Hydrolytic Degradation of Triacylglycerols and Changes in Fatty Acid Composition in Rice Bran During Storage

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ABSTRACT

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Hydrolytic rancidity makes rice bran unsuitable for human consumption, restricting its use to animal feed. To better understand this lipolytic process, bran from rice cultivars ‘Cypress’ and ‘Earl’ differing in oil concentration (23.5 and 18.3 mg of triacylglycerol/100 mg of bran, respectively) was stored at room temperature for five months, and the changes in triacylglycerol content and fatty acid composition, as well as the accumulation of free fatty acids (FFA) were followed. The decomposition of triacylglycerols displayed a decay pattern, with Cypress showing a more elevated degradation rate when compared with Earl. At day 36, both lines reached the same oil concentration, but the triacylglycerol decomposition in Cypress was still higher, indicating that oil concentration may not be a significant factor affecting the intensity of the rancidity process. The higher degradation rate observed in Cypress was apparently caused by higher lipase activity, which was 26% higher ($P < 0.001$). Fatty acid composition of triacylglycerols changed during storage, the palmitic acid percentage was similarly reduced in both lines to ≈80% of its initial concentration. Oleic and linoleic acids remained almost unmodified or slightly increased. The final content of FFA was ≈58% higher in Cypress than in Earl ($P < 0.001$). In conclusion, lipase activity appears to be an important factor determining the intensity of the hydrolytic process, but further research is required to confirm this conjecture. If this hypothesis is verified, a selection for lower lipase activity could be useful for increasing rice bran stability.

Rice (*Oryza sativa* L.) bran is a nutritionally valuable byproduct obtained from the outer layers of the rice kernel during the milling process. It is rich in protein (14–16%), fat (12–23%), crude fiber (8–10%), and minerals and vitamins. When bran layers are removed from the endosperm during the milling of rice, the individual cells are disrupted and the rice bran lipids come into contact with highly reactive lipases (Ramezanzadeh et al 1999). These enzymes are both endogenous to the bran and of microbial origin and initiate hydrolytic deterioration of kernel oil (Champagne et al 1992). The decomposition of bran triacylglycerols into free fatty acids (FFA) by lipases makes them unsuitable for human consumption or for the economical extraction of edible oil (Barnes and Galliard 1991). The FFA increase bran acidity, generate unacceptable functional properties, and produce undesirable taste and odors. As a consequence, freshly milled rice bran has a short shelf-life. The hydrolytic rancidity process can, however, be inhibited by a short-time heat treatment that inactivates lipases, producing stabilized rice bran (Malekian et al 2000). After stabilization, the bran can serve as a good source of protein, essential fatty acids, calories, and vitamins. While much of the world rice crop is processed by very small mills, only large milling operations can justify stabilization systems and extract oil from rice bran at a scale that is economically practical (McCaskill and Zhang 1999). A direct use of rice bran avoiding the requirement of stabilization treatments may tremendously increase rice bran utilization in food applications. For such a goal, a better understanding of the mechanism and factors involved in the lipolytic process of rice bran lipids is required. That knowledge may lead to breeding strategies for creating new varieties with low susceptibility to hydrolytic rancidity. This investigation was undertaken to study the decomposition of triacylglycerols, the release of fatty acids, and the modification of the fatty acid profile in rice bran during storage.

MATERIALS AND METHODS

Plant Material and Sampling

Freshly harvested kernels from a long-grain (Cypress) and a medium-grain (Earl) rice cultivar were used for this experiment. The grains were dehulled with a rice huller (Satake model THO 35A) and then milled (model MC 250, Satake Engineering Co., Ltd., Tokyo, Japan) with an 54.7-g weight in position 4 and 8 for Cypress and Earl, respectively. The collected rice bran was sieved through a 840-µm sieve and immediately placed under storage conditions. The bran was stored in open plastic bags at ambient temperature (20–25°C) for five months. Subsamples of ≈1 g of bran were taken after 0, 4, 12, 17, 23, 29, 36, 50, 57, 112, and 149 days of storage, and frozen (–20°C) until analysis. Two replicates per cultivar were performed.

