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Review

Composition, industrial processing and applications of rice bran γ-oryzanol

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ABSTRACT

Rice bran oil (RBO) (20–25 wt% in rice bran) is a unique rich source of commercially-important bioactive phytochemicals, most of them of interest in nutrition, pharmacy and cosmetics. The unsaponifiable constituents of RBO include mainly tocols (vitamin E, 0.10–0.14%) and γ-oryzanol (esters of *trans*-ferulic acid with sterols and triterpenic alcohols, 0.9–2.9%). The following topics concerning γ-oryzanol are reviewed: analytical methods for characterisation and determination; influence of genetic and environmental factors on the composition of rice bran; extraction approaches, including supercritical CO_2 and subcritical water; and biomedical and industrial applications, including food and pharmaceuticals. Concentration ranges of γ-oryzanol, tocopherols and tocotrienols found in rice bran and RBO from different varieties and geographical areas are summarised. This review focuses on the 2003–2008 period, where an average of 13–14 references per year were published; however, some relevant work reported during the 1998–2002 period is also briefly commented upon.

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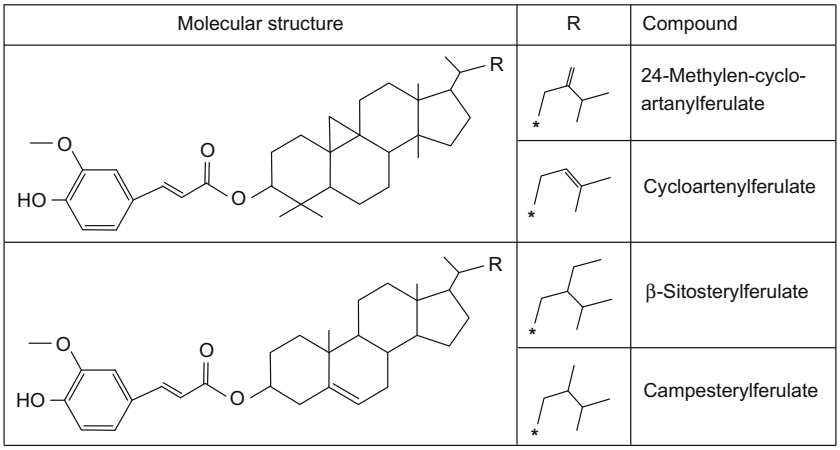

Fig. 1. Chemical structures of the four main components of γ-oryzanol.

1. Introduction

Among edible oils, rice bran oil (RBO, 20–25 wt% in rice bran) is a unique rich source of commercially-important bioactive phytochemicals, most of them of interest in nutrition, pharmacy and cosmetics (Da Silva, Sanches, & Amante, 2005; Danielski, Zetzl, Hense, & Brunner, 2005). The unsaponifiable constituents of RBO include mainly tocopherols and tocotrienols (tocols or vitamin E) and γ-oryzanol, as well as other compounds which are found at lower concentrations, such as lecithin and carotenoids (Chen & Bergman, 2005a; Patel & Naik, 2004; Stoggl, Huck, Wongyai, Scherz, & Bonn, 2005), the flavone tricin (Devi & Arumughan, 2007), long-chain alcohols such as 1-octacosanol, and squalene (Ha et al., 2006). The concentrations of tocols and γ-oryzanol in RBO depend largely on genetic and environmental factors, being within the 0.10–0.14% and 0.9–2.9% ranges, respectively (Diack & Saska, 1994; Lloyd, Siebenmorgen, & Beers, 2000; Patel & Naik, 2004).

γ-Oryzanol is mainly composed of esters of *trans*-ferulic acid (*trans*-hydroxycinnamic acid) with phytosterols (sterols and triterpenic alcohols). Among these, cycloartenol, β-sitosterol, 24-methylenecycloartenol and campesterol predominate (Xu & Godber, 1999; Lloyd et al., 2000). The molecular structures of *trans*-ferulates of these four phytosterols are shown in Fig. 1. γ-Oryzanol also contains lower concentrations of esters of the *trans*-ferulic acid with Δ^7-stigmasterol, stigmasterol, Δ^7-campesterol, Δ^7-sitostenol, campestenol and sitostenol (Xu et al., 1999), as well as esters of *cis*-ferulic (Akihisa et al., 2000) and caffeic acids (Fang, Yu, & Badger, 2003).

Phytosterols carry out functions in plants equivalent to those of cholesterol in animals, being thus required as necessary components of cell membranes and as precursors of important biomolecules, including sex hormones and vitamins. There are about 44 phytosterols known to exist in plants, the most abundant being β-sitosterol, campesterol and stigmasterol. The food sources with the highest total phytosterol contents, as the sum of these three compounds (in mg/100 g), are the oils of rice bran (1055), corn (952), wheat germ (553), flax seed (338), cottonseed (327), soybean (221), peanut (206) and olive (176) (Kritchevsky, 1997). Most phytosterols available today are mainly derived from processing of canola, cottonseed, corn and soybean. An alternative commercial source is tall oil, a by-product of paper mills. Significant amounts of phytosteryl *trans*-ferulates have been also extracted from rye and wheat grains (Hakala et al., 2002) and laminarian seaweeds (Nagasaka, Shinoda, Ushio, & Ohshima, 2008).

Most research has been conducted to take advantage of the high contents in RBO of phytosteryl *trans*-ferulates. The production of both RBO and RBO fractions enriched in γ-oryzanol, as well as the health properties of γ-oryzanol, were reviewed a few years ago (Patel & Naik, 2004). Since then, the interest in the production and applications of RBO, including fractions enriched in phytosteryl *trans*-ferulates, has remarkably increased. In the present work, the following topics concerning γ-oryzanol are reviewed: analytical methods for characterisation and determination; influence of genetic and environmental factors affecting the composition of rice bran; extraction approaches, including supercritical carbon dioxide ($SC–CO_2$) and subcritical water; and biomedical and industrial applications, including food and pharmaceuticals. This review is roughly focused on the 2003–2008 period, comprising 87 references; however, some relevant work out of the ca. 20 references found in the previous 1999–2002 period is also briefly commented upon.

2. Characterisation and determination of γ-oryzanol components

The analytical methods used for the characterisation and determination of γ-oryzanol, tocopherols and tocotrienols, including the nature of the samples, and extraction, separation and detection techniques, are summarised in Table 1. Also, relevant topics, such as the influence of genetic and environmental factors on their concentration levels, are briefly outlined in this table and the text that follows.

2.1. Non-chromatographic spectrophotometric methods

Bucci, Magri, Magri, and Marini (2003) have compared the use of fixed wavelength, second-derivative and multicomponent spectrophotometry applied to the determination of γ-oryzanol in RBO. The absorption bands of γ-oryzanol were red-shifted from 314 to 327 nm when the oil was diluted with isopropanol. Thus, in comparison to *n*-hexane, absorbance measurements were less affected by the interference of the oil matrix when using isopropanol. The interference of oil matrix was fully removed by second-derivative spectrophotometry, and multivariate techniques provided evidence on the presence of unexpected interferences. Thus, either the second-derivative at 330.4 nm or multicomponent data analysis within the 310–360 nm range was recommended to increase accuracy, particularly at low γ-oryzanol concentrations. Descriptions of the IR spectrum of γ-oryzanol, and thermogravimetry and differential scanning calorimetry data, were also given.

Lilitchan et al. (2008) have developed a method based on partial extraction followed by UV–Vis spectrophotometry for the determination of total lipids and γ-oryzanol in rice bran. Two identical rice

Table 1
Characterisation and determination of γ-oryzanol and tocols, and influence of genetic and environmental factors.

