Biosystems Engineering (2004) **88** (4), 453–460
doi:10.1016/j.biosystemseng.2004.04.009
PH—Postharvest Technology

Nutraceutical Concentrations within the Bran of Various Rice Kernel Thickness Fractions

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(*Received 19 August 2003; accepted in revised form 13 April 2004; published online 25 June 2004*)

Several important nutraceutical compounds, such as tocotrienols, tocopherols, and oryzanols, can be extracted from rice bran, a by-product of milling. This study was conducted to not only provide information regarding nutraceutical concentrations within the rice kernel based on bran collected from successive milling, but also to determine levels of nutraceutical concentrations across several different thickness fractions. Nutraceutical compounds were measured in the bran from two long-grain rice varieties, Cypress and Drew. Rough rice was separated into three thickness fractions ($<1{\cdot}84$, 1·84–1·98, and $>1{\cdot}98$ mm) and each fraction milled for three successive 10 s milling durations. Bran was collected from each milling duration of each thickness fraction to allow quantification of the nutraceutical content. Results showed that bran collected from rice milled for longer durations (30 s) had lower levels of tocotrienols and tocopherols compared to bran from shorter milling durations (10 s). The highest concentration of oryzanols was in the rice bran from the first 10 s milling duration. Overall, compared to bran from thinner kernels ($<1{\cdot}84$ mm), the bran from thicker kernel fractions contained a higher content of nutraceuticals.

1. Introduction

Rice (*Oryza sativa L.*) bran, composed of pericarp, seed coat, nucellus, aleurone layers and germ (Juliano & Bechtel, 1985; Orthoefer, 1996), is a by-product of milling, constituting approximately 10% weight of rough rice. During initial milling, the outer layers of the rice kernel, comprised of pericarp, seed coat, and nucellus (Juliano & Bechtel, 1985), are removed first, and any subsequent milling removes the more inner layer, aleurone, and the embryo or germ (Juliano & Bechtel, 1985), leaving polished rice, composed mainly of starchy endosperm. The lipid content of rice bran is approximately 18–22%, with the lipids existing as lipid bodies or spherosomes in the aleurone layer (Tanaka *et al.*, 1973). Additional constituents of the bran include protein, carbohydrates, ash, vitamins, and minerals (Qureshi *et al.*, 2000; Saunders, 1985).

The antioxidative and disease-preventative phytonutrients or nutraceuticals of rice bran have recently gained attention. Phytonutrients in rice bran possessing antioxidant and other reported beneficial physiological properties include: ferulic acid, its esterified derivative (γ-oryzanol), and unsaponifiable components such as tocopherol (vitamin E) and tocotrienol (as a form of vitamin E) (Jariwalla, 2001).

Oryzanol, γ-oryzanol in particular, is a mixture of sterol esters of ferulic acid that have been extensively studied and characterised (Rogers *et al.*, 1993; Rong *et al.*, 1997). γ-oryzanol protects rice bran oil from oxidation, inhibits peroxidation of lipids mediated by iron or UV irradiation (Jariwalla, 2001), and has been shown to lower blood cholesterol (Nicolosi *et al.*, 1993; Seetharamaiah & Chandrasekhara, 1993). For example, addition of 0·5% γ-oryzanol to a cholesterol-enriched diet was shown to be effective in lowering triglycerides, low-density lipoprotein (LDL)- and very low-density lipoprotein (VLDL)-cholesterol in serum, and reducing cholesterol levels in the liver (Seetharamaiah & Chandrasekhara, 1993).

Tocopherols and tocotrienols (tocols, each with four active homologs) are lipophilic antioxidants that protect the cell membranes from lipid peroxidation. Although both of these compounds have similar antioxidant

efficacy, tocotrienols have recently been shown to have additional functions of physiological and clinical importance. These include antitumor action against mammary cancer (Guthrie *et al.*, 1997) and possible beneficial effects on cardiovascular health (Qureshi *et al.*, 1991). Additionally, tocotrienols decrease serum total cholesterol and LDL cholesterol levels (Qureshi *et al.*, 2000) by inhibiting the hepatic enzymic activity of β-hydroxy-β-methylglutaryl coenzyme A (Qureshi *et al.*, 2002) and suppress smooth muscle proliferation in large arteries (Chatelaine *et al.*, 1993). These effects of tocotrienols contribute to the cholesterol-lowering and antiatherogenic qualities of rice bran oil.

