

Effects of Commercial Processing on Antioxidants in Rice Bran¹

B. J. Lloyd,² T. J. Siebenmorgen,^{3,4} and K. W. Beers⁵

ABSTRACT

Cereal Chem. 77(5):551–555

Rice bran contains high amounts of beneficial antioxidants including tocopherols, tocotrienols, and oryzanols. Current rice milling technology produces rice bran from different layers of the kernel caryopsis. Under current practices, these layers are combined and then steam-extruded to form a stabilized rice bran pellet that is storage-safe prior to oil extraction. Each of these rice bran intermediates can vary in antioxidant content. The objective of this study was to investigate the changes in

selected antioxidants in rice bran from both long- and medium-grain rice during commercial milling and bran processing. Rice bran collected from various milling breaks of a commercial system had varying antioxidant levels. Bran collected after milling break 2 had the highest levels of tocopherol and tocotrienol. Oryzanol concentration was significantly higher in outer bran layers. Results also indicate that the long-grain rice bran averaged $\approx 15\%$ more antioxidants than the medium-grain rice bran.

Although it has long been considered an excellent source of vitamins and other nutrients, rice bran is an underutilized coproduct from rice milling. Rice bran contains $\approx 20\%$ lipids (Saunders 1986), which is similar to other oilseeds, but rice bran oil (RBO) contains more unsaponifiable lipids than other common vegetable oil sources (Orthoeffer 1996). RBO contains $\approx 3\text{--}5\%$ unsaponifiable lipids (Sayre 1988), depending on the type of rice (Gaydou et al 1980) and the method used to extract and refine the lipids. The unsaponifiable fraction in RBO contains a unique complex of naturally occurring antioxidant compounds, of which the tocopherols (vitamin E), tocotrienols, and oryzanol compound groups have received the most interest.

The antioxidant compounds in rice bran or RBO have purported health benefits as well as antioxidant characteristics for improving the storage stability of foods. Several studies have reported the effects of RBO on metabolic activities including reduced plasma cholesterol in laboratory animals and humans (Yoshino et al 1989, Qureshi et al 1991, Hegsted et al 1990, Kahlon et al 1992, Hegsted and Windhauser 1993). Oryzanol has been studied for its ability to reduce cholesterol absorption (Rong et al 1997). Komiyama et al (1992) and Nesaretnam et al (1998) reported anticancer activity associated with tocotrienols. Thus, rice bran is viewed as a potential source of these high-value antioxidants for use as additives in foods, pharmaceuticals, and cosmetics.

RBO contains high concentrations of the antioxidant tocopherol compared with other oil seeds (Kao and Luh 1991). Approximately 1.0% (v/v) of the unsaponifiable fraction of RBO is vitamin E (α -tocopherol). Rice bran also contains an analogue to vitamin E known as tocotrienol. Approximately 1.7% (v/v) of the unsaponifiable fraction of RBO is tocotrienol (de Deckere and Korver 1996), where the unsaponifiable fraction is $\approx 4.2\%$ of the total lipid content (Hui 1996). Both tocopherol and tocotrienol contain α , β , and γ isomers, but tocopherol isolated from rice bran also has a δ isomer (Morton 1975). Both tocopherol and tocotrienol possess strong antioxidant activity.

Oryzanol or γ -oryzanol is a mixture of sterol esters of ferulic

acid first isolated by Kaneko and Tsuchiya (1955). Initially oryzanol was thought to be a simple compound but was later found to be a mixture of several different esterified sterols, primarily cycloartenol, β -sitosterol, 24-methylene-cycloartanol, cyclobranol (cycloartenol), and campesterol (4-desmysterols) (Rogers et al 1993). Figure 1 shows one of the esters of oryzanol. The complete oryzanol group is unique to RBO, but the exact composition of oryzanol depends on the rice cultivar. Crude RBO can contain $\leq 2\%$ (v/v) oryzanol (Norton 1995).