Reference	Samples	γ-Oryzanol		Tocopherols (T) and tocotrienols (T_3)			Comments, conclusions
		Method	Content (mg/g)	Method	Content (μg/g)	Content (μg/g)	
Xu and Godber (1999)	RBO extracted without saponification (LA, USA)	Preparative NP-HPLC followed by RP-HPLC with UV–Vis, GC–MS	9.8	–	–	–	Individual components of γ-oryzanol were characterised
Lloyd et al. (2000)	Bran from a mixture of long-grain varieties (LG-1) and a medium-grain variety (AR, USA): (a) LG-1 (b) Bengal	RP-HPLC with UV–Vis	Before/after steam expansion of bran: (a) 74/3.47 (b) 4.00/3.27	RP-HPLC with fluorescence		Before/after steam expansion of bran, T_3: (a) 25/22 (b) 19/16	Extraction with hexane. Upon steam expansion of bran, γ-oryzanol shows a 26% decrease, T + T_3 levels being less affected
Bergman and Xu (2003)	Bran from seven cultivars harvested in 1999–2000 in four states (USA)	NP-HPLC with UV–Vis	2.510–6.864	NP-HPLC with fluorescence	T + T_3: 179–389 α-/γ-T: 27–94/6–59	α-/γ-T_3: 47–165/61–130	Extraction with hexane. Individual data per cultivar, year and state are given (global ranges in this table). Correlation studies are performed. Effects of growing environment *vs.* genotype are evaluated
Bucci et al. (2003)	RBO from: (a) Italy (10 samples) (b) Thailand (10 samples) (c) Switzerland (10 samples)	Derivative UV–Vis, RP-HPLC with UV–Vis and FTIR	(a) 3.8–4.1 (b) 2.0–2.6 (c) 0.89–1.02	–	–	–	Second-derivative and multicomponent (λ = 310–360 nm) UV–Vis methods for γ-oryzanol determination are described
Dunford et al. (2003)	RBO (AR, USA): (a) Crude (b) Raffinate fraction	NP-HPLC with ELSD	(a) 15 (b) 49	–	–	–	Extraction with SC–CO_2. Data concerning free fatty acids, free phytosterols and fatty acid esters of phytosterols are also given
Miller et al. (2003)	Brown rice: (a) Cripto (Germany) (b) Balilla (Germany) (c) Xiushui11 (China)	NP-HPLC with UV–Vis. Fraction transfer to GC–FID and GC–MS	(a) 0.627 (b) 0.393 (c) 0.310	–	–	–	Extraction with dichloromethane–methanol (2:1, v/v). Concentrations of individual steryl ferulates are given
Fang et al. (2003)	Mixture of varieties (AR, USA) (a) Germ (b) Bran without germ	RP-HPLC with UV–Vis and ESI-MS/MS	(a) 0.578 (b) 2.814	RP-HPLC with UV–Vis and ESI-MS/MS	–	α-/β-/γ-/δ-T_3: (a) 457/56/68/31 (b) ND/106/ND/ND	Successive extractions with ethanol and methanol. Concentrations of 17 γ-oryzanol components are given
Khatoon and Gopalakrishna (2004)	RBO from three varieties (India): (a) Basmati (b) Jaya (c) Parboiled commercial variety	RP-HPLC with UV–Vis	(a) 10.7 (b) 14.3 (c) 13.3	RP-HPLC with fluorescence	α-/(β + γ)-/δ-T: (a) 369/439/143 (b) 10/12/4 (c) 0.74/0.99/0.45	α-/(β + γ)-/δ-T_3: (a) 652/60/81 (b) 0.77/1.52/1.57 (c) ND/ND/ND	In addition to RBO, concentrations in milled rice oil, brown rice flour and milled rice flour are given. Concentrations of fatty acids are also given
Rohrer and Siebenmorgen (2004)	Bran from two varieties: (a) Cypress (b) Drew	RP-HPLC with UV–Vis	(a) 2.32–2.52 (b) 1.10–2.02	RP-HPLC with fluorescence	T: (a) 193–286 (b) 98–204	T_3: (a) 660–855 (b) 286–719	Accelerated solvent extraction (10.3 MPa, 50 °C) with hexane. Ranges from different bran thickness fractions are given. Highest γ-oryzanol concentration at 5-10 s milling time
Anwar et al. (2005)	RBO from four varieties (Pakistan): (a) Super Kernel (b) 386 (c) 385 (d) Basmati	RP-HPLC with UV–Vis	(a) 0.802 (b) 0.639 (c) 0.415 (d) 0.779	NP-HPLC with fluorescence	α-/(β + γ)-/δ-T: (a) 284/83/75 (b) 175/99/57 (c) 180/121/39 (d) 300/91/83	α-/(β + γ)-/δ-T_3: (a) 120/196/72 (b) 106/125/20 (c) 95/210/39 (d) 136/276/64	Extraction with *n*-hexane. Oil content: 14.7-19.1%. Concentrations of sterols and fatty acids are given

(*continued on next page*)

Table 1 (*continued*)

Reference	Samples	γ-Oryzanol		Tocopherols (T) and tocotrienols (T_3)			Comments, conclusions
		Method	Content (mg/g)	Method	Content (μg/g)	Content (μg/g)	
Iqbal et al. (2005)	RBO from five varieties (Pakistan): (a) Super Kernel (b) Super-2000 (c) Super-Basmati (d) Super-386 (e) Super-Fine	RP-HPLC with UV–Vis	(a) 0.802 (b) 0.789 (c) 0.698 (d) 0.655 (e) 0.511	RP-HPLC with fluorescence and voltametry on a glassy carbon electrode	T by fluorescence/voltammetry: (a) 512/503 (b) 481/472 (c) 459/451 (d) 392/378 (e) 419/423	T_3: (a) 478 (b) 452 (c) 389 (d) 364 (e) 343	Extraction with *n*-hexane. Growth period and quantity of irrigation water needed for cultivation of a variety has a significant effect on its antioxidant properties
Stoggl et al. (2005)	RBO	RP-HPLC with UV–Vis and APCI-MS	Qualitative study	RP-HPLC with UV–Vis and APCI-MS	Qualitative study	–	Extraction with *n*-hexane. Separation of γ-oryzanol, tocopherols (with resolution of the β/γ pair) and carotenoids in a single chromatogram using a C_{30} column
Chen and Bergman (2005b)	Bran from two varieties (TX, USA): (a) Cypress (b) Bengal	RP-HPLC with UV–Vis	(a) 3.4–3.9 (b) 3.8–4.2	RP-HPLC with fluorescence	α-/(β + γ)-/δ-T: (a) 37–74/10–25 (b) 29–80/12–30/2.3	α-/(β + γ)-/δ-T_3: (a) 27–55/53–164/3–5 (b) 19–54/44–127/5–6	Extractions with isopropanol (I), hexane (H), methanol and 1:1 I–H. Global ranges given in this table (maximal values with I and I–H, and minimal with H)
Miller and Engel (2006)	Brown rice from 30 cultivars harvested in 2000–2002 (Italy, France and Spain)	NP-HPLC–UV–vis coupled to GC-FID	Data per each cultivar are given; global range: 0.262–0.627	–	–	–	Extraction with 2:1 CH_2Cl_2/methanol. Concentrations of γ-oryzanol components are given. Distributions are influenced by environmental conditions, but not by grain maturity
Krishna et al. (2006)	RBO (India): (a) Chemically refined (b) Physically refined	UV–Vis	(a) 1.4–1.8 (b) 5.50–1.39	UV–Vis	T + T_3: (a) 500–570 (b) 480–700	–	Colour intensity, unsaponifiable matter and free fatty acid data are given. RBO is compared to refined groundnut, safflower and sunflower oils
Schramm (2006)	Bran outer layers of two varieties (lab- and pilot-scale studies): (a) Cypress (b) Cheniere	RP-HPLC with UV–Vis	(a) 1.99–2.45 (b) 1.92–2.82 (pilot) (c) 2.05–2.65 (d) 1.84–2.39 (pilot)	NP-HPLC with fluorescence	T + T_3: (a) 177–205 (b) 146–198 (pilot) (c) 209–221 (d) 169–210 (pilot)	–	Global ranges for 5–45 s milling time are given in this table. Highest γ-oryzanol and tocol concentrations occurred at 5–15 s and 10 s milling time, respectively
Schramm et al. (2007)	Bran outer layers of two varieties (LA, USA): (a) Cypress (b) Cheniere	RP-HPLC with UV–Vis	(a) 1.85–2.52 (b) 2.01–2.70	NP-HPLC with fluorescence	T + T_3: (a) 170–218 (b) 204–229	–	Extraction with previous saponification (which causes hydrolysis of γ-oryzanol). Protein and rice bran saccharide data are also given. Global ranges for 5–45 s milling time are given in this table
Ha et al. (2006)	Unmilled brown rice from Odaesan variety (Korea)	UV–Vis	0.199	NP-HPLC with fluorescence	α-/β-/γ-/δ-T: 14.6/0.6/1.3/0.1	α-/γ-/δ-T_3: 8.7/11.9/0.5	Extraction with hexane. The decrease of nutraceuticals as the degree of milling increases is studied. Fatty acid, phytosterols, squalene and octacosanol data are also given.
Britz et al. (2007)	Brown rice from six lines grown in greenhouses: (a) Arborio (*japonica*) (b) Kaybonnet (*japonica*) (c) Jodon (*indica*) (d) Tellahamsa (*indica*) (e) CG-17 (*glaberrima*) (f) Italica (*japonica*)	RP-HPLC (C30 column) with UV–Vis and APCI-MS	(a) 0.277–0.383 (b) 0.252–0.351 (c) 0.259–0.276 (d) 0.228–0.326 (e) 0.180–0.236 (f) 0.220–0.226	RP-HPLC (C30 column) with fluorescence and APCI-MS	T + T_3 in μmol/kg: (a) 91–100 (b) 140–158 (c) 135 (d) 99–104 (e) 97–108 (f) 216–236	T_3 in μmol/kg: (a) 67–75 (b) 113–137 (c) 107–112 (d) 74–81 (e) 67–73 (f) 75–80	Ethanol extracts. Separation of γ-oryzanol, tocols (with resolution of the β/γ pairs) and carotenoids in a single chromatogram. Concentrations of γ-oryzanol and tocol components are given. Study of the influence of temperature during growing season.