Most previously conducted studies on phytonutrients in rice bran have not focused on determining the nutraceutical concentration of the various rice bran constituents. Identifying the layers of bran containing the majority of nutraceuticals would be helpful in maximising the economics of efficiently extracting these compounds. One study, conducted on steryl ferulate esters from corn and rice, quantified esters from the different processing fractions of corn bran, but only quantified esters from composite rice bran and rice bran oil (Norton, 1995) with no direct determination of the esters from the individual rice bran layers. Another study found steryl cinnamic acid derivative esters to be localised in the pericarp and aleurone layers of corn bran (Norton, 1994); however, the study did not measure the esters in individual layers of rice bran. Lloyd *et al.* (2000) collected rice bran from three successive milling breaks of a commercial milling system in order to quantify antioxidant levels from each break, and concluded that tocopherol and tocotrienol levels were highest in rice bran collected from the second milling break (82·6 and 29·4 mg kg^{-1} [bran], respectively), while bran from the first milling break contained the highest oryzanol content (6·42 g kg^{-1} [bran]). However, the collections of bran from each milling break in the commercial system did not necessarily represent the bran from any given kernel bran layer since kernels with various thicknesses within a bulk mill at different rates (Chen & Siebenmorgen, 1997). Therefore, a more controlled procedure in which kernels with various thicknesses are milled separately is needed to more positively identify nutraceutical concentrations in each successive bran layer.

This research was conducted to provide information regarding nutraceutical contents within rice kernels having different kernel thicknesses. Bulk rough rice comprises kernels of various sizes and maturities. Wadsworth *et al.* (1979) indicated that kernel thickness within a bulk is related to kernel maturity. Given the speculation that kernel maturity would affect nutraceutical levels, the concentration of nutraceuticals could then vary with kernel thickness. Thinner kernels generally contain higher contents of protein, vitamins, and lipids, but have a lower starch content than thicker kernels (Chen & Siebenmorgen, 1997; Matthews *et al.*, 1981). The effects of rough rice kernel thickness on milling quality, such as head rice yield has been studied (Sun & Siebenmorgen, 1993), with rough rice separated into several thickness fractions and milled. It was found that kernel thickness had a dramatic influence on head rice yield. Therefore, evaluating the effect that kernel thickness fractions have on nutraceutical levels in bran could be valuable information for isolating bran with maximum nutraceutical concentrations. The rice industry currently processes and mills rice as a bulk without separating into fractions; if the levels of nutraceuticals from various kernel thickness fractions are elucidated, it will determine the value of separating kernel fractions prior to commercial milling from the standpoint of maximising nutraceutical availability.

The objectives were: (1) to determine the nutraceutical concentration in successively removed controlled milling fractions of rice bran as a means of estimating the location of nutraceuticals within the rice bran morphology; and (2) to determine the effect of kernel thickness on rice bran nutraceutical concentrations. These findings could be used to optimise bran procurement for economically maximising the extraction of nutraceuticals from rice bran.

2. Materials and methods

2.1. *Sampling techniques*

Two long-grain rice cultivars, Cypress and Drew, were harvested from the Northeast Research and Extension Center, Keiser, AR, at moisture contents (MC) of 17·2 and 18·5% (expressed on a wet basis), respectively. Immediately after harvest, the rice was cleaned using a dockage tester (Model XT4, Carter-Day Co., Minneapolis, MN) and dried by placing the rice onto screens in a controlled temperature and relative humidity (RH) chamber (21°C, 53% RH) to achieve approximately 12% MC. Following drying, the rice was placed into plastic buckets, sealed, and stored at 7°C for approximately 2 months before sorting.

Using a precision sizer (Style no. ABF2, Carter-Day Co., Minneapolis, MN), the rough rice samples were separated into three thickness fractions ($<$1·84, 1·84–1·98, $>$1·98 mm) by starting with the 1·98 mm screen and continuing to the 1·84 mm screen. After sizing, the thickness fractions were returned to plastic buckets, sealed and stored at 7°C for approximately 2 months before milling.