Much of the previous research on antioxidants in rice bran and RBO has focused on procedures using different solvent systems to extract the maximum amount of unsaponifiable lipids. Recent studies have also focused on the health benefits of these compounds. However, few of these studies have documented how initial processing steps of the postharvest rice bran feed stock affect antioxidant retention. Martin et al (1993) noted that heat processing of rice bran to stabilize it against oxidation reduced the concentrations of many valuable compounds. Nicolosi et al (1994) reported striking differences in levels of tocotrienols and oryzanols from commercially available RBO. They found that 90% of the oryzanol and tocotrienols were lost during oil refining. They suggested that differences in the stabilization procedure could result in variable recoveries of the unsaponifiable compounds. Diack and Saska (1994) found that when separating vitamin E and oryzanol compounds from RBO, the individual concentrations varied substantially according to the origin of the rice bran. Wells (1993) noted that oxidative degradation was likely due to loss of antioxidant activity in tocopherol and tocotrienol from high temperatures during extrusion stabilization, which indicated that these compounds were heat labile.

The overall goal of this study was to establish a better understanding of how commercial milling systems affect the levels of specific antioxidants in rice bran from long- and medium-grain rice cultivars. The intent was to help processors assess the best method for retaining these compounds in rice bran before oil extraction to increase the value of the rice bran.

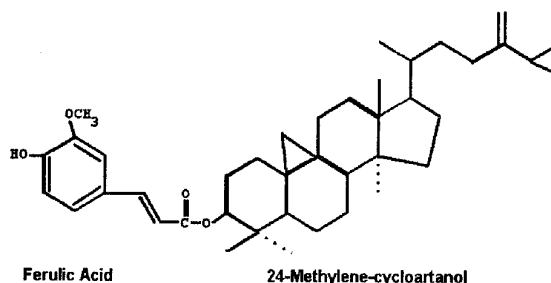


Fig. 1. Molecular structure of ferulic acid esterified with 24-methylene-cycloartanol, one of five compounds of oryzanol found in rice bran.

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² Research assistant, Biological and Agricultural Engineering Department, University of Arkansas, Fayetteville, AR 72701.

³ Professor, Food Science Department, University of Arkansas, Fayetteville, AR 72701.

⁴ Corresponding author. E-mail: tjs@enr.uark.edu Phone: 501/575-2841. Fax: 501/575-6936.

⁵ Research assistant professor and director of Central Analytical Laboratory, Poultry Science Department, University of Arkansas, Fayetteville, AR 72701.

MATERIALS AND METHODS

Long- and medium-grain rice used in this study was obtained from Riceland Foods (Jonesboro, AR) and was harvested from several commercial farms located in Craighead County in northeast Arkansas. The medium-grain rice was a pure cultivar, Bengal, the predominant medium-grain cultivar currently grown in Arkansas. The long-grain rice was an LG-1 class (a rice industry grain classification) that was not a pure cultivar but a mixture of several cultivars of similar size. The LG-1 class used for this study most likely included a mixture of Drew, Kaybonnet, and Allen (W. Carlisle, *personal communication*).

Immediately after harvest, the rice was commercially dried in cross-flow column dryers in the last weeks of September 1998 at Riceland Foods, Jonesboro AR. Approximately 2,750 m³ (78,000 bu) of medium-grain rough rice and ≈2,114 m³ (60,000 bu) of long-grain rough rice were used in the study. Both the medium- and long-grain lots were stored in individual 1,400 m³ (40,000 bu) upright concrete storage silos. The moisture content of each lot was maintained at ≈12.5% by ventilation fans attached to each storage bin. Unless otherwise stated, all moisture contents are expressed on a wet basis.

Milling Procedures

Rice bran samples were procured from a commercial milling system (Riceland Foods). Because the milling system at Riceland Foods is commercial scale, a large amount of rice, ≈211 m³ (6,000 bu), was milled during a given sampling trial. Sampling trials occurred only during a lot change, so the sampling bin rice was the only rice supplied to the continuous-process milling system. A milling run

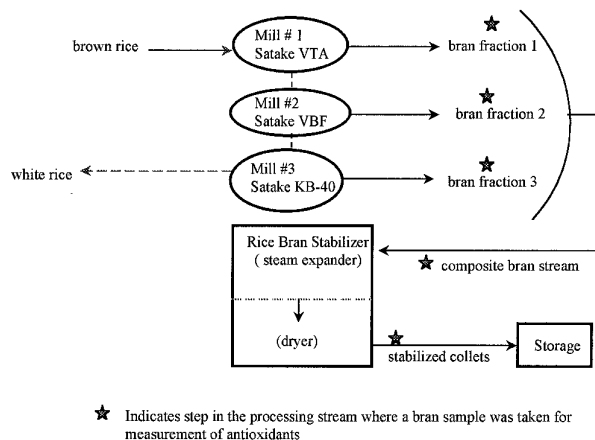


Fig. 2. Simplified schematic of commercial three-break rice milling and bran processing system.