Aguilar-Garcia et al. (2007)	Bran and brown rice powder from three varieties (Venezuela): (a) Cimarrón (b) Zeta 15 (c) FONAIAP-1	RP-HPLC with UV–Vis and fluorescence	Bran/brown rice powder: (a) 1.55/0.328 (b) 2.72/0.388 (c) 2.12/0.201	RP-HPLC–UV–Vis and fluorescence	α-/(β + γ)-T: (a) ND/41 (b) 26/21 (c) 35/26	α-/(β + γ)-/δ-T_3: (a) ND/138/17 (b) 16/140/7 (c) 24/127/6	Linear correlations between the concentrations of the antioxidants are studied using principal component analysis
Finocchiaro et al. (2007)	Brown rice from two cultivars (Italy): (a) S. Eusebio (red) (b) Carnaroli (white)	NP-HPLC with UV–Vis	Dehulled/undermilled/milled raw rice: (a) 0.470/0.255/0.090 (b) 0.744/0.191/0.058	NP-HPLC with fluorescence	T + T_3 in dehulled/undermilled/milled raw rice: (a) 50/31/15 (b) 43/10/10	–	Extraction with methanol. Concentrations of hydroxycinnamic acids, proanthocyanidins, total polyphenols and polyphenolic compounds are given. Concentrations in water-cooked and risotto-cooked rice are also given
Devi and Arumughan (2007)	Defatted rice bran (India)	RP-HPLC with UV–Vis	7.82	NP-HPLC with UV–Vis	138	–	Extraction with methanol. Further enrichment and purification with acetone and mixed solvents. Concentrations of *trans*-ferulic acid are given. Superoxide radical-scavenging activity studies
Devi, Jayalekshmy, and Arumughan (2007)	Defatted rice bran (India)	RP-HPLC with UV–Vis	7.83	NP-HPLC with UV–Vis	146	–	Extraction with MeOH. Further enrichment and purification with acetone and mixed solvents. Concentrations of *trans*-ferulic acid are given
Deepam et al. (2007)	RBO (Kerala, India): (a) Crude (b) Refined	RP-HPTLC with UV–Vis densitometry	(a) 18.8 (b) 13.8	RP-HPTLC with UV–Vis densitometry	T + T_3: (a) 2000 (b) 1400	–	Sterol, squalene and steryl ester data also given
Paucar-Menacho et al. (2007)	RBO from Brasil: (a) Crude (b) Refined	RP-HPLC with UV–Vis	(a) 0.106 (b) 0.098	–	–	–	Physico-chemical indices and fatty acid concentrations are also given
Heinemann et al., 2008	Bran from 32 genotypes of two varieties (Brazil): (a) Japonica (b) Indica	NP-HPLC	(a) 0.246 (b) 0.190	NP-HPLC	T + T_3: (a) 24.2 (b) 17.1	–	Antioxidant concentrations are used to classify japonica and indica rice
Balachandran et al. (2008)	RBO from parboiled rice (India)	RP-HPLC with UV–Vis	11.11 (SC–CO_2) 11.89 (hexane)	NP-HPLC with UV–Vis (CLC–NH_2 column)	α-T: 107	α-T_3/(β-T_3 + γ-T)-/δ-T_3: 120/261/1330	Extraction with SC–CO_2 compared to hexane. Concentrations of γ-oryzanol components, tocols, sterols and fatty acids are given
Zigoneanu et al. (2008)	Bran from Cypress rice (LA, USA)	–	–	NP-HPLC with fluorescence	α-/γ-T: 44–64/5–7 (I) 26–35/3–5 (H)	α-/γ-T_3: 8–54/38–44 (I) 9–184/17–24 (H)	Microwave-assisted extraction with isopropanol (I) and hexane (H) compared to conventional extraction at different temperatures
Chotimarkorn et al. (2008a)	Bran from five varieties (Thailand): (a) Kh. Dawk mali105 (b) Kh. Pathum Thani 60 (c) Kh. Suphan buri 90 (d) Kh. Chinat 1 (e) Kh. Gokho 13	RP-HPLC with UV–Vis	(a) 0.99 (b) 1.08 (c) 0.56 (d) 0.79 (e) 0.61	RP-HPLC with fluorescence	α-/β-/γ-/δ-T: (a) 330/210/100/30 (b) 380/25/130/10 (c) 120/80/120/30 (d) 230/180/60/10 (e) 280/200/80/40	α-/β-T_3: (a) 110/200 (b) 200/260 (c) 210/200 (d) 170/160 (e) 100/120	Extraction with methanol. Concentrations of total phenols and flavonoids are given
Imsanguan et al. (2008)	Bran from Suphanburi rice (Thailand)	RP-HPLC with UV–Vis	11.4 (SC–CO_2) 9.81 (ethanol)	RP-HPLC with UV–Vis	α-T:127 (SC–CO_2) 172 (hexane)		Extraction with SC–CO_2 in comparison to hexane and ethanol (maximal yields given in this table)

(*continued on next page*)

Table 1 (*continued*)

Reference	Samples	γ-Oryzanol		Tocopherols (T) and tocotrienols (T_3)			Comments, conclusions
		Method	Content (mg/g)	Method	Content (μg/g)	Content (μg/g)	
Lilitchan et al. (2008)	Bran from nine varieties (Thailand)	UV–Vis	3.67 (Soxhlet) 3.43 (partial extraction method)	–	–	–	Partial extraction method for the quick determination of total lipids and γ-oryzanol in bran. Results obtained with hexane are given in this table
Wang et al. (2008)	Rice from Taiwan: (a) Bran (b) RBO	RP-HPLC with UV–Vis	SC–CO_2/hexane: (a) 2.24–2.67/3.03 (b) 13.3–15.2/15.2	–	–	–	Extraction with SC–CO_2 in comparison to hexane. Concentrations of free fatty acids and triglycerides are also given
Chen et al. (2008)	RBO (Taiwan)	Semipreparative RP-HPLC with UV–Vis, and ^{1}H NMR	5.8–8.8	–	–	–	Extraction with SC–CO_2 in comparison to hexane. Concentrations of several γ-oryzanol components and fatty acids are given

bran samples (1.0 g) were weighed into two vials and extracted using different volumes (4 and 8 ml) of the same solvent. The solid residues were removed by centrifugation and the absorbance of the two supernatants was measured. Total lipids and γ-oryzanol were then quantified by solving two simultaneous equations, where the values of the two absorbances were given to the independent variable. In comparison to total extraction, the proposed procedure was simpler and quicker, and the consumption of solvents was largely reduced. The procedure also provided measurements of the solid/liquid adsorption coefficients of the solutes. About 92–95% of the lipids and 95–96% of the γ-oryzanol on nine rice varieties were extracted into *n*-hexane at a 10:1 (v/w) ratio of *n*-hexane to bran.

2.2. Chromatographic methods

Deepam, Kumar, Sundaresan, and Arumughan (2007) have developed a fast, simple, accurate, precise and sensitive HPTLC method with spectrophotometric detection for the simultaneous estimation of unsaponifiable components in RBO. The method involves a two-stage separation on a pre-coated silica gel 60 F_{254} TLC plate. The first TLC run separates phytosterols, γ-oryzanol and tocols, and the second one separates phytosteryl esters, wax and squalene. Recovery values ranged from 93.5% to 102%. The LODs of γ-oryzanol and tocols were 1 and 11 μg/ml, respectively. Repeatability was within the 0.30–1.18% range.

γ-Oryzanol in RBO was pre-separated and quantified by HPLC–UV, and its profile was established on-line by GC (Miller, Frenzel, Schmarr, & Engel, 2003). Identification of 24-methylenecycloartenyl *trans*-ferulate, campesteryl *trans*-ferulate, β-sitosteryl *trans*-ferulate and campestanyl *trans*-ferulate was confirmed by off-line GC–MS.

Fang et al. (2003) have used direct RBO analysis by RP-HPLC–MS/MS to address inconsistencies in the reported number and structures of ferulates. Nine novel phytosteryl esters were characterised by their chromatographic retention and both negative- and positive-ion mode ESI-MS. Evidence for the presence of hydroxylated ferulate esters and caffeate esters as part of γ-oryzanol was obtained. The method enabled rapid and direct on-line characterisation of phytosteryl esters in oils. Application to the identification and quantification of polar metabolites of phytosterols in biological fluids after oil consumption was suggested.

Several studies have shown the feasibility of modifying the total contents and profiles of tocols and γ-oryzanol in rice bran by breeding; however, rapid and reliable analytical methods are necessary to support breeding programs. Chen and Bergman (2005b) developed a simple, one-step equilibrium extraction procedure coupled with RP-HPLC. At a 1:60 (w/v) ratio of bran to solvent, isopropanol and methanol were better than *n*-hexane. Using methanol, 92–102% of the phytochemicals were recovered in a 1-min extraction. After gradient elution, tocols were detected by fluorescence and γ-oryzanol by UV–Vis spectrophotometry.

Stoggl et al. (2005) have compared the use of silica, C_{18} and C_{30} stationary phases for the RP-HPLC–UV separation of tocopherols, carotenoids and γ-oryzanol in a single run. The highest resolution was obtained with a C_{30} column. The elution strength of methanol was increased by the addition of *tert*-butyl methyl ether. On-line APCI-MS detection was also used.

Yu, Nehus, Badger, and Fang (2007) have used HPLC–UV–ESI-MS/MS for the rapid and direct identification and quantification of tocols and γ-oryzanol in rice bran and germ. The concentration of tocols in rice germ was found to be 5 times higher than in rice bran, whereas γ-oryzanol was 5 times more concentrated in rice bran. Also, the major tocol component was α-tocopherol in rice germ and γ-tocotrienol in rice bran. These data suggested that rice bran and germ have significantly different profiles of tocols and γ-oryzanol.

Foodstuff authentication according to geographical, botanical or varietal origin, or according to the history of the sample (including industrial processing), can be accomplished by application of multivariate supervised pattern recognition to analytical data. Marini, Balestrieri, Bucci, Magri, and Marini (2003) have used linear discriminant analysis and artificial neural networks to discriminate RBOs according to their geographical origin; RBOs manufactured in Italy, Thailand and Switzerland were included in the training set. The predictor variables, which were measured by GC–FID, were FAMEs and silylated phytosterols.

Finally, as far as we know, the atmospheric pressure photoionisation (APPI) interface, which yields very low limits of detection for low polarity compounds, has been not applied yet to RBO-related research. Free and esterified sterols were determined in human serum by HPLC-APPI (Lembcke et al., 2005).

2.3. Determination of antioxidant and antiradical activities

Using DPPH and chemiluminescence, Akiyama et al. (2001) studied the free radical-scavenging activities of the four major components of γ-oryzanol. The authors demonstrated that chemiluminescence was rapid and convenient for screening radical-scavenging activities of rice products. Xu and Godber (2001) studied the antioxidant activities of cycloartenyl, 24-methylenecycloartenyl and campesteryl *trans*-ferulates, α-tocopherol and free *trans*-ferulic acid. For this purpose, the hydroperoxide isomers of linoleic acid were measured using NP-HPLC–UV. All these compounds showed antioxidant activity.

The inhibition of cholesterol autoxidation by the non-saponifiable fraction of RBO was studied in an aqueous system (Kim, Godber, King, & Prinyawiwatkul, 2001). The loss of cholesterol, the formation of 7-ketocholesterol, and the changes in levels of tocols and γ-oryzanol, were monitored. Reductions of 92%, 82% and 64% 7-ketocholesterol were produced in the presence of 2100, 1400 and 700 μg RBO fraction/g, respectively. Without the non-saponifiable RBO fraction, the samples showed almost complete degradation of cholesterol. Tocol concentrations decreased during the treatments, but the γ-oryzanol concentrations remained the same.

In a comparative study, the highest antioxidant activity in the inhibition of cholesterol oxidation was found for 24-methylenecycloartenyl *trans*-ferulate, other γ-oryzanol components also having activities higher than those of the tocols (Xu, Hua, & Godber, 2001). Thus, γ-oryzanol may relevantly contribute to the potential hypocholesterolaemic property of rice bran (see also Section 4.1).

