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Partial extraction method for the rapid analysis of total lipids and γ-oryzanol contents in rice bran

Supathra Lilitchan [a,*], Cholticha Tangprawat [a], Kornkanok Aryusuk [b], Sumalee Krisnangkura [c], Salisa Chokmoh [b], Kanit Krisnangkura [b]

[a] *Department of Nutrition, Faculty of Public Health, Mahidol University, Rachathewi, Bangkok 10400, Thailand*
[b] *Biochemical Technology Division, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkhuntien, Bangkok 10150, Thailand*
[c] *Department of Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University, Dusit, Bangkok 10300, Thailand*

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Abstract

Total lipids and γ-oryzanol in rice bran were determined by a partial extraction method. The results agreed well with the conventional total extraction methods. The proposed method uses fewer hazardous organic solvents, takes a shorter extraction time and requires no special extraction apparatus. Total lipids and γ-oryzanol in nine rice bran varieties were analysed by the developed technique. Daw Dum 5647 had the highest total lipids and γ-oryzanol while the lowest content was found in KD XBT 313-19-1-1 and SP XBT 43-7, respectively. The adsorption coefficient (K_d) of the lipids and γ-oryzanol, between hexane and bran, at 30 °C are between 1.16 and 2.00 and 2.02 and 2.65, respectively (depending on the moisture content of the bran). From the K_d values, it was estimated that about 92–95% of the lipids and 95–96% of the γ-oryzanol were extracted into hexane at a 10:1 (v/w) ratio of hexane to bran. The effect of solvents on the extraction of γ-oryzanol from rice bran was also studied. It was found that isopropanol was the most suitable solvent for extraction and determination of γ-oryzanol in rice bran. It showed better agreement with the total extraction method.

Keywords: Total lipids; γ-Oryzanol; Rice bran; Adsorption coefficient; Partial extraction method

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* Corresponding author. Tel.: +66 2354 8539; Fax: +66 2640 9839.
E-mail address: phsll@mahidol.ac.th (S. Lilitchan).

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1. Introduction

Rice (*Oryza sativa*) bran is a by-product produced in the rice milling industry. It possesses approximately 10% weight of the total rice grain. Rice bran (RB) is an excellent source of lipids, containing from 18% to 22% oil, especially unsaturated fatty acids (McCaskill & Zhang, 1999; Orthoefer, 1996; Tanaka, Yoshida, Asada, & Kasai, 1973). Recently, rice bran oil (RBO) has received attention because of its unique health benefits (Nicolosi, Rogers, Ausman, & Orthoefer, 1994). It contains a high level of several phytochemicals, e.g. γ-oryzanol (Xu & Godber, 1999), tocopherols and tocotrienols (Shin & Godber, 1994).

Gamma oryzanol in rice bran was about 13 to 20 times (w/w) greater than in tocopherol and tocotrienols (Bergman & Xu, 2003). Gamma oryzanol is a complex mixture of ferulate, esterified with sterols or triterpene alcohols (Rogers et al., 1993). Gamma oryzanol was shown to be able to reduce cholesterol absorption (Rong, Ausman, & Nicolosi, 1997). It was appropriate for the treatment of the inflammatory process (Akihisa et al., 2000) and it could inhibit linoleic acid and cholesterol oxidation (Xu & Godber, 2001; Xu, Hua, & Godber, 2001). In addition, it is a potential antioxidant for food, pharmaceutical and cosmetic industries (Nanua, McGregor, & Godber, 2000; Iqbal, Bhanger, & Anwar, 2005).