lasted ≈2 hr due to the large capacity of the milling system. The LG-1 samples were milled to retail-packaged rice specifications. This rice underwent a three-break milling treatment in three successive and separate commercial-sized milling machines (Satake Houston, TX) (Fig. 2) that individually removed bran from the rice kernels. Multibreak milling is the most common method of commercial rice milling. The first mill was the Satake VTA, which is an abrasion-type mill; the second mill was the Satake VBF, which is a friction-type mill; the third mill was the KB-40, which is a water-mist polisher mill with very little friction (Fig. 3). The medium-grain samples were milled to cereal customer specifications and were milled in only the first two machines.

Bran was collected from each milling machine and from a composite bran stream. Bran was also collected at the outlet of a high-temperature steam-injected expander used at the Riceland location to stabilize the bran by heating the bran to temperatures (≈120°C) sufficient to prevent lipid oxidation and resultant oil rancidity. The expander uses low-pressure steam as the primary method of enzymatic degradation. The resultant bran is formed into a collet, which is a large pellet ≈2.5 cm long and 1.5 cm in diameter. Figure 2 shows the collection locations of rice bran samples from the commercial processing system.

Sampling Techniques

During each milling, bran was collected at different processing locations by inserting a plastic cup into the proper bran stream and

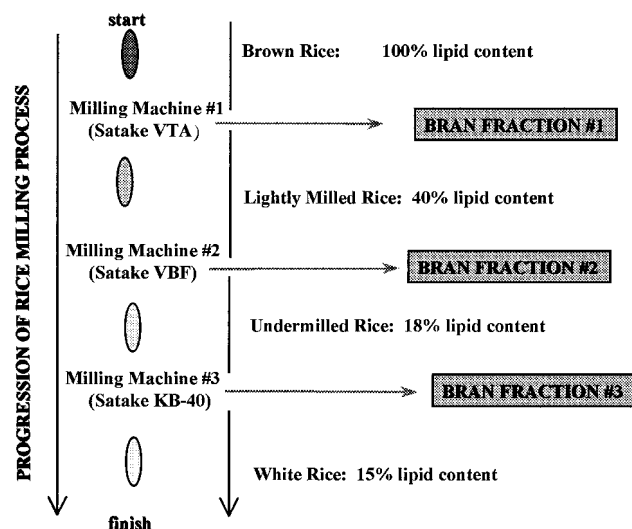


Fig. 3. Successive removal of surface lipids from a rice kernel using a commercial three-break milling system. Lipid content (%) average remaining on milled rice kernels measured from three samples.

TABLE I
Antioxidant Levels in Rice Bran for Long- and Medium-Grain Rice Collected from Different Processing Locations Within a Commercial Rice Milling and Bran Processing Facility^a

Antioxidant	Rice Type	Bran				Stabilized Collets
		From Mill Break 1	From Mill Break 2	From Mill Break 3 ^b	Composite Bran from All Mill Breaks	
Tocopherol, mg/kg bran	Long-grain	75.4	82.6	33.8	77.7	66.2
	Medium-grain	51.4	97.3	None	71.2	56.7
	LSD ^c	23.7	24.4	None	23.7	29.7
Oryzanol, g/kg bran	Long-grain	6.42	3.13	1.09	4.74	3.47
	Medium-grain	5.17	2.58	None	4.00	3.27
	LSD	1.074	1.106	None	1.074	1.346
Tocotrienol, mg/kg bran	Long-grain	23.3	29.4	12	24.9	21.6
	Medium-grain	18	19.6	None	19.4	15.8
	LSD	8.3	8.5	None	8.3	10.4

^a Each value represents average of six measured subsamples.

^b No bran samples collected for mill break 3 for medium-grain rice.