Using brown rice, Akiyama, Hori, Takahashi, and Yoshiki (2005) extracted the four major γ-oryzanol components, which were identified by NMR and MS. All four constituents and free *trans*-ferulic acid displayed similar scavenging activities for DPPH radicals. Thus, it was confirmed that the 4-hydroxyl group of *trans*-ferulic acid was the active unit for DPPH radical-scavenging activity. Similarly, Nystrom, Makinen, Lampi, and Piironen (2005) studied the antioxidant activity of phytosteryl ferulate extracts from wheat and rye bran. Their capability to inhibit hydroperoxide formation in bulk methyl linoleate and methyl linoleate emulsion, and their activity to scavenge DPPH radicals, was analysed. The activities were compared to those of synthetic steryl ferulates, rice phytosteryl ferulates, *trans*-ferulic acid, and α-tocopherol. The phytosteryl ferulate extracts exhibited good antioxidant activity, especially in the bulk lipid system. The radical-scavenging activity was similar to that of *trans*-ferulic acid, indicating that the acid moiety is responsible for the antioxidant properties.

Devi and Arumughan (2007) have evaluated the antioxidant potential of extracts of defatted rice bran. Sequential extractions with methanol, acetone and *n*-hexane, and again with acetone, were performed. The antioxidant activities of the extracts, as estimated by the Schaal oven stability test, were compared to those of BHT and TBHQ. All the extracts were effective in inhibiting lipid oxidation, as assessed by DPPH and other methods. The extracts proved to be effective even at the high temperatures employed in differential scanning calorimetry.

Chotimarkorn, Benjakul, and Silalai (2008a) have investigated the antioxidant activity and phytochemical contents in five varieties of long-grained rice bran, which are the most commonly cultivated varieties in Thailand. The antioxidant activity of methanolic rice bran extracts was investigated by using DPPH, ferrous ion and lipid peroxidation inhibition. The total phenolic, flavonoid, γ-oryzanol and tocol contents of the extracts were determined by colouimetric assays and HPLC.

2.4. Influence of genetic and environmental factors

The effects of genetics *vs.* environment on the tocols and γ-oryzanol contents of southern US rice, and associations between the levels of these phytochemicals, have been quantitatively studied (Bergman & Xu, 2003). In general, growing environment had a greater effect on tocols and γ-oryzanol levels than genotype. Therefore, rice breeders selecting genotypes with optimised levels of tocols and γ-oryzanol will need to grow their breeding material across multiple years and locations.

The tocols, γ-oryzanol and fatty acid composition of Basmati and Jaya rice, as well as parboiled rice, were investigated (Khatoon & Gopalakrishna, 2004). Parboiling of paddy rice affected the tocol contents adversely, but the γ-oryzanol contents remained unchanged. In comparison to Jaya brown rice, Basmati brown rice had higher tocol contents and lower γ-oryzanol contents.

Anwar, Anwer, and Mahmood (2005) have investigated the contents of γ-oryzanol and tocols in four rice varieties from Pakistan. The major phytosterol fractions of the oils were found to consist of campesterol (10–19%), stigmasterol (14–19%), β-sitosterol (49–58%), and Δ^5-avenasterol (8–13%). Iqbal, Bhanger, and Anwar (2005) evaluated the antioxidant activity of five rice bran varieties of Pakistan. The total phenolic content, antioxidant activity in linoleic acid system, reducing power, metal-chelating ability, and scavenging capacity by DPPH and ABTS radicals and conjugated dienes, were measured. The tocols and γ-oryzanol contents were determined by RP-HPLC. The antioxidant power was correlated with growth period and irrigation water demand, according to the variety. Super Kernel which, in comparison to other varieties, had the longest growth period and took the lowest amount of water, also exhibited the highest antioxidant activity, the reverse behaviour being observed for Super Fine rice.

Miller and Engel (2006) used on-line coupled HPLC–GC to determine total γ-oryzanol and the composition of phytosteryl ferulates in 30 European cultivars of brown rice grown at different sites and in different seasons. Cycloartenyl *trans*-ferulate and 24-methylenecycloartenyl *trans*-ferulate were the major γ-oryzanol components, followed by campesteryl *trans*-ferulate, campestenyl *trans*-ferulate and β-sitosteryl *trans*-ferulate. The proportions of the individual phytosteryl ferulates showed an enormous variability. However, irrespective of the great variations observed for single phytosteryl ferulates, the proportions of the sum of 4,4′-dimethylsteryl ferulates (cycloartenyl *trans*-ferulate and 24-methylenecycloartenyl *trans*-ferulate) and the sum of 4-desmethylsteryl ferulates (campesteryl *trans*-ferulate, campestenyl *trans*-ferulate and β-sitosteryl *trans*-ferulate) varied little. The natural variability observed for γ-oryzanol contents and phytosteryl ferulate distributions were shown to be influenced by environmental conditions, but not by the degree of maturity of rice grains.

Krishna, Hemakumar, and Khatoon (2006) have compared the physical properties and composition of refined RBO with those of

groundnut, sunflower and safflower refined oils. Refined RBO showed the highest values of colour intensity (7.6–15.5 *vs.* 1.5–2.0 Lovibond units for the other oils), unsaponifiable matter (2.5–3.2% *vs.* 0.15–1.40%), tocopherols (480–700 mg/kg *vs.* 300–600 mg/kg) and free fatty acids (0.14–0.55% *vs.* 0.05–0.10%). Among the four oils, only RBO contained γ-oryzanol.

Britz et al. (2007) have evaluated the impact of temperature on the accumulation of tocols and γ-oryzanol. Seeds from six rice lines grown in greenhouses were analysed. Temperatures were maintained near ambient at one end of each greenhouse, and 4.5 °C above ambient at the other end. γ-Oryzanol and tocols were extracted from brown rice and analysed by HPLC–MS. Tocols varied widely between lines, but changed only slightly with respect to temperature. In general, the proportions of α-tocotrienol and/or α-tocopherol increased at elevated temperature, whereas γ-tocopherol and γ-tocotrienol decreased. The most abundant γ-oryzanol component was 24-methylenecycloartenyl *trans*-ferulate (40–62% of total). Its levels increased 35–57% at elevated temperature, thus suggesting that the physiological action of individual ferulated phytosterols should be investigated, because their relative proportions in γ-oryzanol can change with temperature during the growing season.

Aguilar-Garcia, Gavino, Baragano-Mosqueda, Hevia, and Gavino (2007) have evaluated the relationship between antioxidant capacity and levels of various antioxidants in rice bran and brown rice powder. The antioxidant capacity of three different varieties of Venezuelan rice was measured using FRAP, ABTS and ORAC. Principal component analysis and multiple regression indicated that FRAP was sensitive to polyphenols and total tocotrienols, while ORAC was sensitive to polyphenols and total tocopherols. ABTS was the least sensitive of all assays tested. Thus, results from antioxidant capacity assays must be interpreted with caution, particularly in complex systems, further study being necessary to define more precisely the nature of the chemical reactions involved.

The total antioxidant capacity and chemical composition of tocols, γ-oryzanol and polyphenols of white rice and the less common red rice were compared (Finocchiaro et al., 2007). The effect of milling and cooking on the antioxidants was also investigated. Dehulled red rice showed an antioxidant capacity more than three times greater than dehulled white rice. This capacity was essentially characterised by the presence of proanthocyanidins and associated phenolics. Milling caused a significant loss of antioxidant capacity, although red rice maintained higher values. Cooking caused a further loss of antioxidants, but when there was a full uptake of cooking water by the grains (''risotto'') this loss was limited. Thus, the consumption of whole or partially milled rice cooked as risotto would be preferred to preserve its nutritional properties.

Heinemann, Xu, Godber, and Lanfer-Marquez (2008) have compared the concentrations of tocols and γ-oryzanol in 32 genotypes of the *indica* and *japonica* subspecies of brown rice. The aim of the study was the development of a method to distinguish between these two subspecies. The compounds were analysed by NP-HPLC. The mean content of γ-oryzanol and tocols across all samples was higher in *japonica* than in *indica* rice. Further, in *japonica* rice, α-tocopherol, α-tocotrienol and γ-tocotrienol were the most abundant tocols, while in *indica* rice the most abundant were γ-tocotrienol, α-tocopherol and α-tocotrienol. Both α-tocopherol and α-tocotrienol levels were positively correlated between themselves and with respect to total tocol and γ-oryzanol contents.

3. Industrial processing of rice bran, in order to obtain phytochemical-rich fractions

γ-Oryzanol is an important value-added co-product of rice bran processing. Therefore, research to improve the recovery of γ-oryzanol and other phytochemicals, in order to obtain fractions enriched in a particular compound or group of compounds, has been conducted. Within this concern, particular attention has been paid to brown rice milling and RBO extraction and refining by either physical or chemical techniques. Relatively novel procedures involve the use of SC–CO_2, subcritical water and enzymes.

3.1. Influence of milling

Rice bran has been traditionally processed as a homogenous material; however, it has been shown that high-value components in the rice bran layer vary according to kernel thickness, bran fraction, rice variety, and environmental conditions during the growing season (Schramm, Abadie, Hua, Xu, & Lima, 2007). Thus, current rice milling technology produces rice bran from different layers of the kernel caryopsis. The phytochemical contents of each of these fractions can vary widely, the γ-oryzanol concentration being higher in the outer bran layers (Lloyd et al., 2000). Rice bran fractionation is advantageous for two reasons: (1) some fractions contain higher concentrations of components of interest, with respect to the overall bran layer average, and (2) less bran needs to be processed to obtain components of interest (Schramm et al., 2007).

Rohrer and Siebenmorgen (2004) have collected information regarding nutraceutical concentrations in bran obtained after successive milling stages. In this way, nutraceutical concentrations across several different thickness fractions were established. Thus, bran collected from rice milled for longer durations (30 s) had lower levels of tocols compared to bran from shorter milling durations (10 s). The highest concentration of γ-oryzanol was in the rice bran from the first 10 s milling duration.