Each thickness fraction for both Cypress and Drew was milled to obtain milled rice and bran. The milling procedure consisted of dehulling approximately 150 g of rough rice using a Satake Rice Machine (Type THU, Satake Engineering Co., LTD, Tokyo, Japan). Roller clearance was maintained across all thickness fractions at 0·483 mm. The resulting brown rice was milled in a laboratory mill (McGill No. 2, RAPSCO, Brookshire, TX) for bran removal. Placing a 1·5 kg weight on the lever arm 15 cm from the middle of the mill chamber controlled the pressure on the rice during milling. Samples of each thickness fraction were milled for three consecutive 10 s milling durations to remove the bran for subsequent analysis. The samples were first milled for 10 s (t_1) and the bran collected. The milled rice was removed from the mill and sifted using a screen tray so that any bran passing through the screen would be combined with the respective bran collected during milling. The rice was placed back in the mill chamber and milled for an additional 10 s to constitute 20 s (t_2) milled rice and bran. These same steps were again followed for the 30 s (t_3) bran collection. The mill was thoroughly cleaned between each milling by brushing the dust and broken kernels from the screen and removing excess bran from the rotor. Bran samples were then placed in plastic freezer storage bags, purged with nitrogen, and stored at -10°C until subsequent extraction and analysis.

2.2. *Lipid extraction for nutraceuticals*

2.2.1. *Chemicals and materials*

All solvents were high-performance liquid chromatography (HPLC) grade. Hexane was obtained from VWR Scientific Products (West Chester, PA), and methanol and acetonitrile were obtained from EM Science (Gibbstown, NJ).

2.2.2. *Accelerated solvent extraction*

The nutraceuticals of interest (tocols and oryzanols) were extracted from rice bran samples using an Accelerated Solvent Extractor (ASE 200, Dionex, Sunnyvale, CA). The pressure during extraction was maintained at 10·3 MPa with a constant temperature of 50°C. Each bran sample (approximately 2·5 g) was placed in an extraction cartridge, loaded onto the extraction unit, ASE 200, and extracted for three cycles using hexane with a static duration of 10 min per cycle. The total extraction duration of each sample was 30 min. Due to the limited bran quantities obtained from each milling duration of each thickness fraction, each bran sample was only extracted in duplicate. After collection of the solvent containing the lipid extract, the hexane was evaporated using an Automatic Environmental SpeedVac® System (Savant Instruments, Inc., Holbrook, NY) to concentrate the extract. The lipid material from the extracted bran was weighed in order to obtain the mass in g of the lipid content per mass of the bran in g. This was utilised to calculate the average lipid content extracted from each bran sample. The rice bran lipid extract was then redissolved in 4 m*l* of acetonitrile:methanol (75:25%), and then centrifuged at 200 min^{-1} for 10 min. The supernatant was collected and filtered through a 0·45 µm filter prior to liquid chromatography analysis.

2.3. *High-performance liquid chromatography analysis*

Rice bran lipid extract from the ASE was analysed for tocotrienols, tocopherols, and oryzanols using a reverse-phase high-performance liquid chromatography (HPLC) method (Lloyd *et al*., 2000). Briefly, a symmetry C_{18} column (5 µm, 25 cm by 4·6 mm) (Waters Corp., Milford, MA) interfaced with a fluorescence and photodiode-array detectors in tandem were employed in the HPLC system (Waters 2690 Alliance Milford, MA). The initial mobile phase conditions were acetonitrile, methanol, and water (60:35:5), which changed to a gradient mobile phase in 1 min to acetonitrile, methanol, and water (60:40:0). The mobile phase then changed linearily to acetonitrile, methanol, and water (20:80:0) over the next 14 min was held for 5 min before returning to initial conditions, for a total run time of 30 min. The excitation and emission wavelengths of the fluorescence detector were set at 298 and 328 nm, respectively, for detection of tocotrienols and tocopherols, while oryzanols were detected using UV with a wavelength of 325 nm.