The quantification of γ-oryzanol in RB can be performed by many methods that involve extraction of RBO from the bran, followed by analysis of the amount of γ-oryzanol in the RBO by HPLC. In order to determine the amount of γ-oryzanol in RBO it is very important to completely extract this fraction from the oil. Various extraction techniques have been used for the analysis of γ-oryzanol in RBO such as liquid–liquid extraction, solid phase extraction, supercritical fluid extraction (SFE) and direct solvent extraction (Chen & Bergman, 2005; Hu, Wells, Shin, & Godber, 1996; Shin, Godber, Martin, & Wells, 1997; Xu & Godber, 2000). These extraction techniques have several significant disadvantages. The major disadvantage of liquid–liquid extraction is the use of large volumes of expensive, toxic, high-purity organic solvent. Also, it is extremely time-consuming. The requirements for solid phase extraction solvents are less stringent than those for liquid–liquid extraction (Desideri, Lepri, Heimler, Giannessi, & Checchini, 1984). Due to the disadvantages of the conventional extraction techniques, solvent free sample preparation methods or those employing less organic solvent are becoming more important. In the field of SFE, various researchers proposed the use of supercritical carbon dioxide in order to separate waxes, oryzanol and free fatty acid fractions from RBO (García et al., 1996; Kuk & Dowd, 1998; Xu & Godber, 2000). Although, SFE has the advantage that the requirements for SFE solvent are inertness, non-corrosion, non-flammable and non-toxic properties, a special apparatus is required. Recently, the use of direct solvent extraction has been reported for determination of RBO and γ-oryzanol contents in RB, which uses the rapid equilibrium extraction method to give the RBO and γ-oryzanol from RB (Chen & Bergman, 2005; Proctor & Bowen, 1996; Proctor, Jackson, Scott, & Clark, 1994). This extraction method has the following advantages over the currently available methods: speed, no special extraction instrumentation is needed but if the extraction solvent capacity is lower, this method must use a large volume of solvent. Solid–liquid extraction is an alternative extraction method. Many researchers employed solid–liquid extraction to extract natural antioxidants and investigated their properties from grape seed (Jayaprakasha, Selvi, & Sakariah, 2003; Shi, Yu, Pohorly, & Kakuda, 2003; Yilmaz & Toledo, 2004) and from other plant materials (Bandonienė, Pukalskas, Venskutonis, & Gruzdienė, 2000; Moure et al., 2001; Škerget et al., 2005) and used it as a tool for their identification (Guendez, Kallithraka, Makris, & Kefalas, 2005; Tsao & Deng, 2004).

Solid–liquid extraction is defined by the solid–liquid equilibrium (SLE), which is characterised by the distribution or adsorption coefficient of a solute between a solid phase and a solvent phase. The adsorption coefficient (denoted by K_d) is the ratio of the solute concentration in the liquid phase to that in the solid sample at equilibrium. SLE can also be expressed mathematically as shown

$$K_d = \frac{C_m}{A_s} \tag{1}$$

where C_m is the concentration of the solute in organic solvent and A_s is the amount of the solute being adsorbed by one gram of the adsorbent (rice bran).

Defining the two concentration as

$$C_m = M_m/V_m \tag{2}$$

$$A_s = M_s/g_s \tag{3}$$

where M_m is the amount of the solute in organic solvent (g), M_s is the amount of the solute in solid phase (g), V_m is the volume of organic solvent (ml) and g_s is the weight of rice bran (g).

The adsorption coefficient can be expressed as

$$K_d = \left(\frac{M_m}{V_m}\right) \Big/ \left(\frac{M_s}{g_s}\right) \tag{4}$$

If K_d, V_m and g_s are known, Eq. (4) can be readily used to quantify unknown concentrations of the target analyte by measuring the M_m. However, establishing a K_d value can be a tedious and time-consuming.

In the gas/product partition coefficient (K_p) it can be found that two widely used indirect methods are the equilibrium partitioning in closed system (EPICS) method, originally proposed by Lincoff and Gosset (1984) and the phase ratio variation (PRV) method described by Ettre, Welter, and Kolb (1993). In the EPICS method, two sample vials with different solution volumes, but the same amounts of volatile compound, are used. The K_p is obtained by measuring the equilibrium vapour concentration ratio in the two vials by gas chromatography (GC). In the PRV method, several volumes of a solution with the same initial concentration are introduced in the vials and the equilibrium gas concentration is also measured by GC. This method has been recently used in the case of an aroma compound in a mixture (Athes, Pena Y Lillo, Bernard, Perez-Correa, & Souchon, 2004; Bylaite, Ilgunaite, Meyer, & Adler-Nissen, 2004; Jouquand, Ducruet, & Giampaoli, 2004; Savary, Guichard, Doublier, & Cayot, 2006) or in the case of other volatile organic compounds (Peng & Wan, 1997). Therefore, a simple yet accurate and universally reproducible approach to establishing K_d values would be highly desirable with such a system, as that described in this paper. In this study, total lipid and γ-oryzanol in rice brans were determined by two solid–liquid partial extractions with different volumes of solvents but the same weight of sample is used and followed by solving two simultaneous equations.