Chen and Bergman (2005a) have studied the influence of the milling degree using rough rice from different cultivars. The differences in bran removal among cultivars decreased as milling time increased, thus, samples that were milled for 30–40 s showed no differences in the concentrations of tocols. Also, immature thin kernels had significantly lower contents of phytochemicals than the mature ones.

According to Ha et al. (2006), the lipid, tocols, γ-oryzanol, squalene and octacosanol contents of both brown and milled rice decreased as the degree of milling increased; however, the phytosterol profile remained the same, β-sitosterol being the most abundant (50–56% of total phytosterols). Milled and brown rice showed also differences in the relative percentages of α-tocopherol, and α- and γ-tocotrienols.

Schramm et al. (2007) have quantified the amounts of rice bran removed at milling times between 3 and 40 s, and have correlated those amounts with the concentrations of tocols, γ-oryzanol, saccharides and proteins. The highest γ-oryzanol and protein concentrations were found in the outer portion of the bran layer, while the highest saccharide concentration was found in the inner portion. Thus, to extract high γ-oryzanol and protein concentrations, only the outer portion of the bran layer required processing, whereas to extract high concentrations of rice bran saccharides, only the inner portion should be processed. The concentrations of tocols showed no difference across the bran layer, though the highest concentration occurred within the first 10 s milling.

3.2. Chemical and physical refining of rice bran oil

γ-Oryzanol and most other phytochemicals are largely lipophilic, and thus are extracted with the RBO; however, differently from tocols, γ-oryzanol is transferred to soapstock during the neutralisation step of chemical refining of RBO (Narayan, Barhate, & Raghavarao, 2006). Alternatives to conventional chemical refining, including physical refining and the use of membrane technology (Manjula & Subramanian, 2008) have been proposed. The bran pro-

cessing techniques for the production of quality RBO with low loss of phytochemicals have been reviewed (Ghosh, 2007).

The isolation of γ-oryzanol, and the effect that impurities have on its extraction and purification, in relation to industrial unit operations, have been reviewed (Narayan et al., 2006). Solid–liquid, liquid–liquid, crystallisation and precipitation methods were discussed. Problems related to prior processing and compositional variation in soapstock, resistance to mass transfer, moisture content and the presence of surface-active components, which cause emulsion formation, were examined. Emphasis was placed on engineering inputs required for solving problems such as saponification, increasing mass transfer area, and drying methods. Those processes having the potential to work on a large scale were presented.

Rodrigues, Filho, and Meirelles (2004) have studied the distribution equilibria for liquid–liquid systems containing RBO, free fatty acids, ethanol, water, γ-oryzanol and tocols at 298.2 K. Model systems were used for adjusting NRTL and UNIQUAC interaction parameters between γ-oryzanol and the other compounds. UNIQUAC interaction parameters between tocols and the other components were also determined. The interaction parameters obtained were capable of correctly predicting the equilibrium for systems containing crude RBO and aqueous ethanol.

The effect of different processing steps on retention of γ-oryzanol in refined RBO, and the γ-oryzanol composition of 18 Indian cultivars and commercial products, were investigated (Krishna et al., 2001). Degumming, dewaxing and alkali treatment of RBO removed 1.1%, 5.9% and 93.0–94.6% γ-oryzanol, respectively. Physical refining reduced the γ-oryzanol content of the oil from 1.63–2.72% to 1.1–1.74%, whereas alkali-refined oil contained 0.19–0.20%. Bleaching and deodorisation did not affect the content of γ-oryzanol appreciably. Krishna, Prashanth, Pragasam, Raghavendra, and Khatoon (2003) further investigated the content and composition of unsaponifiable matter in refined RBO produced in India. Chemically and physically refined RBO contained 2.6% and 4.5% unsaponifiable matter, respectively.

In a patented procedure (Indira et al., 2004), a γ-oryzanol-enriched fraction is obtained by saponification of a hot dispersion of RBO soapstock with sodium hydroxide, followed by dehydration, leaching with ethyl acetate and/or acetone in a packed bed, decanting the leached extract, and dissolving the decanted extract. Also in a patent filed (Kim et al., 2004–2005), γ-oryzanol is extracted from RBO with isopropanol. The procedure was applied to the production of mayonnaise, whose oxidation was prevented by the presence of γ-oryzanol.

High-purity γ-oryzanol can be obtained by crystallisation (Du, Zhang, & Feng, 2004; Zullaikah, Melwita, & Ju, 2008). Also, Lai, Hsieh, and Chang (2005) developed an efficient chromatographic separation method for the production of pure γ-oryzanol. First, crude RBO was obtained by Soxhlet extraction. Then, γ-oryzanol was purified by preparative NP-HPLC on a silica gel packed column. The three-step gradient elution was carried out with *n*-hexane/ethyl acetate mobile phases. A >90% γ-oryzanol recovery, with a 90–98% purity, was achieved, but the productivity was only about 10 mg per injection. Hence, further scaling-up work was needed.

Yu et al. (2006) investigated the independent and sequential fractionations of RBO with acetone at low temperatures. The aim was to prepare oil fractions enriched in unsaturated fatty acids (UFAs). The liquid fraction from independent fractionation at −35 °C was the best (89.6% UFAs). However, when weight yield was considered, the largest amount of UFAs was obtained by sequential fractionation at 0 °C (87.5 wt% recovered in a fraction containing 71.5% UFAs). As the fractionation temperature was lowered, the content of γ-oryzanol in the liquid fractions gradually increased.

Van Hoed et al. (2006) examined the effects of each individual step of chemical refining of RBO on its major and minor components. Large γ-oryzanol losses and a change in the individual phytosterol composition were produced by either alkalinisation or neutralisation. After bleaching, some isomers of 24-methylenecycloartanol were detected. Due to their relatively high volatility, free phytosterols and tocotrienols were stripped off from RBO during deodorisation, and thus concentrated in the deodoriser distillate, but the RBO γ-oryzanol concentration did not change upon deodorisation.

Rodrigues, Onoyama, and Meirelles (2006) studied the influence of RBO deacidification by liquid–liquid extraction on the losses/transfer of fatty compounds. The aim was to maximise the transfer of free fatty acids and minimise the losses of neutral oil plus minor compounds. Multivariate non-linear regression was used to optimise the operation conditions. Also, the UNIQUAC equation was used to predict the transfer/losses of fatty and nutraceutical compounds to the alcohol phase.

To preserve γ-oryzanol, a physical RBO refining technique was proposed (Paucar-Menacho, da Silva, Santana, & Goncalves, 2007). Inactivated and extruded rice bran obtained by the production of parboiled rice was used to extract crude RBO by the expeller method. Refining consisted of acid degumming (with 85% H_3PO_4), centrifugation, clarification, deodorisation, and winterisation. 97% of the γ-oryzanol was preserved by the proposed procedure, and its presence improved the oxidative stability of the oil, compared with that of commercial RBO.

Zigoneanu, Williams, Xu, and Sabliov (2008) have investigated the microwave-assisted extraction of rice bran with isopropanol and *n*-hexane at increasing temperatures. The increase in tocols with temperature, from 40 to 120 °C, was 59.6% for isopropanol and 342% for *n*-hexane; however, isopropanol was better than *n*-hexane for the extraction of γ-tocopherol and γ-tocotrienol, also leading to higher oil yields at high temperatures. Further, fractions extracted with isopropanol at 120 °C had the highest antioxidant activity. Also, Cravotto, Binello, Merizzi, and Avogadro (2004) investigated rice bran extraction using high-intensity ultrasound. Upon sonication, bran wax was hydrolysed, yielding policosanol (a mixture of C_{24}–C_{34} linear fatty alcohols). Li, Pordesimo, Weiss, and Wilhelm (2004) used both ultrasound and microwaves to enhance oil extraction yields from soybeans. As far as we know, similar approaches have not yet been applied to rice bran.

γ-Oryzanol enrichment in RBO was attempted using hydrophobic polymeric membranes (Manjula & Subramanian, 2008). Owing to partial rejection of γ-oryzanol by the membranes, enrichments higher than 300% and 50% were achieved with refined and crude RBO, respectively. Processing of crude and model RBO yielded *ca.* 50% enrichments. Oil dilution with *n*-hexane largely improved the oil flux, but reduced selectivity.

3.3. Extraction with supercritical–CO_2

Owing to the low viscosity and high diffusivity of SC fluids, highly efficient extraction procedures can be developed. Further, from the environmental viewpoint, SC–CO_2 is much better than organic solvents. Xu and Godber (2000), compared liquid organic solvents with SC–CO_2 relative to efficiency for extracting lipids and γ-oryzanol from rice bran. Among the solvents tested, a 50:50 *n*-hexane/isopropanol mixture at 60 °C for 45–60 min produced the highest γ-oryzanol yield. Without previous saponification, the yield of γ-oryzanol was approximately two times higher than that with saponification. However, using SC–CO_2 the yield of γ-oryzanol was approximately four times higher than the highest yield obtained by extraction with liquid organic solvents.

Several aspects of industrial RBO fractionation with SC–CO_2 were investigated (Dunford & King, 2000, 2001; Dunford, Teel,

& King, 2003). First, SC–CO_2 fractionation was evaluated as a means to reduce the free fatty acid content and to minimise phytosterol losses (Dunford & King, 2000). Low-pressure and high-temperature isothermal conditions were found to be favourable for minimising triglycerides and phytosterol losses during the free fatty acid removal from crude RBO. Second, application of a temperature gradient along the column was found to be beneficial in reducing the triacylglycerol lost in the extract (Dunford & King, 2001). The use of a high temperature in the stripping section improved free fatty acid removal from crude RBO, while increasing the SC–CO_2 flow rate did not affect the extract composition. RBO fractions with total phytosterol ester contents similar to that of commercially available phytosterol ester-enriched margarines/spreads were obtained.