Quantification of tocopherols and tocotrienols was accomplished by comparison to those of authentic standards. Tocotrienol and tocopherol were obtained from Merck KGa (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, MO), respectively. Total oryzanol in each sample was compared to an authentic standard donated by Dr. J. Samuel Godber of the Department of Food Science, Louisiana State University, Baton Rouge, LA and to a purchased standard (American Tokyo Kasel, Inc., Portland, OR). The total concentration of tocotrienols and tocopherols were derived by the summation of the α, β, γ, and δ forms (Lloyd *et al*., 2000). Total oryzanol in each rice bran sample was determined in a manner similar to that used for tocotrienols and tocopherols. The concentration ranges of calibration standards were 2·0–50, 25–200, and 50–600 µg ml^{-1} for tocotrienols, tocopherols, and oryzanols, respectively.

3. Results and discussion

3.1. *Levels of nutraceuticals in bran fractions from three milling durations*

3.1.1. *Tocotrienol levels*

Table 1 shows the nutraceutical levels and corresponding lipid contents of the bran from thickness-fractionated Cypress rice, with each fraction milled for three successive durations. There were no significant differences in tocotrienol concentrations among milling durations except for the bran from the t_3 milling duration of the mid-thickness fraction (1·84–1·98 mm), which was significantly lower in tocotrienol levels as determined by Student's *t*-test probability $P<0{\cdot}05$ using one-way analysis of variance (JMP IN 5·0., SAS Inc., Cary, NC). The mass total of tocotrienols across all thicknesses at t_3 was lower (634 mg kg^{-1} [bran]) than t_1 and t_2, (831 and 797 mg kg^{-1} [bran], respectively). In the current study, the 10 s milling duration was designed to simulate the bran collected from the second break of a commercial milling system. Other investigators (Lloyd *et al.*, 2000) concluded that tocotrienol concentrations were greater in long-grain rice bran taken directly from the second break of a commercial milling system. No statistical difference was found for the tocotrienols from the t_2 milling duration in the current study; however, a greater mass total of tocotrienols across all thicknesses at t_2 were noted compared to t_1 and t_3 (Table 1). The values in the current study are within the range of 72–1157 mg kg^{-1} which agree with those reported by Rogers *et al.* (1993).

Table 2 shows nutraceutical and corresponding lipid levels for Drew rice bran among the three milling durations and across the three thickness fractions. These findings are comparable to those found for Cypress bran, in that the tocotrienol levels from the t_3 milling duration were significantly lower for the thinnest and mid-thickness range kernels. It is reasoned that during this milling duration, not only residual layers of bran but also some endosperm were removed. Since lower lipid values were measured in the longest milling durations (Tables 1 and 2), the bran collected would be expected to have low levels of tocotrienol compounds. In addition, the mass total of tocotrienol content across all thicknesses was lower from t_3, 405 mg kg^{-1} [bran], compared to t_1, 629 mg kg^{-1} [bran], and t_2, 653 mg kg^{-1} [bran].

It was reasoned that the tocotrienol content was related to the amount of extracted lipid from the bran, which was apparent by comparing lipid contents (%) and the corresponding levels of tocotrienols, such that, in milling durations containing high levels of extracted lipids, the tocotrienol content was also high for both Drew and Cypress rice bran. For example, the highest level of extracted lipid, 28·2%, from the bran of the t_2 milling duration from mid-thickness kernels for Cypress rice, also contained the greatest level of tocotrienols, 330 mg kg^{-1} [bran] (Table 1).

Table 1
Nutraceutical levels in bran from three thickness fractions of Cypress rice milled for three durations[†]