^c Least significant difference ($P < 0.05$).

filling the cup with bran. Bran (≈ 1 kg) was then placed in a plastic self-sealing bag and then immediately sealed in a bag made of an oxygen-impermeable plastic film. To minimize lipid oxidation, these bags were vacuum-sealed with a Food Savor Compac II sealer that evacuated the bag to a vacuum of ≈ 1 atm according to manufacturer's specifications. The sealed bran was then wrapped in aluminum foil to protect the vitamins in the rice bran from light degradation. Each processing location was sampled six times on two separate dates in 1999 (March 4 and May 4). Six analytical preparations of each sample were measured and averaged together. Data represents the least significant means of each sample determined by SAS/STAT (vers. 7, SAS Institute, Cary, NC) in the general linear means procedure using analysis of variance.

The sealed packages were then placed in an insulated cooler of ice ($\approx 0^\circ\text{C}$) for transportation to the University of Arkansas Fayetteville. Bran samples were immediately stored in a freezer at -20°C , except for a few samples that were stored in a refrigerator at 4°C . These samples were kept at a temperature consistent with the temperature of the insulated ice cooler so that any deviations of the samples stored at 4°C from identical samples stored at -20°C could be attributed to the higher temperature of the ice cooler. All samples remained in cold storage until laboratory analysis was completed during late April and early May 1999.

Lipid Extraction

Bran samples were extracted as collected except for the collet samples, which were ground in a small electric coffee grinder. This ensured that each sample had a finely ground texture. Total lipid was extracted from rice bran samples using hexane by a modified method of Hu et al (1996). Hexane (2 mL) containing $0.3 \mu\text{g/mL}$ of tocol as an internal standard was added to 0.5 g of rice bran in a 16×100 -mm borosilicate glass tube. The sample was vortexed for at least 30 sec, then centrifuged for 5 min at $750 \times g$. The clarified upper hexane fraction was transferred to a 13×100 -mm borosilicate glass tube. Rice bran was reextracted to ensure complete lipid recovery. The hexane fractions were combined and evaporated to dryness using a nitrogen-evaporation apparatus with supplemental heat. The rice bran lipid was redissolved in 4 mL of acetonitrile and methanol (75:25), then centrifuged (10 min at $1,000 \times g$) before transfer to HPLC autosampler vials.

HPLC Analysis

The total lipid fraction of rice bran was analyzed by reverse-phase HPLC using a modified method of Rogers et al 1993. The system consisted of an HPLC (Waters 2690 Alliance Milford, MA) connected to both fluorescence and UV detectors allowing simultaneous measurement of tocotrienols, tocopherols (fluorescence, $\text{Ex} = 298$; $\text{Em} = 328$), and oryzanols (UV 325 nm). A Waters Symmetry analytical column (C18, 150×2.1 mm, $5 \mu\text{m}$) and gradient mobile phase (0.3 mL/min) were used to separate analytes. Initial mobile phase conditions were acetonitrile, methanol, and water (60:35:5) which changed in 1 min to 0% water, 40% methanol, and 60% acetonitrile. The mobile phase then changed linearly to acetonitrile, methanol, and water (20:80:0) over the next 14 min and held for 5 min before returning to initial conditions. Total HPLC run time was 30 min. The resulting chromatographic separation was very similar to that achieved by Rogers et al 1993. Baseline separation was achieved for all tocopherol compounds, while slight shouldering occurred for the tocotrienols and oryzanols.

For quantification of tocotrienols and tocopherols, sample peak areas were compared to those of authentic standards. Total tocotrienol and tocopherol levels in rice bran (Table I) were derived by the summation of α , β , γ , and δ forms where β and γ isomers were cumulative and known to coelute. Tocotrienol and tocopherol standards were purchased from Merck KGaA, (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO), respectively. Total oryzanol in each rice bran sample was determined in a manner similar to that used for tocopherol. The oryzanol standard was obtained

from Amsino Intl. (Minneapolis, MN). The concentration ranges of calibration standards were 2.5 – $0.02 \mu\text{g/mL}$, 6.0 – $0.25 \mu\text{g/mL}$, and 600 – $24 \mu\text{g/mL}$ for tocotrienols, tocopherols, and oryzanols, respectively. In each set of compounds, the lowest limit of detection was ≈ 10 -fold lower than the lowest calibration standard.