Finally, the potential of continuous countercurrent SC–CO_2 fractionation for deacidification of crude RBO was examined (Dunford et al., 2003). A pilot-scale packed column was used. It was shown that fractionation at low pressure and high temperature effectively removed free fatty acids from crude RBO without any γ-oryzanol loss in the extract. γ-Oryzanol content of the refined fraction was three times higher than that of the feed material. Phytosterol ester content of the refined fraction also increased. Danielski et al. (2005) also used SC–CO_2, both to extract RBO from rice bran, and to deacidify oil in a countercurrent column. RBO with <1% free fatty acid was obtained.

An integrated approach to extraction and refining of RBO using SC–CO_2, designed to preserve the phytochemicals, has been recently reported (Balachandran et al., 2008). Using a pilot model system, the RBO extraction yield increased with temperature and time, and was also favoured by the presence of structured stainless streel rings. Under optimum conditions, the yield was comparable with that obtained with *n*-hexane (22.5%). The RBO had negligible phosphatides, wax and oxidation-promoting metal ions, and was far superior in colour quality when compared with RBO extracted with *n*-hexane.

Also using SC–CO_2 extraction of γ-oryzanol from powdered rice bran, Wang et al. (2008) obtained an 18.1% RBO yield. The extraction efficiencies of γ-oryzanol and triglycerides were 88.5% and 91.3%, respectively. The concentration factors in the resulting oil were higher than using Soxhlet extraction with *n*-hexane. Pressure was more effective than temperature in enhancing the extraction efficiency and concentration factor of γ-oryzanol. Response surface methodology was employed to determine the optimal pressure (30 MPa) and temperature (40 °C) for increasing the γ-oryzanol concentration in the extracted oil.

According to Imsanguan et al. (2008) the best conditions for α-tocopherol extraction were batch + continuous operation at 48 MPa and 55 °C, whereas for γ-oryzanol the best conditions were continuous extraction at 48 MPa and 65 °C. SC–CO_2 provided higher yields and rates for extracting both α-tocopherol and γ-oryzanol than extraction with *n*-hexane and ethanol. Finally, Chen et al. (2008) have studied the deacidification of RBO by using SC–CO_2 at both laboratory and pilot-scale.

3.4. Extraction with subcritical water

At temperatures and pressures close to that of its critical point (374 °C, 22 MPa), water behaves like a highly hydrophobic solvent, thus being useful to extract lipophilic substances from solid and semi-solid matrices. After cooling and depressurising, a lipophilic and a hydrophobic fraction can be obtained. High recoveries in extraction times much shorter than using SC–CO_2 can be achieved. Today, however, subcritical water extraction for rice bran processing has been still scarcely investigated, and as far as we know, scaled-up processes of industrial interest have not yet been described.

Wiboonsirikul, Hata, Tsuno, Kimura, and Adachi (2007) have treated black rice bran with water and subcritical water up to 260 °C. The extracts were analysed for their radical-scavenging activity, protein and carbohydrate contents, molecular–mass distribution, antioxidant activity, emulsifying activity, and emulsion-stabilising activity. The radical-scavenging activity and the protein content of the extracts increased with the extraction temperature. The carbohydrate content also increased at increasing temperatures up to 200 °C, and steeply decreased at higher temperatures. The extracts obtained at 260 °C exhibited a suppressive activity toward auto-oxidation of linoleic acid. The extracts prepared at 40–200 °C showed emulsifying and emulsion-stabilising activities.

Hata, Wiboonsirikul, Maedab, Kimura, and Adachi (2008) have also treated defatted rice bran with subcritical water in the 180–280 °C range. The total sugar concentration was the highest for the extracts at 200 °C. The protein concentration and radical-scavenging activity increased at increasing temperatures. The extracts obtained below 200 °C showed emulsifying and emulsion-stabilising activities.

3.5. Enzymatic processing techniques

The use of enzymes in rice bran processing, including enzymes specifically modified by genetic engineering, is still today a new and relatively unexplored technology. Potential applications are the development of improved food products and novel products of pharmaceutical interest. A processing technology to polish rice in a selective way with the help of xylanases and cellulases has been developed (Das, Banerjee, & Bal, 2008a; Das, Gupta, Kapoor, Banerjee, & Bal, 2008b). Enzymes produced by *Aspergillus* sp. and *Trichoderma* sp. acted upon the non-starch polysaccharides of the bran layers of moistened brown rice, releasing their monomeric sugar constituents, as detected through HPLC. Surface degradation of the rice grain was also studied by scanning electron microscopy (Das et al., 2008b). Selective degradation of bran layers facilitated the retention of phytochemicals. Antioxidant activity followed the order brown rice > enzyme-treated rice > milled rice. In comparison to mechanically-milled rice, bio-polished rice had better cooking attributes and higher antioxidant concentrations (Das et al., 2008a).

Jahani, Alizadeh, Pirozifard, and Qudsevali (2008) have used response surface methodology to determine the optimum processing conditions for enzymatic degumming of RBO. Reaction time, enzyme dosage, water content and temperature were investigated with respect to phosphorus and free fatty acid contents.

4. Biomedical applications

4.1. Hypocholesterolaemic–hypolipidaemic activity

Cholesterol is transported in the blood plasma of all animals by lipoproteins, which have a wide range of molecular sizes, including VLDL, IDL, LDL and HDL. Hyperlipoproteinaemias are heritable disorders associated with increased plasma concentrations of cholesterol, higher LDL–cholesterol than normal, implying a high risk of cardiovascular disease. The first (and sometimes the only) therapeutic approach to hyperlipoproteinaemias is diet. In this concern, RBO and its main components have demonstrated an ability to improve the plasma lipid pattern of rodents, rabbits, non-human primates and humans, reducing total plasma cholesterol and triglyceride concentrations, and increasing the HDL cholesterol level. Other potential properties of RBO and γ-oryzanol, studied both in *in vitro* and in animal models, include modulation of pituitary secretion, inhibition of gastric acid secretion, antioxidant action

and inhibition of platelet aggregation. The pharmacology and toxicology of RBO and its main components, with particular attention to plasma lipid altering effects, were reviewed by Cicero and Gaddi (2001).

Using monkeys, Wilson, Ausman, Lawton, Hegsted, and Nicolosi (2000) studied the effects of different unsaturated vegetable oils on serum lipoprotein levels. An RBO diet produced similar reductions in serum total cholesterol (−25%) and LDL cholesterol (−30%). Further, these reductions were not accompanied by reductions in HDL cholesterol, as occurred with canola and corn oil diets. The lowering capabilities of the RBO diet exceeded those predicted from the fatty acid composition of RBO. Despite the lower contents in saturated fatty acids and higher contents in monounsaturated fatty acids of canola oil, and the higher contents of corn oil in PUFAs, RBO exhibited comparable cholesterol-lowering properties. This was attributed to the greater content in RBO of unsaponifiables.

Tsuji, Takahashi, Kinoshita, Tanaka, and Tsuji (2003) studied the effects of different contents of γ-oryzanol in RBO on serum cholesterol levels of rats fed with a hypocholesterolaemic diet (HCD). Dietary lipid sources were lard in the control group, and normal RBO, with either low or high levels of γ-oryzanol, in the test groups. Serum total cholesterol levels increased in the control group, and clearly decreased in the RBO-fed groups.

The effects of policosanols and phytosterols on lipid profiles, cholesterol biosynthesis, and tissue histopathological changes in hamsters were studied (Wang, Jones, Pischel, & Fairow, 2003). Hamsters were given diets supplemented with Octa-6 (a policosanol mixture from sugar cane), Ricewax (a policosanol mixture from rice wax), Cholestatin (a nutritional supplement containing mainly β-sitosterol, campesterol and stigmasterol), and Ricewax plus Cholestatin. Phytosterols reduced both total and HDL cholesterols without a significant effect on triglycerides and non-HDL cholesterol, as compared to the control. Ricewax plus phytosterols had effects similar to those with phytosterols alone. The inconsistency between this and previous investigations was attributed to multiple factors, including the particular lipid metabolism of hamsters and the formula/delivery form of policosanols.

Wilson, Nicolosi, Woolfrey, and Kritchevsky (2007) also conducted a study using hamsters, to separately establish the relative cholesterol-lowering activities of RBO, γ-oryzanol and *trans*-ferulic acid. Hamsters were fed with an HCD for 2 weeks, at which time they were bled and segregated into groups with similar plasma cholesterol concentrations. Group I (control) continued on the HCD, group 2 was fed with the HCD containing 10% RBO, group 3 was fed the HCD plus 0.5% *trans*-ferulic acid, and group 4 was fed with the HCD plus 0.5% γ-oryzanol. After 10 weeks on the diets, plasma total cholesterol and VLDL + LDL cholesterol concentrations, were significantly lower in the groups fed with the RBO (−64% and −70%, respectively), *trans*-ferulic acid (−22% and −24%, respectively) and γ-oryzanol (−70% and −77%, respectively) diets, compared to the control. The levels of plasma triglycerides, lipid hydroperoxides and aortic cholesterol ester accumulation were also much lower in the groups fed with the RBO and γ-oryzanol diets. On the other hand, hamsters fed with the control and *trans*-ferulic acid diets had higher plasma tocol concentrations, compared to the RBO and γ-oryzanol diets. The study suggested that γ-oryzanol has a greater effect on lowering plasma VLDL + LDL cholesterol levels, and raising plasma HDL cholesterol, than *trans*-ferulic acid, possibly through an increase of faecal excretion of cholesterol and its metabolites. However, *trans*-ferulic acid may have a greater antioxidant capacity *via* its ability to maintain serum tocol levels.

Accinni et al. (2006) assessed the effects and advantages of a combined dietary supplement with omega-3 PUFAs, tocols, niacin and γ-oryzanol on lipid profile, inflammatory status and oxidative balance. Dyslipidaemic volunteers were randomly assigned to receive placebo (**A**), omega-3 PUFA and tocols (**B**), and the same as **B** plus γ-oryzanol and niacin (**C**). Lipid profile, reactive oxygen species, total antioxidant capacity, tocols, interleukin, tumour necrosis factor and thromboxane B_2 were determined. At baseline, all dyslipidaemic subjects showed oxidative stress. After four months, all biochemical markers improved in groups treated with dietary supplementation, particularly in group **C**.