Nutraceutical	*Milling duration*[‡], *s*	*Thickness fractions*[**], *mm*			
		<1·84	*1·84–1·98*	*>1·98*	*Total*
Tocotrienols[*], mg kg^{-1} [bran]	t_1	231^{B} (15·2%)[§]	308^{Aa} (25·0%)	258^{B} (23·1%)	797
	t_2	241 (18·1%)	330^{a} (28·2%)	260 (23·9%)	831
	t_3	188 (9·68%)	217^{b} (17·3%)	229 (20·0%)	634
	Total	660	855	747	
Tocopherols, mg kg^{-1} [bran]	t_1	76·0	95·5^{a}	80·0	252
	t_2	69·3	119^{a}	98·0	286
	t_3	47·7^{B}	67·3A^{Bb}	88·7^{A}	204
	Total	193	282	267	
Oryzanols, g kg^{-1} [bran]	t_1	7·47^{Ba}	9·53^{Aa}	8·18^{ABa}	25·2
	t_2	1·35^{Cb}	3·52^{Ab}	2·59^{Bb}	7·46
	t_3	0·260^{Bc}	0·225^{Bc}	1·83^{Ab}	2·32
	Total	9·08	13·3	12·6	

[†] Each value represents an average of duplicate samples extracted by accelerated solvent extraction.
[‡] t_1 = first 10 s milling duration, t_2 = 20 s milling duration, t_3 = 30 s milling duration.
[*] Significantly different Student's *t*-test (probability $P<0{\cdot}05$) values across columns of thickness fractions within each nutraceutical for each milling duration are indicated by different capital letters.
[**] Significantly different Student's *t*-test ($P<0{\cdot}05$) values among milling durations for each nutraceutical within each thickness fraction are indicated by different lowercase letters.
[§] Average lipid content (%) extracted from the bran for nutraceutical quantification; all nutraceuticals have the same corresponding lipid content for each milling duration of each thickness fraction.

Table 2
Nutraceutical levels and corresponding lipid contents (%) in bran from three thickness fractions of Drew rice milled for three durations†

Nutraceutical	*Milling duration‡, s*	*Thickness fractions**, mm*			
		<1·84	*1·84–1·98*	*>1·98*	*Total*
Tocotrienols*, mg kg^{-1} [bran]	t_1	113^{Ba} (10·7%)§	281^{Aa} (23·7%)	235^{A} (19·9%)	629
	t_2	103^{Ba} (8·60%)	311^{Aa} (26·9%)	239^{A} (20·8%)	653
	t_3	$69·8^{Bb}$ (5·00%)	127^{Bb} (17·9%)	208^{A} (16·6%)	405
	Total	286	719	682	
Tocopherols, mg kg^{-1} [bran]	t_1	$42·9^{Ba}$	$61·1^{A}$	$49·8^{Ba}$	154
	t_2	$32·9^{Bab}$	$74·8^{A}$	$66·8^{ABa}$	175
	t_3	$22·0^{Bb}$	$67·6^{A}$	$36·3^{Bb}$	126
	Total	97·8	204	153	
Oryzanols, g kg^{-1} [bran]	t_1	$4·82^{Ba}$	$7·85^{Aa}$	$7·56^{Aa}$	20·2
	t_2	$1·64^{Bb}$	$4·32^{Ab}$	$1·73^{Bb}$	7·69
	t_3	$0·118^{c}$	$0·240^{c}$	$0·740^{b}$	1·10
	Total	6·58	12·4	10·0	

† Each value represents an average of duplicate samples extracted by accelerated solvent extraction.
‡ t_1 = first 10 s milling duration, t_2 = 20 s milling duration, t_3 = 30 s milling duration.
* Significantly different Student's *t*-test (probability $P<0·05$) values across columns of thickness fractions within each nutraceutical for each milling duration are indicated by different capital letters.
** Significantly different Student's *t*-test ($P<0·05$) values among milling durations for each nutraceutical within each thickness fraction are indicated by different lowercase letters.
§ Average lipid content (%) extracted from the bran for nutraceutical quantification; all nutraceuticals have the same corresponding lipid content for each milling duration of each thickness fraction.

3.1.2. *Tocopherol levels*

The levels of tocopherols for Cypress rice bran for all three thickness fractions milled for three successive durations are given in Table 1. The trends were similar to the results from the tocotrienols, in that the bran from the t_3 milling duration was lower in tocopherol content when compared to the bran from the t_1 and t_2 milling durations in the mid-thickness fraction. A lower tocopherol content in bran removed after long milling durations (*i.e.* t_3) is anticipated due to the similarity in structure and function to tocotrienols, therefore, their location in the bran should be parallel.