Surface Lipid Analysis

The rice from each milling treatment was collected and analyzed to determine the percentage of lipids remaining on kernels after each milling break. These lipids were extracted using a Soxtec System HT 1043 extraction unit (Tecator, Sweden) on six samples per run using petroleum ether as the solvent. Milled rice samples were not ground before extraction. Conversely, the brown, nonmilled rice samples (rice entering the milling system) were ground in an Udy cyclone mill to a powder (#20 mesh, 0.841 mm) before extraction with petroleum ether. The brown rice was the only sample ground. This prevented the waxy seed coat layer of the brown rice kernels from shielding the solvent from the underlying lipid-rich aleurone layer. Approximately 5 g of brown rice flour or milled whole kernel rice was placed in a porous extraction thimble. A small piece of glass wool was placed above the sample to prevent the rice sample from boiling out and to allow the solvent to drip freely onto the sample. The samples were then placed in a drying oven at 100°C for 1 hr. Each sample was then extracted with 50 mL of petroleum ether for 30 min. The sample thimbles were removed from the ether bath and rinsed with condensed ether for another 30 min, then the condensing solvent was collected for 15 min. The remaining cup of lipids was allowed to air-dry for 2 min, and then placed in a drying oven at 100°C for 30 min to evaporate any remaining solvent. Finally, the cup was placed in a desiccator to cool for 30 min, and the weight of the remaining oil was weighed on an analytical balance. The reported lipid content was the mass of the extracted lipids divided by the total amount of starting rice material. This procedure was followed for all rice samples. The percentage of lipids removed by the milling treatments from the long-grain samples is shown in Fig. 3.

RESULTS AND DISCUSSION

Tocopherol Levels

Figure 4 shows the measured levels of tocopherol for the processing steps in the commercial milling and bran processing system. A significant difference in tocopherol levels across some processing steps was observed for both long- and medium-grain rice bran using a multiple *t*-test with $\alpha = 0.05$ in the general linear models pro-

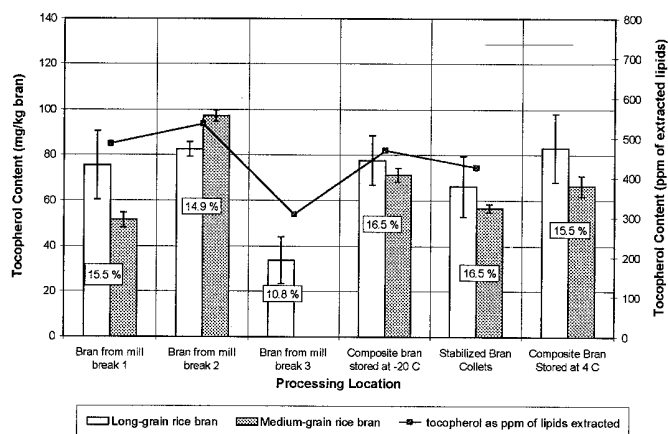


Fig. 4. Tocopherol concentrations for rice bran taken at different locations from a commercial rice processing facility. Data points for a given location represent the mean of six subsamples collected on two dates. Error bars show standard error of each mean value. Lipid content (%) average amount extracted from respective long-grain rice bran samples.

cedure (SAS Institute, Cary, NC). These results (Table I) indicate that tocopherol levels in rice bran varied according to the degree of milling of the rice kernel, as indicated by varying levels across the three milling breaks, as well as the processing step in the commercial stream. The high variability of the mean tocopherol levels (error bars in Fig. 4) limited the statistical inference for significant differences at 95% confidence, but the overall trends indicated changes in tocopherol throughout the processing system. The bran from milling break 2 had the highest tocopherol content for long- and medium-grain rice of any sampling location. This might be attributed to inclusion of the highly concentrated lipid aleurone layer removed primarily during milling break 2 (Fig. 3) that would tend to remove most of the aleurone layer. Milling break 3 removes the final 3% of the bran layer, some of which is starch ridges and pieces. The cells making up the aleurone layer, which is the innermost of the four layers of the rice bran, contain the highest amount of caryopsis lipid bodies (Bechtel and Pomeranz 1977). These cellular lipid bodies are believed to house the lipid-soluble tocopherol and tocotrienol antioxidants because their natural role as antioxidants is to protect the maturing rice kernel lipid layers from oxidation by sequestering free radicals (Suarna et al 1992). Tocopherol content was lowest in bran collected from milling break 3, which was the final milling operation. This milling break removed the final traces of the bran layer as well as kernel endosperm, as indicated by a lower extracted lipid content (Fig. 3).