4.2. Anti-inflammatory activity and miscellaneous biomedical applications

Using methanol extracts of RBO, Akihisa et al. (2000) isolated six novel ferulates of phytosterols, namely, the *trans*-ferulates of cycloeucalenol and 24-methylenecholesterol, and the *cis*-ferulates of cycloartenol, 24-methylenecycloartanol, 24-methylcholesterol and sitosterol. These compounds, jointly with the already known *trans*-ferulates of cycloartenol, 24-methylene-cycloartanol, 24-methylcholesterol, sitosterol and stigmastanol, and eight other synthetic *trans*- and *cis*-ferulates of phytosterols, along with the corresponding free alcohols, were evaluated with respect to their anti-inflammatory activity against induced inflammation in mice. All the ferulates plus cycloartenol and 24-methylenecycloartanol showed marked inhibitory activity.

More recently, Islam et al. (2008) have investigated the anti-inflammatory effects of γ-oryzanol, cycloartenyl *trans*-ferulate and *trans*-ferulic acid (as a possible metabolite of γ-oryzanol *in vivo*). Severe colitis was induced in mice, and a series of indices were monitored. Both γ-oryzanol and cycloartenyl *trans*-ferulate markedly inhibited the inflammatory reactions. Thus, phytosteryl ferulates could be effective as therapeutic and/or preventive agents for gastrointestinal inflammatory diseases. Further studies on the mechanism of the ameliorative effect of γ-oryzanol on intestinal inflammation have been reported (Hori et al., 2008).

Oxidative stress is considered to be a key factor in the development of diabetes and its complications. Kanaya et al. (2004) have examined the antioxidative effects of a crude lipophilic rice bran extract, Ricetrienol (Tsuno Rice Fine Chemicals, Wakayama, Japan), in obese diabetic and non-diabetic mice. Ricetrienol contains phytosterols (15%), tocotrienols (15%), tocopherols (3%), squalene (8%) and other compounds. A number of biochemical parameters in plasma, kidney and urine were measured. The results suggested that Ricetrienol exerts a protective effect against oxidative damage in diabetes mellitus.

Chotimarkorn and Ushio (2008) have investigated the effects of the oral administration of *trans*-ferulic acid and γ-oryzanol to mice with ethanol-induced liver injury. Prevention of liver injury was reflected in decreases of activities of plasma aspartate aminotransferase and alanine aminotransferase, and hepatic lipid hydroperoxide and TBARS levels. Mice treated with *trans*-ferulic acid and γ-oryzanol also recovered from the decrease in hepatic glutathione level together with enhanced superoxide dismutase activity. Thus, both *trans*-ferulic acid and γ-oryzanol exerted a protective action on liver injury induced by chronic ethanol ingestion.

Luo, Li, Yu, Badger, and Fang (2005) extracted three novel phytosteryl *trans*-ferulates from rice bran. Two of them, as well as cycloartenyl *trans*-ferulate and 24-methylenecycloartenyl *trans*-ferulate, showed moderate cytotoxicity against human breast adenocarcinoma MCF-7 cells. Parrado et al. (2006) described the production and stabilisation of a water-soluble extract obtained by enzymatic treatment of rice bran. The physicochemical composition and biological properties of the extract, including the anti-proliferative activity on cancer cells, were studied. The extract contained peptides and free amino acids, fat and γ-oryzanol. Preliminary studies on the anti-proliferative effect on leukaemia tumour cell growth *in vitro* were reported.

5. Industrial applications

5.1. Stabilisation of fats, frying oils and fried products

The stabilising effects of RBO on fats and oils are mainly due to the antioxidant and radical scavenging properties of γ-oryzanol. These effects are important in relation to both the traditional use of RBO in many Asian countries as a cooking and salad oil (Ghosh, 2007), and the manufacturing of industrial products of improved stability. Normally, less stable liquid oils are hydrogenated to enhance their oxidative stability for deep-fat frying purposes; however, considerable amounts of nutritionally undesirable *trans* and positional isomer fatty acids are formed during hydrogenation. An alternative to hydrogenation is blending with polyunsaturated oils containing natural antioxidants, such as virgin olive and sesame seed oils, and RBO; the stability of snacks fried in these oils substantially increased (Kochhar, 2000).

To assess oil stability, the polymeric triglycerides formed at frying temperatures were determined by size-exclusion HPLC (Gertz, Klostermann, & Kochhar, 2000). Furthermore, the stabilising effects of tocopherols, tocopherol esters, phytosterol fractions, γ-oryzanol, *trans*-ferulic acid, and other natural and synthetic antioxidants when added to the oils were measured. Ascorbic acid 6-palmitate and some phytosterol fractions showed the greatest antioxidant activity. Also, non-refined oils were more stable at elevated temperature than refined oils. A relationship between the unsaponifiable matter content and oxidative stability of the oil was shown. A radical peroxidation mechanism was hypothesised to predominate at lower temperatures; however, when a large volume of oil is heated and the oxygen supply is poor, non-radical reactions such as acid-catalysed dehydration or nucleophilic substitution may take place.

Compositional changes of rice germ oils prepared at different roasting temperatures and times were evaluated and compared with those of unroasted oil (Kim et al., 2002). The colour, phosphorus content, and α- and γ-tocopherol levels increased as roasting temperature and time increased, whereas the fatty acid and γ-oryzanol concentrations did not change.

The antioxidant effects of RBO and partially hydrogenated soybean oil, when used in the preparation of French fries, were investigated (Abidi & Rennick, 2003). Polar fractions of the three oils were analysed for non-volatile components by size-exclusion HPLC with ELSD. Thermal degradation *via* hydrolysis and the extent of polymer formation were much greater in partially hydrogenated soybean oil than in RBO. The amounts of various polymeric species, including trimers and higher polymers, were lower in high-oryzanol RBO than in RBO.

To investigate the molecular mechanism(s) of the antioxidant activity of γ-oryzanol, scavenging of DPPH**.**, OH**.** and O_2**.**-radicals, and 2,2′-azobis(2,4-dimethylvaleronitrile)-initiated lipid peroxidation, were used (Juliano, Cossu, Alamanni, & Piu, 2005). The effect of γ-oryzanol on the oxidative stability of vegetable oils was evaluated in an accelerated oxidation test, and compared with the effects of BHA and BHT. The results demonstrated that γ-oryzanol is an organic radical scavenger able to prevent lipoperoxidation. Moreover, γ-oryzanol showed a dose-dependent increase of the induction times; in particular, it improved the oxidative stability of oils with high contents of PUFAs, which are prone to peroxidation.

Sunflower, groundnut and mustard oils, and palm olein, were fortified with both RBO and crude sesame oil, and the stability of the mixtures was ascertained by deep-frying potato bhaji (potato slices sandwiched with Bengal gram flour) (Nasirullah & Rangaswamy, 2005). Absorption of fat by the moist product made the oil media vulnerable to oxidation. The *p*-anisidine values indicated the higher stability of fortified palm olein. Hydroperoxide and conjugated dienes were assessed by UV at 230 nm. The absorbance of both leftovers and oil absorbed by the product decreased in the order sunflower > groundnut > mustard = palm olein blends. No losses of γ-oryzanol and sesamol (3,4-methylenedioxyphenol) produced by deep-frying were observed.

The capacity of sitostenyl *trans*-ferulate and α-tocopherol to prevent polymerisation of high oleic sunflower oil at frying temperatures was studied (Nystrom, Achrenius, Lampi, Moreau, & Piironen, 2007). Both antioxidants reduced polymer formation, though no synergistic effect was demonstrated. In addition, sitostenyl *trans*-ferulate was degraded at a lower rate than α-tocopherol.

The effect of γ-oryzanol microencapsulation on the stability against heat-induced lipid oxidation was studied (Suh, Yoo, & Lee, 2007). Using TBARS, lard treated with microencapsulated γ-oryzanol displayed greater oxidative stability than lard treated with γ-oryzanol. During heating, a substantially larger amount of γ-oryzanol remained in the treated lard as well. Apparently, microencapsulation could be used to protect γ-oryzanol from the heat-induced loss of its antioxidant effect.

The effects of the addition of methanolic extracts of crude long-grain rice bran to tuna oil were studied (Chotimarkorn, Benjakul, & Silalai, 2008b). Changes in fatty acid composition, oxygen consumption, peroxide and *p*-anisidine values, residual tocopherol, phenolic and γ-oryzanol contents during storage were compared to those achieved by stabilising tuna oil with BHT. Lipid peroxidation was significantly retarded by the addition of rice bran extracts, and its oxidative stability increased by increasing the concentration of the added extracts. Lipid peroxidation resulted in losses of tocopherols and total phenolics, but the γ-oryzanol contents did not decrease.

The capability of rice bran powder in inhibiting lipid peroxidation of fried rice flour dough during storage was evaluated (Chotimarkorn & Silalai, 2008a). Rice flour dough containing rice bran powder was fried in soybean oil and stored at 60 °C. PUFAs decreased rapidly in fried dough without rice bran powder. Also, in fried dough containing rice bran powder the absorption of oxygen in vial headspace and the lipid hydroperoxide and TBARS values were lower, and tocopherol degradation was slower. During storage, the γ-oryzanol contents of fried dough remained unaltered.

The effect of the soybean oil/RBO ratio on lipid peroxidation inhibition in fried rice-flour dough during storage was also investigated (Chotimarkorn & Silalai, 2008b). Rice flour dough was fried in 100% soybean oil, and in mixtures containing 25%, 50% and 75% RBO, and stored at 60 °C. Dough fried in 100% soybean oil and in mixtures having 25% RBO showed a rapid decrease of headspace oxygen concentration, total tocopherols and PUFAs, and an increase of saturated fatty acid contents, whereas dough fried in oil mixtures with 50%, 75% and 100% RBO showed lower increases of peroxide and *p*-anisidine values. Thus, the oil mixtures with large percentages of RBO retarded significantly the progress of oxidative and hydrolytic rancidity in fried dough during storage.