Table 2 shows the tocopherol content for Drew rice bran for all three thickness fractions milled for three successive durations. As noted for the tocotrienols, a trend of lower tocopherol content in the bran from the t_3 milling durations compared to t_1 was apparent; in particular, the thinnest (<1·84 mm) and thickest (>1·98 mm) kernels had significantly lower tocopherol contents in the t_3 milling duration bran compared to the t_1 milling duration.

3.1.3. *Oryzanol levels*

Table 1 shows the oryzanol content for Cypress rice bran for all three thickness fractions milled for three successive durations. Oryzanol content was notably higher (>7·40 g kg^{-1} [bran]) in the bran collected from the t_1 milling than from successive milling durations. The mass total of oryzanols across all thicknesses at t_1 was greater than t_2 and t_3 (7·46 and 2·32 mg kg^{-1} [bran], respectively). There was a consistent significant decrease in oryzanol levels as the durations of milling successively increased for the thinnest and mid-thickness kernels of Cypress rice.

The trends for Drew were similar to the results for Cypress rice, in that significantly greater ($P<0·05$) concentrations of oryzanols were found for the bran from t_1 milling durations, which were greater than the t_2 and t_3 milling durations in all thickness fractions (Table 2). The mass total of oryzanols across all thicknesses were also greater at t_1 (20·2 g kg^{-1} [bran]) than t_2 and t_3 (7·69 and 1·10 g kg^{-1} [bran], respectively). This finding concurs with those of other investigators who noted oryzanol was highest in commercially milled bran that had been collected after the first break (Lloyd *et al.*, 2000). In addition, the high levels of oryzanols in the first 10 s milling duration indicate that the ferulate esters that comprise oryzanol are located primarily in the outer pericarp, seed coat, and nucellus layer (Bechtel & Pomeranz, 1977), which are the outer bran layers of the brown rice kernel that are presumably removed first during milling.

The average concentration of oryzanols extracted from rice bran have been reported to be 9·8 g kg^{-1} [bran]

(Xu & Godber, 2000) using hexane extraction without saponification, and 6·42 g kg^{-1} [bran] from a mixture of several varieties of long-grain rice from the first milling break (Lloyd *et al*., 2000), which are comparable to the mass average oryzanol values across all thickness fractions at t_1 milling measured in this study (8·39 mg kg^{-1} [bran] in Cypress rice bran and 6·74 mg kg^{-1} [bran] in Drew rice bran).

3.2. *Kernel thickness effects on nutraceutical concentrations*

3.2.1. *Tocotrienol levels*

Table 1 shows that the tocotrienol content of bran from the mid-thickness fraction of Cypress rice milled for only 10 s (t_1) was significantly higher than from thinner (< 1·84 mm) and thicker (> 1·98 mm) kernels at the same milling duration. There were no significant differences in tocotrienols across thickness fractions for the t_2 and t_3 milling durations, although a trend towards greater tocotrienol content for bran from the mid-thickness kernels at the t_2 milling duration for Cypress and Drew was noted. Additionally, the mass total of tocotrienols across milling durations was greater from mid-thickness kernels (855 mg kg^{-1} [bran]) compared to thinner (660 mg kg^{-1} [bran]) and thicker (747 mg kg^{-1} [bran]) kernels.

In general, tocotrienol levels were significantly lower in the bran from thinner kernel fractions of Drew than those from thicker kernels (> 1·98 mm) at all milling durations (Table 2), as well as a lower mass total in thinner kernels across all milling durations (286 mg kg^{-1} [bran]) compared to thicker kernels (682 mg kg^{-1} [bran]). The lipid content of the bran from Drew rice was also lower from the thinner kernels than the mid-thickness or thicker kernels, which indicates that the rice kernel size prior to milling is an important factor influencing tocotrienol concentrations in the lipid bodies of the rice bran layers (Vasan *et al*., 1979).