GC Analysis of Triacylglycerol Content and Fatty Acids

The triacylglycerol content and fatty acid composition of rice bran oils were simultaneously determined by gas-liquid chromatography of fatty acid methyl esters (FAME), using triheptadecanoin as an internal standard. To prepare FAME, 100 mg of rice bran was weighed and 1 mL of 2 g/L of triheptadecanoin in isooctane was added. The solvent was evaporated in vacuum, and the samples were extracted and transmethylated for 30 min at 20°C with 1 mL of a 0.5*M* solution of sodium methylate in methanol. Isooctane (1 mL) and 0.5 mL of 5% (w/v) of $NaHSO_4$ in water were added in that order, and samples were mixed. The tubes were then centrifuged for 5 min at 4,000 rpm and 2 µL of the isooctane phase was injected into the gas chromatograph. Analysis was performed in a gas chromatograph (model 5890 Series II, Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector (FID) and a fused-silica capillary column FFAP, 25 m × 0.25 mm × 0.25 µm thickness (Macherey & Nagel GmbH+Co KG, Düren, Germany). The carrier gas was helium at a pressure of 120 kpa. For the oven temperature program, initial temperature (180°C) was linearly increased to 225°C at 10°C/min, and the final temperature was kept for 10.5 min. The injector and detector temperatures were 230 and 250°C, respectively. The samples (2 µL) were injected at a split rate of 45.5:1. Fatty acids were identified and quantified using known reference compounds. Triacylglycerol content was expressed as mg of triheptadecanoin equivalent per 100 mg of fresh bran, and fatty acid composition as % of total fatty acids. Two replicates were done for this analysis.

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Determination of FFA

The procedure used for measuring FFA was a modification of the method of Kwon and Rhee (1986). FFA were measured in duplicate using cupric acetate-pyridine as a color development reagent and caprylic acid (C8:0) as an external standard. Rice bran (≈100 mg) was weighed and 3 mL of isooctane was added. The samples were extracted for 5 min using a shaker (Innova 2000, New Brunswick Scientific Co., NJ) at 250 rpm. The tubes were then centrifuged for 5 min at 4,000 rpm and 2 mL of the supernatant was pipetted into a new tube containing 1 mL of reagent (3%, v/v, pyridine in a 5%, w/v, aqueous cupric acetate solution). The mixtures were mixed using the shaker at 250 rpm for 5 min. After sedimentation of the aqueous phase, an aliquot of the isooctane phase was measured at 715 nm. FFA were expressed as mg of C8:0 per 100 mg of fresh rice bran.

Lipase Assay

Lipase activity was determined in quadruplicate in fresh bran samples (day 0) using an olive oil and Tween 20 emulsion as enzyme and substrate. Rice bran (≈200 mg) was extracted with 2 mL of 50 m*M* potassium phosphate buffer, pH 7.2, for 1 hr using a shaker at 250 rpm. The samples were then centrifuged for 5 min at 4,000 rpm and 1 mL of the aqueous layer was pipetted into a tube containing an emulsion of olive oil and Tween 20 in water (30 mg each per mL of water). The samples were incubated at 35°C for 18 hr. After that, the released fatty acids were extracted with 3.5 mL of isooctane and measured as indicated above. Lipase activity was expressed as mg of C8:0 equivalent per g of fresh rice bran.

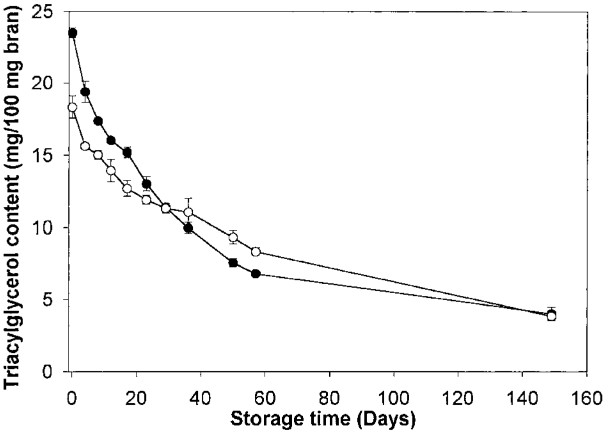

Fig. 1. Evolution of triacylglycerol content (mg of triacylglycerol/100 mg of bran) during storage. Cypress (●); Earl (○).