2. Materials and methods

2.1. Materials

Gamma oryzanol were gifts from the Vegetable Oil Refinery (Bangkok, Thailand). Solvents (analytical and HPLC grade) were purchased from LabScan (Bangkok, Thailand).

All varieties of rice (i.e. Kor Khor 6, SP XBT 60-12, SP XBT 43-7, SP XBT 52-18, SP XBT 56-39, KD XBT 313-19-1-1, Hom Nin, Daw Dum 5647, Daw Dum 5645, Hom Supan) were obtained from the Center of Excellence on Rice Molecular Breeding and Product Development, Kasetsart University KamPhang Saen Campus, Thailand. The brans were collected from the same milling system and stored in polyethylene bags at 4 °C in a refrigerator. Moisture content of the bran was determined by drying at 100 °C until constant weight was obtained. This analysis was duplicated and all the results were expressed on a dry matter basis.

2.2. Extraction of total lipids and γ-oryzanol

2.2.1. Classical extraction

The extraction of the rice bran oil was carried out according to AOAC. Exactly 1.0 g of bran sample was weighted and extracted with petroleum ether in a Soxhlet for 16 h at a condensation rate of 2–3 drops/s. The solvent was evaporated to dryness in a vacuum rotary evaporator and the residue was dried at 100 °C, cooled and weighed.

2.2.2. Soxtec apparatus extraction

Exactly 1.0 g of bran sample was weighted and extracted with petroleum ether (50–75 ml) in a dried extraction cup and put on the heater of the Soxtec apparatus system for 20 min. The sample thimbles were removed from petroleum ether and rinsed for 30–45 min. The extraction cup was removed, dried at 100 °C for 30 min, cooled and weighed.

2.2.3. Determination of total lipids and γ-oryzanol by partial extraction

Two identical rice bran samples (1.0 g) were weighed into two identical vials and extracted with the same solvent using different volumes (4 and 8 ml) by vigorous mixing on a vortex mixer for 1 min, at room temperature. The solid brans were removed by centrifugation for 10 min at 2,500 rpm. The absorbance of the two supernatants was measured using a DR-4000 UV–vis spectrophotometer (Hach, Colorado, USA). The lipid and γ-oryzanol contents in the extracts were quantified against the standard curve. Total lipids and γ-oryzanol were calculated by solving two simultaneous equations. Expanding Eq. (1)

$$K = \left(\frac{x}{V}\right)\left(\frac{w}{y-x}\right) \tag{5}$$

where x is the amount (g) of lipid or γ-oryzanol in the extract, y is the total amount of lipid or γ-oryzanol in the bran. V is the volume (ml) used for extraction and w is the weight of bran used for extraction.

There are two unknowns (K and y) in Eq. (5), thus extraction of two identical samples with different volumes of solvent are necessary. In order to simplify the analysis and the calculation, the solvent used for the second extraction is double ($V_2 = 2V_1$) and the amount of solute in the two extracts are x_1 and x_2. Substituting these values into Eq. (5), Eqs. (6) and (7) are obtained

$$K_1 = \left(\frac{x_1}{V_1}\right)\left(\frac{w}{y-x_1}\right) \tag{6}$$

and

$$K_2 = \left(\frac{x_2}{V_2}\right)\left(\frac{w}{y-x_2}\right) \tag{7}$$

Although the K value for solid extraction is slightly varied, as the amount of solvent used for extractions is different, it is assumed that the change does not affect the accuracy of this study. Thus, by assuming $K_1 = K_2$

$$\left(\frac{x_1}{V_1(y-x_1)}\right) = \left(\frac{x_2}{V_2(y-x_2)}\right) \tag{8}$$

rearranging Eq. (8),

$$y = \frac{x_1 x_2}{2x_1 - x_2} \tag{9}$$

Therefore, the total amount of solute (y) can be calculated from Eq. (9). Substitution y into Eq. (6) or (7) K is obtained.