The high extracted lipid values observed in the bran from mid-thickness kernels at the t_2 milling duration for Cypress and Drew rice, 28·2 and 26·9%, respectively, could be attributed to this bran containing a higher proportion of the aleurone layer, which is comprised of lipid bodies that are believed to house antioxidants (Juliano & Goddard, 1986; Suarna *et al*., 1992). This milling step could be postulated to remove most of the aleurone layer and any successive millings would remove the remaining bran (5%), most of which is amyloplasts containing starch granules. The aleurone layer varies from one to five cell layers and is rich in lipid bodies (Juliano & Goddard, 1986). The lipid bodies (along with the embryo) are a rich source of oil and vitamins (Vasan *et al*., 1979). These lipid bodies would be expected to contain large amounts of lipid-soluble tocopherol and tocotrienol antioxidants since their role is to protect the lipids from oxidation during rice kernel development.

3.2.2. *Tocopherol levels*

The tocopherol content of the bran from Cypress rice was significantly greater in the thickest kernels (> 1·98 mm) than from the thinnest kernels (< 1·84 mm) at the t_3 milling duration.

For Drew rice, the bran from mid-thickness kernels at the t_2 and t_3 milling durations had significantly greater ($P < 0·05$) tocopherol content than the thinnest kernels and across all thicknesses, the mass total of tocopherols was greater from t_2 (175 mg kg^{-1} [bran]) than t_1 and t_3, (154 and 126 mg kg^{-1} [bran], respectively) (Table 2). One speculation for the high tocopherol content in the bran of t_2 milling duration for thicker kernels (> 1·84 mm), in addition to the previously stated speculation for high tocotrienol content in bran from mid-thickness fractions at t_2 milling duration, may be attributed to the embryo contained in the bran. Since the embryo is a valuable part of the seed (Kumaravel *et al*., 1985) and accounts the for more than 95% of the total tocopherols of the oil content in the rice grain (Gopala Krishna *et al*., 1984), it would seem plausible that the high tocopherol content observed in the bran from the t_2 milling duration could be due to the embryo being removed during this milling duration; since during the milling process the embryo becomes mixed with the bran (Vasan *et al*., 1979). Although the collected bran from each milling duration was not analysed or inspected for embryo content, the bran from the t_2 milling may have contained the embryo, particularly in the mid-thickness fractions.

3.2.3. *Oryzanol levels*

Across the three thickness fractions for Cypress rice bran, the levels of oryzanols were notably greater (> 9·50 g kg^{-1}) in the bran from mid-thickness kernels at the t_1 milling duration compared to thinner kernels (< 1·84 mm) at the same milling duration. Variations in oryzanols contents were found in the bran across the three thickness fractions for the t_2 and t_3 milling durations, however, the bran from these milling durations were significantly lower in oryzanols in the bran from the t_1 milling duration and were not considered a major source of oryzanols.

The oryzanol content was significantly lower in the bran from thinner kernels of Drew rice at the t_1 milling duration (4·82 g kg^{-1} [bran]) when compared to the bran from mid-thickness and thicker kernels at this same milling duration (7·85 and 7·56 g kg^{-1} [bran], respectively). The oryzanol concentration was significantly greater in the bran from the mid-thickness Drew kernels

at the t_2 milling duration than the thinner and thickest kernels at the same milling duration, which would imply that the concentration of oryzanols could vary depending on kernel thickness.

4. Conclusions

Milling durations influenced the nutraceutical content of bran, such that longer milling durations generally resulted in lower levels of tocotrienols, tocopherols, and oryzanols. This is speculated to be due to the fact that bran collected from the longer milling durations contained more endosperm and less of the nutraceutical-rich aleurone layer and the germ. The aleurone layer and the germ tend to be removed earlier in the milling operation, since the nutraceuticals tended to be primarily located in bran from the first 10 and 20 s milling durations. This suggests that the selection of specific milling durations can maximise the nutraceutical-rich bran that is collected to be used for further isolation of the desired nutrients.

Results also showed that similar trends in nutraceutical locations were found between the two varieties of rice studied. Thicker kernels of both rice varieties tended to contain higher levels of tocotrienols, tocopherols, and oryzanols when compared to thinner kernels. This finding will lend credence to the concept of fractionating rice kernels prior to milling as compared to milling as an unfractionated bulk, in order to obtain greater quantities of nutraceuticals from the rice bran. Therefore, it is more advantageous, when desiring large concentrations of nutraceuticals, to use shorter milling durations with the thickest rice kernels in a bulk.

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