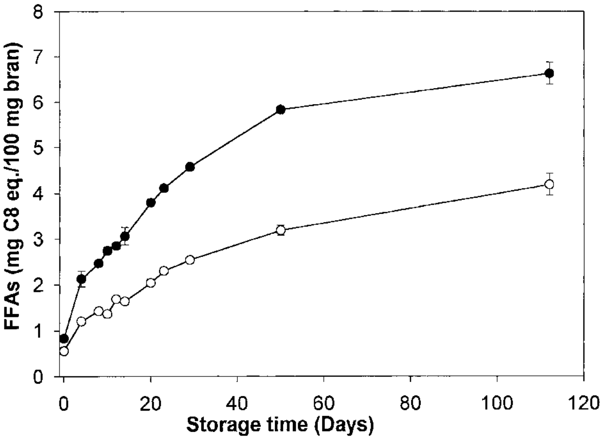

Fig. 2. Accumulation of FFA (mg of C8:0 equivalent/100 mg of bran) during storage. Cypress (●); Earl (○).

Statistical Analysis

A one-way analysis of variance was performed to statistically compare the data. All statistical analysis were done using statistical software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Degradation of Triacylglycerols

Rice triacylglycerols were strongly depleted during storage, following a decay pattern of decomposition (Fig. 1). The shape of the degradation curve suggests that triacylglycerol depletion is

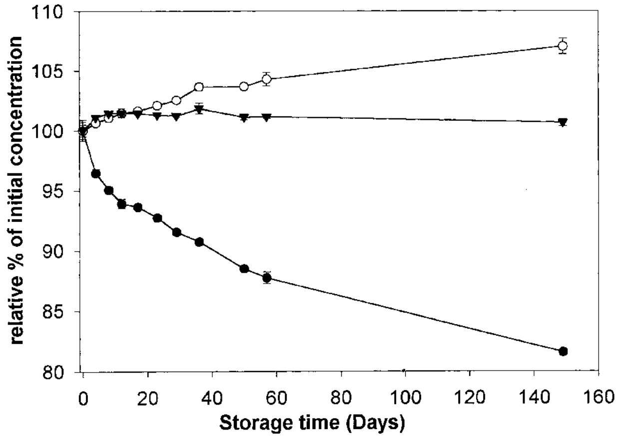

Fig. 3. Changes in fatty acid composition of bran triacylglycerols during storage in Cypress (initial conc. of each fatty acid = 100%). Palmitic acid (●); oleic acid (○); linoleic acid (▼).

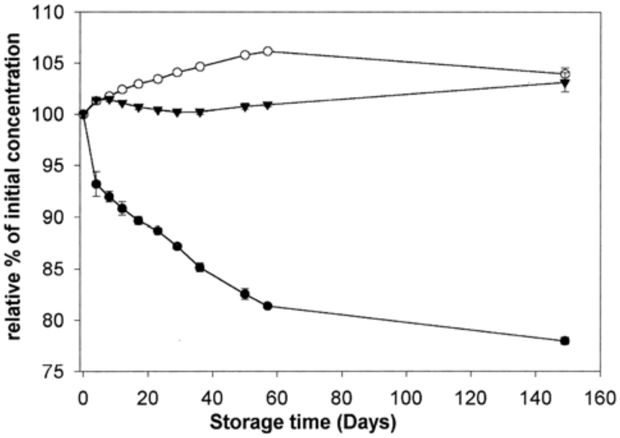

Fig. 4. Changes in fatty acid composition of bran triacylglycerols during storage in Earl (initial conc. of each fatty acid = 100%). Palmitic acid (●); oleic acid (○); linoleic acid (▼).

TABLE I
Initial Triacylglycerol Content and Main Fatty Acid Profile in Rice Bran

		Fatty Acid[b] Composition (% of total fatty acids)				
Cultivar	**TAG[a]**	**C16:0**	**C18:0**	**C18:1**	**C18:2**	**C18:3**
Cypress	23.5	14.9	1.9	43.8	36.0	1.4
Earl	18.3	16.3	2.4	42.2	35.6	1.3

[a] TAG, Triacylglycerol content, expressed in mg of triheptadecanoin equivalent per 100 mg fresh rice bran.

