longer extraction times. Figure 3 shows the effect of extraction temperature on the increase in FFA in flours prepared from brown rice kernels extracted for 60 min. Following 6 mon of storage at 36°C, the FFA contents of flours prepared from kernels extracted at 24°C, 46°C, 54°C and 70°C were approximately 32%, 18%, 12%, and 6%, respectively. Reducing the extraction time from 60 min to 20 min decreased the stability of the flours; FFA levels in flours prepared from kernels extracted for 20 min at 24°C, 46°C, 54°C, and 70°C were approximately 46%, 35%, 27%, and 7%, respectively, following 6 mon of storage at 36°C (data not shown). Following this storage time, flours prepared from kernels extracted at 70°C for 10 min had an FFA content of approximately 13%. These results indicate that an extraction temperature higher than 54°C is required to produce stabilized flours from EtOH-extracted kernels. Flours prepared from kernels extracted at 70°C had high stability to FFA formation.

The higher the temperature of extraction, the more susceptible the kernels were to oxidative deterioration during storage. Extraction time was not a factor. Conjugated diene hydroperoxide (CDHP) contents were determined as a measure of oxidative deterioration of unsaturated lipids in brown rice. As shown in Figure 4, the higher the

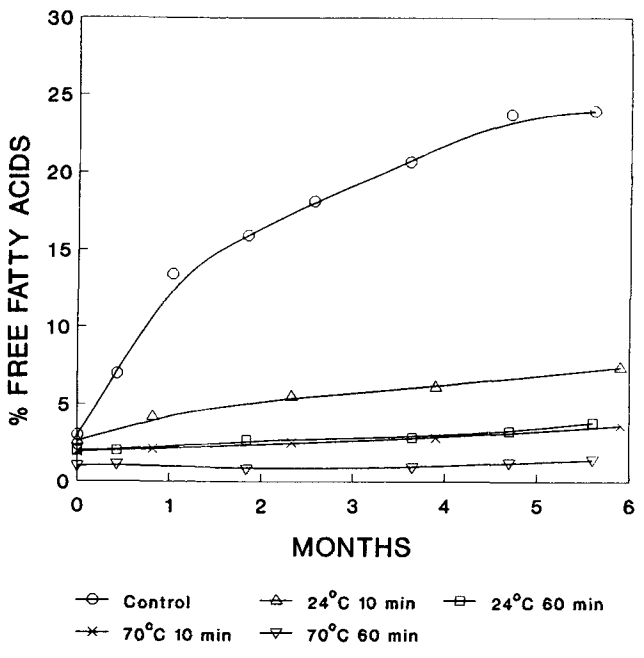

FIG. 2. Effect of ethanol extraction temperature and time on free fatty acids (FFA) levels in brown rice kernels stored at 36°C. Values plotted are means of analyses on two batches of rice. FFA are expressed as percent of kernel oil.

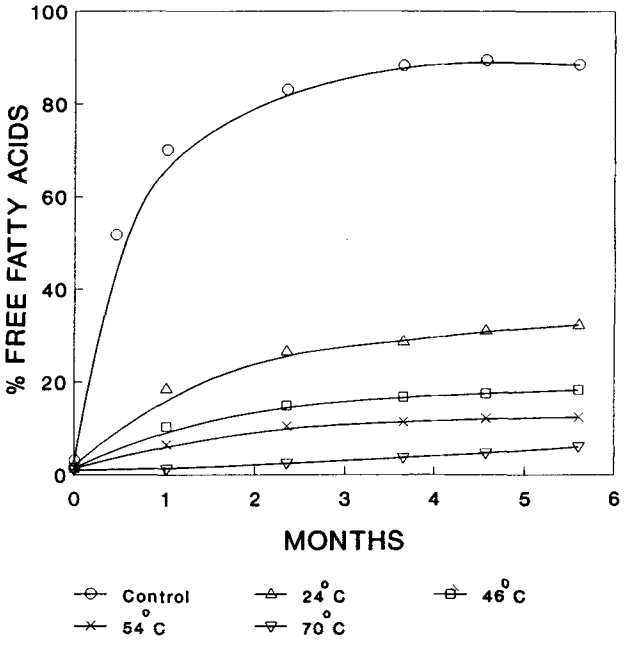

FIG. 3. Effect of ethanol extraction temperature on free fatty acids (FFA) levels in flours prepared from brown rice kernels extracted for 60 min. Flours were stored at 36°C. Values plotted are means of analyses on two batches of rice. FFA are expressed as percent of kernel oil.