3. Results and discussion

3.1. Partial extraction method validation

The average moisture content of rice bran (Kor Khor 6 variety) was $8.42 \pm 0.05\%$. For the simultaneous analysis of the total lipids and γ-oryzanol contents in a rice bran (RB) sample, by using the partial extraction method, hexane was chosen as the extraction solvent. The amounts of lipid and γ-oryzanol in each extraction were measured by UV spectrophotometers at 210 and 314 nm, respectively. Hexane was chosen because its cut-off wavelength varied from 190 to 195 nm and it was the most commonly used extraction solvent for total lipids in RB in literature (Fornari, Bottini, & Brignole, 1994; González, Resa, Ruiz, & Gutiérrez, 1996; McCaskill & Zhang, 1999; Proctor et al., 1994). The total lipids and γ-oryzanol contents in RB were compared with those obtained by the classical method. Results of triplicate measurements are listed in Table 1.

The lipids and γ-oryzanol obtained from each extraction of RB samples were quantified by a UV spectrophotometric method using external standard calibration. The calibration curves are linear between 40 and 280 μg/ml for total lipids and 3–20 μg/ml for γ-oryzanol. The regression coefficients are >0.999. The results showed that the amount of total lipids and γ-oryzanol, determined by using the partial extraction method, was approximately the same as the classical method. Thus, the partial extraction method was reliable and might be used to determine total lipids and γ-oryzanol contents in RB. The partial extraction method consumes less organic solvents (only 10–15 ml), requires a smaller amount of sample size (1 g or less) and takes much less analysis time (<15 min) than the classical extraction methods. The method may be able to extend to the determination of total lipids and γ-oryzanol in different food formulas.

3.2. Total lipids and γ-oryzanol in the brans from different rice varieties

Table 2 summarises the total lipids and γ-oryzanol in the brans from different rice varieties by using the partial extraction method.

Table 1
Comparison of the total lipids and γ-oryzanol contents in rice bran (Kor Khor 6 variety) obtained by the classical and the partial extraction methods with hexane

Extraction method	Total lipids % (dry weight basis)	γ-oryzanol (mg/g)
1. Classical method		
• Soxhlet extraction	21.3 ± 0.38	3.67 ± 0.07
• Soxtec apparatus	20.8 ± 0.51	–
2. Partial extraction (with hexane)	20.7 ± 0.87	3.43 ± 0.14

Data (values) are mean value of triplicate measurements ± SD ($n = 3$).

Table 2
Quantitative determination of total lipids and γ-oryzanol contents in different rice bran varieties using the partial extraction method with hexane

Sample ID	Moisture (% w/w)	Content	
		Total lipids (%)	γ-oryzanol (mg/g)
1. SP XBT 60–12	10.2	19.5	2.03
2. SP XBT 43–7	10.3	20.1	1.95
3. SP XBT 52–18	9.67	19.6	1.97
4. SP XBT 56-39	8.93	19.7	2.04
5. Hom Nin	8.31	19.9	2.66
6. Daw Dum 5647	8.49	21.2	3.07
7. Daw Dum 5645	8.39	18.8	2.35
8. KD XBT 313-19-1-1	9.62	18.2	2.62
9. Hom SuPan	9.88	19.1	2.12

The moisture contents of RBs of different varieties were 8–11%. Total lipids contents were in the range of 18.2–21.2% (dry weight basis). They were in agreement with those reported by Tanaka et al. (1973, approximately 18–22%); Amissah, Ellis, Oduro, and Manful (2003, 13.3–19.8%) and Chokmoh, Krisnangkura, Lomsugarit, and Krisnangkura (2005, 18.89%), using a Soxhlet extraction method. The highest total lipids content was observed for Daw Dum 5647 (21.2%) while the lowest was found in KD XBT 313-19-1-1 (18.2%). The differences were probably due to the difference in rice varieties. Gamma-oryzanol contents were 1.95–3.07 mg/g (dry weight basis). The amount of γ-oryzanol from different rice varieties are slightly lower than those of CPRS and BNGL (3.4–3.9 mg/g and 3.8–4.2 mg/g, Chen & Bergman, 2005) and US long grain and Bengal (4.74 mg/g and 4.0 mg/g, Lloyd, Siebenmorgen, & Beers, 2000). Potential reasons for these variations include differences in genotypes, growth period, the milling techniques and the stabilisation techniques, etc. (Malekian et al., 2000; Iqbal et al., 2005; Rohrer & Siebenmorgen, 2004). However, γ-oryzanol was estimated from all the rice varieties suggesting its potential uses for determination in the function foods and nutracueticals industries.