Long-grain rice bran that had been steam-extruded into stabilized collets showed minor changes in tocopherol content compared with bran from the composite sample. The steam-expansion process was expected to reduce tocopherol levels due to the high temperature exposure (120°C), but no significant difference was observed between the composite bran sample and the steam-stabilized collet sample. Peterson (1994) noted no significant reduction in tocopherol levels in barley after a malting process that included a maximum temperature of 85°C.

The long-grain rice bran collected from milling break 1 had a significantly higher tocopherol content than did the medium-grain bran. Long-grain rice bran collected from milling break 1 averaged 30% more tocopherol than medium-grain rice bran. No significant difference ($P > 0.05$) was found between the tocopherol levels of medium- and long-grain rice bran collected from milling break 2, the composite bran, or the stabilized collets. Table I shows the average difference and the least significant difference (LSD) in the antioxidants for long- and medium-grain rice, which indicated that the rice grain type (long vs. medium) is an important factor affecting tocopherol concentration in RBO depending on the particular layer of bran being re-

moved. For this study, the difference was evident in samples taken from milling break 2.

Tocopherol content was not a function of amount of extracted lipid. This was determined by comparing the relative concentration of tocopherol with the amount of extracted oil, each measured at the respective processing steps. Figure 4 shows the concentration expressed as a fraction of the extracted lipids (ppm) of tocopherol. Each location varied in tocopherol concentration in the same fashion as the actual measured amounts of lipids extracted. The total extracted lipids from the bran collected at each processing step varied up to four percentage points (Fig. 3). Hu et al (1996) reported slightly higher tocopherol concentrations in expansion-stabilized rice bran than the values shown in Table I. These higher values may have been due to the more exhaustive lipid extraction protocol with longer extraction times of 30 min and a higher solvent temperature of 60°C.

The difference in tocopherol content for the collected bran samples stored at 4°C versus the composite bran samples stored at -20°C was not significant ($P > 0.05$). This indicated that storing samples at ≈0°C in the insulated ice cooler immediately after collection and during transit did not alter tocopherol content before transfer into the -20°C storage (the assumed safe storage temperature). This ensured that respiration and oxidation rates were sufficiently lowered by cooling and antioxidant levels of the bran samples were maintained in the insulated ice cooler during the 484 k (301 mi) transit at these temperatures.

Tocotrienol Levels

Figure 5 illustrates the changes in tocotrienol levels during rice bran processing. Like tocopherol, tocotrienol concentration was highest for both long- and medium-grain rice bran taken immediately after milling break 2. Rogers et al (1993) quantified tocotrienols from several commercially refined rice bran oils and found a range of 72–1,157 ppm. The high variability was attributed to different bran sources and different commercial refining methods. The average concentration across processing steps measured for this study was 150 ppm. The level of tocotrienol across processing steps changed less than the other tested antioxidants.

A small decrease in tocotrienol occurred due to the steam-expansion process but the effect was minimal and not significant. The overall difference in tocotrienol content across processing steps measured in this study was 1.56 mg of tocotrienol/kg of bran, which was only a 10% difference. This relative change was much less than that of the other antioxidants measured. This would tend to indicate that tocotrienol was more uniformly distributed through the bran layer surrounding the kernel than tocopherol or oryzanol. This finding

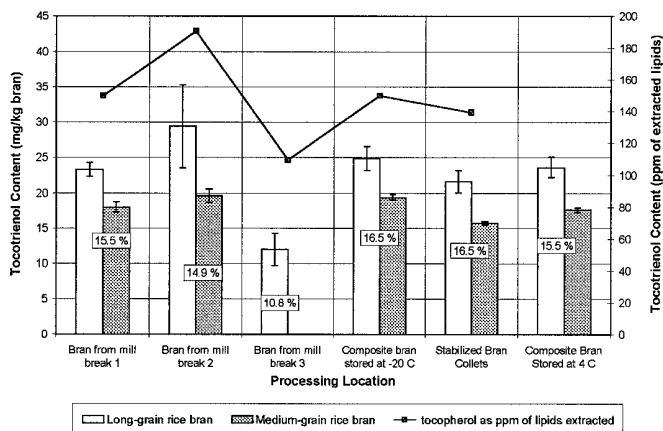


Fig. 5. Oryzanol concentrations for rice bran taken at different locations from a commercial rice processing facility. Data points for a given location represent the mean of six subsamples collected on two dates. Error bars show standard error of each mean value. Lipid content (%) average amount extracted from respective long-grain rice bran samples.