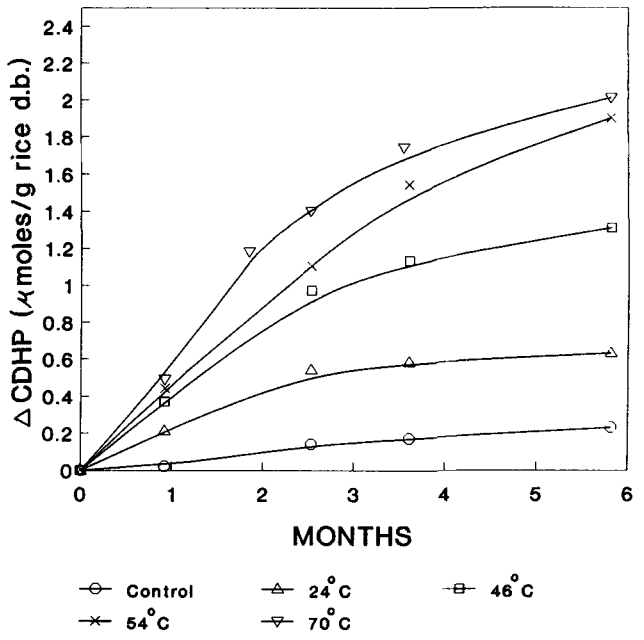

FIG. 4. Effect of ethanol extraction temperature on the change in conjugated diene hydroperoxides (Δ CDHP) contents of brown rice kernels stored at 36°C. Kernels were extracted for 60 min. Values plotted are differences of means calculated from analyses on two batches of rice.

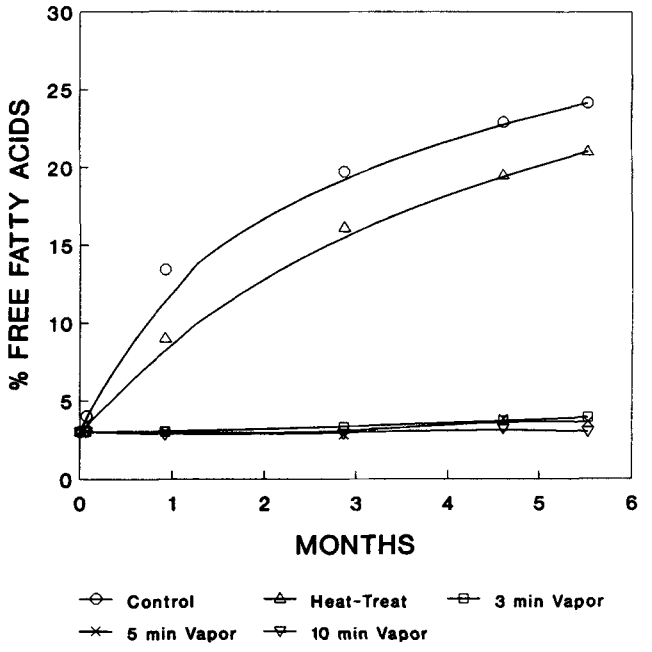

FIG. 5. Effects of storage time at 36°C on free fatty acids (FFA) levels in ethanol vapor-treated, heat-treated, and control brown rice kernels. Kernels were heated to 78°C prior to vapor treatment. "Heat-treated" kernels were heated at 78°C for 10 min without vapor treatment. Values plotted are means of analyses on two batches of rice. FFA are expressed as percent of kernel oil.

temperature of extraction, the larger the increase in CDHP levels in kernels during storage. As discussed earlier, ethanol and heat denature the hemoproteins catalase and peroxidase, which allows the heme iron to initiate oxidation. This iron-initiated oxidation proceeds more rapidly than the enzymatic oxidation of oil with active lipoxygenase. EtOH also disrupts and increases the porosity of the caryopsis coat, leaving oil exposed to oxygen. Differential scanning calorimetry (DSC) endotherms and scanning electron microscopy (SEM) micrographs indicated that the higher the temperature of the EtOH, the greater was its penetration into the kernel and the more disruptive it was to the caryopsis coat (17). Thus, the susceptibility of the kernels to oxidation would increase with higher extraction temperatures.

Stabilization by ethanol vapor treatment. Vapors from boiling aqueous EtOH (95%, v/v) stabilize brown rice kernels to lipolytic hydrolysis. During 6 mon of storage at 36°C, FFA increased little or none in brown rice kernels treated with EtOH vapors for 3 to 10 min, as shown in Figure 5. Flours produced from kernels treated with EtOH vapors had low residual lipase activities. EtOH vapors extracted surface water from 12.8%-moisture kernels and condensed. The treatment lowered the water content of 12.8%-moisture brown rice kernels approximately 1.5%; loss of kernel oil was less than 3%. When the water content of the kernels was lowered to 8% prior to vapor treatment, in order to bring it into equilibrium with the water content of the EtOH, the EtOH vapors did not condense; the water content of the kernels did not change, no oil was lost, and the kernels were stable to FFA formation. Thus, stabilization by EtOH vapors was not dependent on the vapors condensing to a liquid.

EtOH vapors act by denaturing and inactivating endogenous lipases. Since the endogenous lipases are so close to the kernel surface, denaturation by EtOH vapors is probable. The action of EtOH vapors is also attributed to ethanolic denaturation of lipase-producing bacteria and mold on the kernel surfaces, which kills the organisms. EtOH vapor treatment lowered total plate and mold counts to 10/g or less.