3.3. Adsorption coefficient of total lipids and γ-oryzanol

The partial extraction method can also be applied to determine the K_d values of the total lipids and γ-oryzanol. Generally, the K_d value of a solute is not known, but it can be determined by using two partial extractions and solving two simultaneous equations, as described in the experimental section. Table 3 summarises the K_d values of total lipids and γ-oryzanol (in hexane) of nine rice varieties at 30 °C.

The K_d values of total lipids and γ-oryzanol between RB and hexane at 30 °C were 1.16–2.00 and 2.02–2.65, respectively. The K_d values decrease as the moisture content of the RB is increased, as shown in Fig. 1. Thus the slight variation of the K_d values can be ascribed to the differences in the moisture contents in the RB samples. Hexane is a non-polar solvent and moisture would be a good barrier for

Table 3
Adsorption coefficient (K_d) and percent extraction[a] of total lipids and γ-oryzanol from different rice bran varieties using the partial extraction method with hexane

Sample ID	Total lipids		γ-Oryzanol	
	K_d	% Extraction	K_d	% Extraction
1. SP XBT 60–12	1.58	94	2.32	96
2. SP XBT 43–7	1.65	94	2.40	96
3. SP XBT 52–18	1.49	94	2.22	96
4. SP XBT 56–39	1.51	94	2.29	96
5. Hom Nin	1.81	95	2.45	96
6. Daw Dum 5647	1.29	93	2.14	96
7. Daw Dum 5645	1.16	92	2.02	95
8. KD XBT 313-19-1-1	1.89	95	2.48	96
9. Hom SuPan	2.00	95	2.65	96

[a] Percent extraction was calculated from the K_d value using Eq. (4).

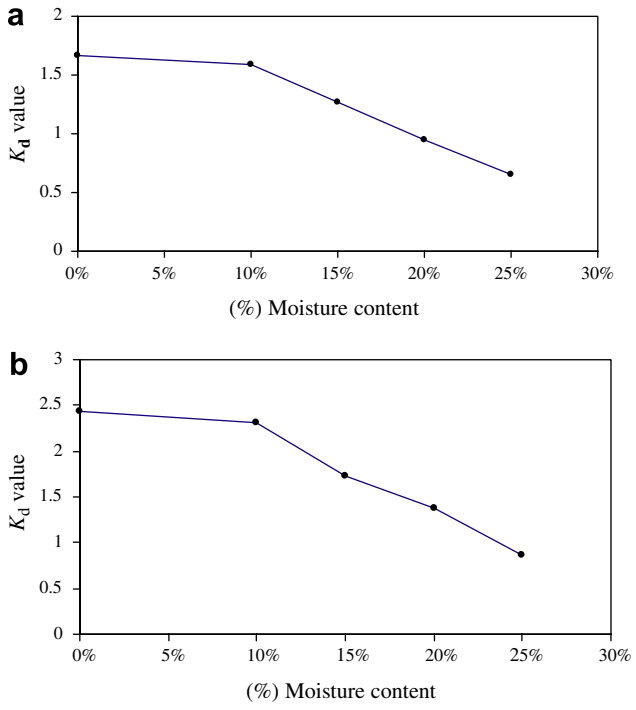

Fig. 1. The relationship between the K_d value of total lipids (a) and γ-oryzanol (b) and moisture content in the rice bran (Kor Khor 6 variety) by partial extraction with hexane.

hexane penetration into the RB during extraction. It is observed that at a higher moisture content in the RB, the K_d values, for both total lipids and γ-oryzanol in RB at 30 °C were decreased (Fig. 1). Therefore, if the K_d value of a solute is known, the amount of that solute can be determined in only one step of extraction under the same condition, that is, temperature and moisture content.