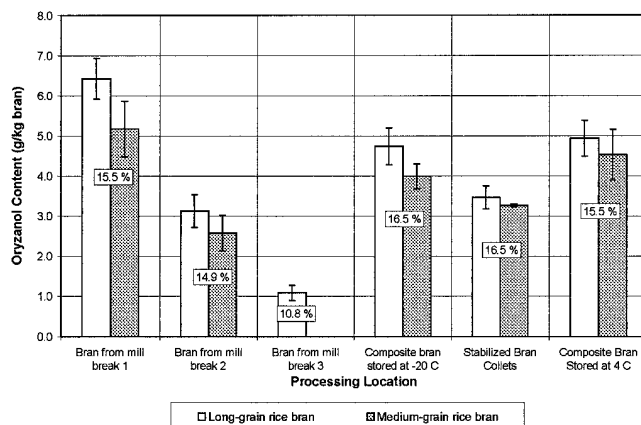


Fig. 6. Tocotrienol for rice bran taken at different locations from a commercial rice processing facility. Data points for a given location represent the mean of six subsamples collected on two dates. Error bars show standard error of each mean value. Lipid content (%) average amount extracted from respective long-grain rice bran samples.

was similar to that of Peterson (1995), who reported that tocotrienol concentration in oat kernels was uniformly distributed throughout the endosperm.

Like tocopherol and oryzanol, long-grain rice bran averaged more tocotrienol than medium-grain rice bran. However, no significant difference was found between long- and medium-grain rice bran, except that long-grain rice bran from milling break 2 averaged 33% more tocotrienol. Also, there was no significant difference ($P > 0.05$) between the composite bran samples stored at 4 and -20°C .

Oryzanol Levels

Figure 6 shows oryzanol levels across processing steps. Bran collected directly after milling break 1 had the highest oryzanol content at 6.42 g of oryzanol/kg of bran for long-grain rice bran and 5.17 g of oryzanol/kg of bran for medium-grain rice bran. This suggests that the ferulate esters that compose oryzanol predominantly resided in the outer pericarp, seedcoat, and nucellus layers, which are the outermost layers of a brown rice kernel (Bechtel and Pomeranz 1977).

There was a significant difference in oryzanol content between the composite bran samples and the heat-stabilized collets. A 26% decrease in oryzanol content of the composite rice bran occurred after the bran had been steam-extruded. This confirmed the assessment of Martin et al (1993) that heat processing will lower the concentration of beneficial antioxidants such as oryzanol in rice bran. No significant difference in oryzanol content existed between the composite bran sample stored at 4 and -20°C .

The oryzanol values measured in this study were higher than the 2.4–2.9 g/kg of dried bran reported by Hu et al (1996). Norton (1995) reported 3.4 mg of ferulate esters/g of dried bran. These differences in concentration may be attributed to the different methods of lipid extraction used in the studies. Hu et al (1996) extracted rice bran using hexane and isopropanol at different temperatures and solvent ratios; Norton (1995) extracted ferulate esters using hexane at a 5:1 solvent-to-bran ratio.

Oryzanol content was significantly ($P < 0.05$) higher in long-grain rice bran from milling break 1 than in medium-grain rice bran from milling break 1. Long-grain rice bran averaged 19.5% more oryzanol for the bran collected from milling break 1 (the milling operation that removed the outermost bran layers. There were no other significant differences between long- and medium-grain rice bran, although the average oryzanol content of long-grain rice bran averaged 15% more oryzanol than medium-grain rice bran.

SUMMARY AND CONCLUSIONS

Rice bran processing had a significant influence on antioxidant levels in bran collected from a commercial rice milling system. Tocopherol and tocotrienol levels were highest in long- and medium-grain rice brans from milling break 2. Bran taken from milling break 1 had the highest oryzanol content. Also, there was no pronounced decrease in tocopherol or tocotrienol levels after steam expansion of rice bran, but oryzanol showed a 26% decrease due to steam stabilization. Results also show that long-grain rice bran had higher overall tocopherol, oryzanol, and tocotrienol contents than medium-grain rice bran.

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[Received October 25, 1999. Accepted May 3, 2000.]