EtOH vapor-treated kernels were more susceptible to oxidative deterioration than untreated kernels, as indicated by increases in CDHP content during storage (Fig. 6). As was the case for extracted kernels, EtOH vapor treatment results in ethanolic- and heat-denaturation of hemoproteins, which then act as iron catalysts. The EtOH vapor treatment also increased kernel porosity, as indicated by DSC results, leaving the oil more susceptible to oxidation.

Brown rice kernel and flour products produced with EtOH. Brown rice kernels stable to lipolytic hydrolysis are produced by either extracting kernels with liquid EtOH or by contacting the kernels with EtOH vapor. Full-fat (<3% oil loss) products are produced by extracting brown rice with EtOH at 24°C or at higher temperatures with recycled ethanol (oil-saturated) and by treating brown rice with vapors from boiling EtOH. Extraction with fresh EtOH at temperatures higher than 24°C removes up to 15% of the kernel oil. Thiamine was retained in kernels extracted at 54°C and less and in kernels treated with EtOH vapors. Sixty-three percent of the thiamine in kernels extracted at 70°C was lost. Little or no loss of protein, dietary fiber, carbohydrates, or minerals occurred during extraction or vapor treatment. Bacterial and mold populations on EtOH-extracted and vapor-treated brown

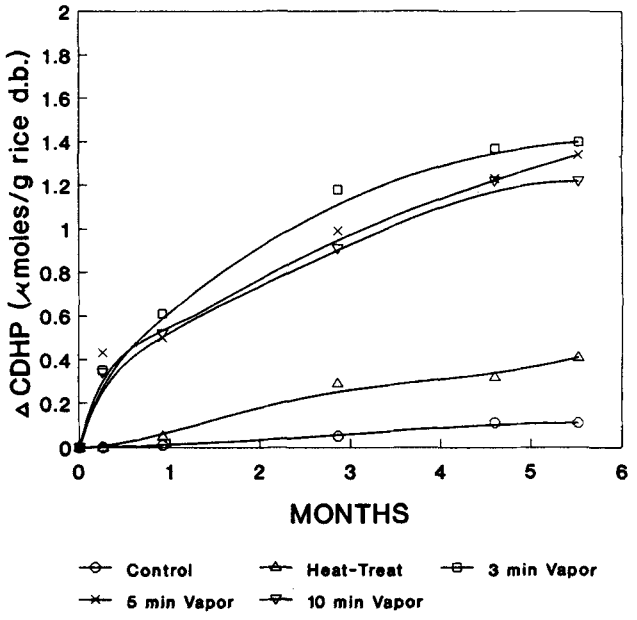

FIG. 6. Change in conjugated diene hydroperoxides (Δ CDHP) contents in ethanol vapor-treated, heat-treated, and control brown rice kernels during storage at 36°C. Values plotted are differences of means calculated from analyses on two batches of rice.

rice were low or not countable. Kernel starch was not gelatinized during processing with ETOH-stabilized brown rice has the appearance and cooking properties of natural brown rice. In contrast to stabilizing brown rice with EtOH, other stabilizing methods (*e.g.*, moist or dry heat, parboiling, precooking, solvent extraction of oil) can gelatinize kernel starch, alter kernel appearance and texture of cooked product and remove oil and valuable nutrients.

Stabilized, ungelatinized brown rice flour is produced by grinding kernels that were extracted at elevated temperatures or treated with EtOH vapors. Brown rice flours produced from rice stabilized by methods that render the starch gelatinized have functional properties unlike raw rice flours. Pregelatinized brown rice flours have limited use in fermented baked products, due to high water uptake at the dough-mixing stage, and can have a negative effect on texture in products not fermented (*e.g.*, cakes, cookies) (26). Brown rice flours produced from EtOH-stabilized brown rice kernels do not have these drawbacks.

Food-quality stabilized bran is produced by milling kernels that are extracted at elevated temperatures or are treated with EtOH vapors. This stabilized bran is suitable for obtaining oil of good quality. Stabilizing brown rice by EtOH extraction also offers a way of removing FFA and lipid oxidation products from rancid rice, thus restoring its low FFA content and eliminating off-odors and off-flavors.

As with processes designed to stabilize brown rice or rice bran by heat, stabilization with ethanol increases the product's susceptibility to oxidation. However, kernels extracted with EtOH at 24°C showed either no increase or just a small increase in susceptibility to oxidation, depending on kernel surface appearance. It may be possible to slow nonenzymatic oxidation in EtOH-stabilized brown rice by using EtOH as a carrier for an iron chelator. Chelating iron would prevent it from initiating oxidation. Studies are currently underway to evaluate the effectiveness of various chelators in decreasing the susceptibility of EtOH-stabilized brown rice products to oxidative deterioration.

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