5.2. Stabilisation and development of other food products

Brown rice, rice bran and normal and enriched RBO have been used to stabilise a variety of food products, being also attractive candidates to develop functional foods (Bergman & Xu, 2003). The oxidation of low-heat whole milk powder during storage was reduced by adding 0.1% RBO (Nanua, McGregor, & Godber, 2000). When compared with control milk powder, consumers could not detect any effect on the flavour of reconstituted whole milk powder containing 0.1% RBO. Also, beef patties containing γ-oryzanol had higher oxidative stability during storage than did beef patties with other antioxidants (Kim, Suh, Yang, & Lee, 2003). The cooked beef containing γ-oryzanol gave the lowest TBARS values, warmed

over flavour scores, and C_7-oxidised cholesterol, hydroperoxide and hexanal levels.

The use of rice bran in Kung-wan, an emulsified pork meatball, was investigated (Huang, Shiau, Liu, Chu, & Hwang, 2005). Protein and fat contents, whiteness index, hardness, gumminess and chewiness of this product decreased as the amount of added bran increased. The sensory scores of taste, texture and overall acceptability of meatballs with less than 10% added bran showed no significant difference from those for meatballs without bran. Also, meatballs enriched with smaller bran particles possessed higher texture profile analysis indices and sensory scores than those with larger added particles.

The effect of cooking rice bran with various levels of added water on the quality of bran and extracted oil was studied (Kumar, Khatoon, Prabhakar, & Krishna, 2006). Tocol contents largely decreased when bran was boiled with water, but the γ-oryzanol contents increased slightly.

A novel foodstuff was obtained from pre-germinated brown rice by soaking in water and drying (Ohtsubo, Suzuki, Yasui, & Kasumi, 2005). Total dietary fibre, total *trans*-ferulic acid and γ-aminobutyric acid contents were higher than those of ordinary brown rice or polished rice. Then, the pre-germinated brown rice was processed with a twin-screw extruder. The puffed pre-germinated brown rice contained more γ-oryzanol, inositol, total *trans*-ferulic acid and total dietary fibres compared with the unpuffed polished rice. The product prepared by coextrusion of pre-germinated brown rice (90%) and beer yeast (10%) contained more free amino acids than polished rice, brown rice and puffed pre-germinated brown rice. Also, extrusion cooking was shown to sterilise the germinated brown rice. The wheat bread prepared with 30% of the puffed pre-germinated brown rice contained more γ-aminobutyric acid and free sugars than ordinary wheat bread. The extrudate bread was shown to be sweeter and equally palatable.

The structure of many food products is based on networks of crystalline fat, including high levels of saturated fatty acids. The use of ingredients capable of structuring fats could lead to the development of more healthful food products (Pernetti, Malssen, van Flöter, & Bot, 2007). Examining their use as oil-structuring agents, the gel-forming capability of mixtures of phytosterols and their *trans*-ferulates was investigated (Bot & Agterof, 2006); under some ratios, these mixtures were found to form transparent gels with dihydrocholesterol, cholesterol, β-sitosterol and stigmasterol.

5.3. Miscellaneous industrial applications

RBO is a relatively inexpensive raw material for the production of biodiesel, with the advantage of the potential production of high value-added byproducts (Ju & Vali, 2005). Thus, related research, also including the development of enzyme-mediated processes, has been carried out. Further, less viscous and more stable biodiesel has been obtained using RBO (Wang, Hicks, & Moreau, 2002). The recovery of γ-oryzanol, tocols and other phytochemicals from the residue has been investigated (Kasim, Chen, & Ju, 2007; Zullaikah, Lai, Vali, & Ju, 2005).

RBO extracts are used in cosmetics, in the treatment of skin-related disorders (e.g. melanin-related disorders) and for minimising wrinkles (Indira et al., 2004). Mainly due to its antioxidant activity, but also because it absorbs UV radiation, γ-oryzanol is used as a sunscreen in cosmetic formulations (Juliano et al., 2005). The effects of *trans*-ferulic acid and γ-oryzanol diet supplementation on cultured red sea bream were examined (Takashi et al., 2008). Commercial brown fish meal diets supplemented with either *trans*-ferulic acid (0.01–0.5%) or γ-oryzanol (0.05–0.5%) were given to zero-year, cultured red sea bream for 98 days. The brightness of the integument colour of administrated fish was higher than that of control fish. Furthermore, TBARS in the liver of administrated fish was lower than in control fish. These results indicated that *trans*-ferulic acid and γ-oryzanol suppressed not only dark-colour pigmentation but also oxidative stress in cultured red sea bream.

6. Conclusions and current trends

Among the advances in analytical methods, the total phytochemical concentration in rice bran can be assessed by the partial extraction-UV method of Lilitchan et al. (2008) with considerable savings of both time and solvents. Selectivity could be improved by combining partial extraction with second-derivative UV-scans and multicomponent techniques (Bucci et al., 2003). Advantages could be also derived from a better knowledge of the liquid–solid distribution equilibria of phytochemicals (Rodrigues et al., 2004, 2006). The concentration profile of the γ-oryzanol components can be assessed with superior selectivity by a variety of chromatographic techniques (Table 1). Concerning HPLC with MS detection, attention should be paid to the APPI interface, which can yield very low LODs for phytosterols and other low polarity compounds (Lembcke et al., 2005). Considerable work has been also addressed to estimate the radical-scavenging and antioxidant activities of both RBO and their extracts; however, more attention to the test sensitivities according to the nature of both the substrates and involved chemical reactions is needed (Aguilar-Garcia et al., 2007).

Bergman and Xu (2003) have demonstrated the greater effect of growing environment on the total tocol and γ-oryzanol contents in relation to genotype. The relative proportions in γ-oryzanol can change with the growing temperature (Britz et al., 2007), and probably also with other factors, such as irrigation regime. Concerning the degree of maturity of rice grains, contradictory results have been reported (Chen & Bergman, 2005a; Miller & Engel, 2006). Other authors have investigated the antioxidant concentrations and activity of bran extracts from different rice varieties (Table 1).

Both the tocol and γ-oryzanol concentration profiles have been used to predict the variety of brown rice (Heinemann et al., 2008). Other trace components of brown rice should be investigated as predictor candidates, maybe to be used together with tocols and phytosteryl ferulates, in the prediction of rice origin, variety and other properties, by application of multicomponent statistical techniques (Marini et al., 2003).

The phytochemical contents of rice bran layers vary widely, the γ-oryzanol concentration being higher in the outer layers (Lloyd et al., 2000; Rohrer & Siebenmorgen, 2004). Thus, rice bran fractionation rather than all-bran processing is advantageous because smaller amounts of richer fractions are processed (Schramm et al., 2007). Information regarding nutraceutical concentrations in bran obtained after successive milling stages, also including different varieties and maturity stages, has been collected (Chen & Bergman, 2005a; Ha et al., 2006; Rohrer & Siebenmorgen, 2004; Schramm et al., 2007).

Several patents oriented to obtain enriched RBO fractions, and to recover γ-oryzanol from RBO soapstock, have been filed. Enriched RBO can be efficiently obtained by using membrane technology (Manjula & Subramanian, 2008). Chromatographic procedures are very efficient, but scaling-up work regarding industrial exploitation is still needed (Lai et al., 2005); application of new chromatographic stationary phases, including both silica-based and polymeric monoliths, could help. Advantage should be taken from the actual development of monolith technology, which tends to blur the borders between columns and membranes.

The influence of microwaves on the extraction efficiency of oil from rice bran was recently investigated (Zigoneanu et al., 2008); however, the enhancement of oil extraction from rice bran, and the possibility of modifying other RBO processing steps by the

simultaneous or sequential application of ultrasound and microwaves has still been scarcely studied (Cravotto et al., 2004).

Industrial RBO fractionation with SC–CO_2, which was investigated by Xu and Godber (2000), Dunford and King (2000, 2001), Dunford et al. (2003) and other authors, has continued in attracting much interest. Subcritical water extraction is also promising, due to its rapidity, versatility in selecting the extraction conditions, and high recoveries of lipophilic compounds; however, in comparison to SC–CO_2, the scarce research and lack of scaled-up procedures involving the use of subcritical water (Hata et al., 2008; Wiboonsirikul et al., 2007) are probably due to the higher installation and operation costs.

Improved food products and enriched fractions for miscellaneous uses are potential benefits of the development of enzymatic procedures for brown rice processing. Future work should consider bio-polishing (Das et al., 2008a,b) as a substitute or a complement to mechanical milling, as well as in the improvement of other stages of RBO processing (Jahani, Alizadeh, Pirozifard, & Qudsevali, 2008), and in the specific production of extracts of pharmaceutical interest (Parrado et al., 2006).

The use of γ-oryzanol-rich diets for the treatment of hyperlipoproteinaemias has been extensively investigated. Research to separately distinguish the effects of phytosteryl esters, ferulic acid and other compounds has been conducted (Wilson et al., 2007); however, little work has been done to evaluate the separate effects of the different phytosterols and phytosteryl ferulates. On the contrary, the anti-inflammatory effects of a number of individual phytosterols and phytosteryl *trans*- and *cis*-ferulates were evaluated (Akihisa et al., 2000). The protective effects of lipophilic rice bran extracts against intestinal inflammation (Hori et al., 2008; Islam et al., 2008), and damage caused by diabetes (Kanaya et al., 2004) and by chronic ethanol ingestion (Chotimarkorn & Ushio, 2008), have been also demonstrated. Research on the anti-cancer properties of rice bran derivatives also deserves more attention (Luo et al., 2005; Parrado et al., 2006).

Much attention has been paid to the stabilising effects of RBO and rice bran powder when added to frying oils and other food products, including fried dough (Chotimarkorn & Silalai, 2008a, 2008b), milk powder (Nanua et al., 2000), cooked beef (Kim et al., 2003) and pork meatballs (Huang et al., 2005). Finally, among other miscellaneous applications, RBO is of interest in the production of biodiesel (Ju & Vali, 2005; Wang et al., 2002), and to supplement diet in aquaculture (Takashi et al., 2008).

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