[b] Fatty acids: palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acid.

concentration-dependent, the lipolytic activity was maximal at the beginning of storage, when the triacylglycerol content is the highest, and decays gradually as the concentration decreases. Considering this, it could be assumed that lipid decomposition in bran with a higher fat concentration might be faster than in that showing a lower lipid content. Indeed, the rate of triacylglycerol depletion was higher in Cypress as compared with Earl. Cypress had a higher triacylglycerol content (Fig. 1). However, at day 36, both lines showed equal contents of triacylglycerols and the rate of triacylglycerol decomposition was still higher in Cypress. That indicates that lipase activity was higher in Cypress than in Earl and that oil concentration is not necessarily a decisive factor affecting the rate of triacylglycerol degradation. The analysis of lipase activity in both lines confirmed this observation. Lipase activity was ≈26% higher in Cypress than in Earl ($P < 0.001$) (11.9 and 9.5 mg of C8:0 equivalent/g of bran, respectively). These results also suggest that genetic variation may exist in rice for lipase activity. Tsuzuki et al (1994) also found cultivar variation for esterase activity in rice bran.

For assessing the relative significance of both oil content and the lipase activity on the susceptibility to rancidity, further studies using a factorial design that includes cultivars showing different combinations of triacylglycerol content and lipase activity are required nevertheless. At the end of a five-month storage time, the cultivars reached a similar concentration of triacylglycerols (4.0 and 3.9 mg of triacylglycerols/100 mg of bran in Cypress and Earl, respectively).

Changes in FFA Concentration

The FFA increased during storage following an exponential rise to maximum curve (Fig. 2). Similar results have been reported by other investigators (Saunders 1985; Champagne et al 1992; Ramezanzadeh et al 1999). In Cypress, the release of FFA was significantly faster and more elevated than in Earl. This result was expected because Cypress showed both higher concentration of triacylglycerols and lipase activity than Earl. It is remarkable that the differences between both lines in FFA accumulation could be observed from the beginning (within the first days of storage). At day 4 for example, Cypress accumulated about twofold more FFA than Earl (2.1 and 1.2 mg of C8:0 equivalent/100 mg of bran for Cypress and Earl, respectively). At the end of the storage time, both lines strongly differed for FFA concentration ($P < 0.001$), Cypress was ≈58% higher than in Earl (6.6 and 4.2 mg of C8:0 equivalent/100 mg of bran for Cypress and Earl, respectively).

Modification of Fatty Acid Profile of Triacylglycerols

Table I presents the initial fatty acid composition of the two studied cultivars. Both lines showed a very similar fatty acid composition pattern. The changes in fatty acid composition of bran triacylglycerols are displayed in Fig. 3 (Cypress) and Fig. 4 (Earl). To compare all fatty acids on the same basis, the evolution of each major fatty acid during storage was represented considering initial values as 100%. Both lines showed similar changes in fatty acid profile. Palmitic acid was strongly and similarly reduced in both lines, the final values representing ≈80% of its initial content. Oleic and linoleic acids remained almost unmodified or slightly increased. The data indicate that lipase has more affinity for cleaving the palmitic acid and glycerol ester bond than do any of the other fatty acids. Rice bran contains several types of lipases that are site-specific and cleave the 1,3-site of triacylglycerols (Takano 1993). By analyzing the lipids of rice bran for triacylglycerol composition, Glushenkova et al (1998) found that 90.3% of the sn-2 positions are occupied by unsaturated fatty acids, whereas the sn-1 and sn-3 positions are mostly occupied by saturated fatty acids. Therefore, the higher release of palmitic acid by rice lipases apparently is due to the preference of these enzymes to cleave the sn-1 and sn-3 positions and not because of a higher affinity to this fatty acid. As a consequence, fatty acid composition may not affect the magnitude of rice hydrolytic rancidity.

CONCLUSIONS

The intensity of the hydrolytic rancidity process in the analyzed cultivars appears to be primarily related to the lipase activity of the bran. Both lines differed strongly for lipolytic degradation, suggesting that genetic differences may exist between cultivars. If so, new varieties showing a reduced susceptibility to hydrolytic rancidity could be developed by selecting for reduced lipase activity.

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