Hexane, at 10 volumes, can extract about 92–95% of the lipids in the RB of different varieties. They are comparable to the 90% reported by (Proctor et al., 1994) and the 89% reported by Proctor and Bowen (1996). In their studies, the equilibrium extraction methods were carried out at ambient temperature (22 °C) and a hexane to bran ratio (v/w) of 10:1. The differences in percent of lipids extraction might be due to the effects of temperature and moisture contents of the bran. For γ-oryzanol, about 95–96% was extracted by hexane, in a singly step, of a 10:1 (v/w) ratio of hexane to RB. Although moisture and temperature affects the K_d value of γ-oryzanol, it is speculated that the lipid (oil) content of the bran should strongly affect the K_d value as well. Results in Table 3 show that the K_d value of γ-oryzanol, from different varieties, are varied between 2.02 and 2.65.

Fig. 2 demonstrates the amount of γ-oryzanol extracted by different volumes. It was estimated that more than 99% of γ-oryzanol in rice bran was extracted by hexane at 40:1 ratio (v/w). Therefore, γ-oryzanol in rice bran can be quantitated by a single step extraction by using hexane at 40 volumes or higher.

3.4. Solvent effects on the accuracy of γ-oryzanol determination

The γ-oryzanol levels determined by two partial extractions with hexane are slightly lower than the classical extraction method (Table 1). The difference may have been due to the effect of the extraction solvent and the accurate K_d value. As described above, the K_d value is based on the phase ratio in the vial (or it is based on the volume of liquid phase/mass of solid phase). Solvent of high volatility may be lost during transfer, which affects the exact volume of the solvent and the concentration of the solute. Thus, solvent of moderate or low volatility such as isopropanol or di-*n*-butyl ether may be advantageous, especially at high room temperature. Currently, alternative solvents, such as isopropanol, are being considered as a vegetable oil extraction solvent to avoid the flammability problems associated with hexane (Lusas et al., 1994). Therefore, different solvents were compared for their accuracy in determination of γ-oryzanol levels. These solvents include hexane, ethyl acetate, di-isopropyl ether, di-*n*-butyl ether, ethanol, isopropanol and butanol. The maximum adsorption wave-

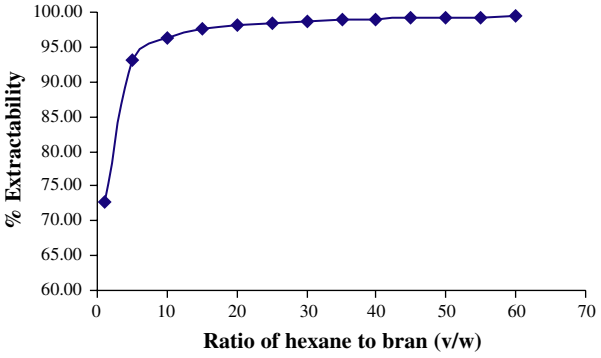

Fig. 2. Illustration of the γ-oryzanol extraction from Hom SuParn rice variety (The K_d value was 2.65) with variable the ratio of hexane to rice bran (v/w).

Table 4
Standard calibration curves of γ-oryzanol and maximum wavelengths (λ_{max})

Solvents	Calibration curves		λ_{max} (nm)
	Regression equation[a]	R^2	
Hexane	$y = 36.372x$	0.9999	314
Ethyl acetate	$y = 35.850x$	0.9999	320
Di-isopropyl ether	$y = 36.819x$	0.9999	320
Di-*n*-butyl ether	$y = 32.268x$	0.9999	320
Ethanol	$y = 33.273x$	0.9996	326
Isopropanol	$y = 33.281x$	0.9999	326
Butanol	$y = 36.861x$	0.9994	326

[a] y = absorbance, x = concentration (μg/ml).

lengths (λ_{max}) of γ-oryzanol in different solvents are slightly shifted, according to their polarities and they are summarised in Table 4, together with the linearity of standard curves. The concentration ranges of the calibration standards were 3–20 μg/ml for all of the organic solvents. All of them showed excellent linearity with correlation coefficients (R^2) greater than 0.999, in the range studied.

Table 5 shows the amount of γ-oryzanol in the RB extracted by different organic solvents. It was found that the γ-oryzanol levels determined by two partial extractions were slightly lower than the classical extraction method, except those extracted by di-*n*-butyl ether and ethanol. Chen and Bergman (2005) reported that isopropanol and methanol could extract higher amount of tocopherols, tocotrienols and γ-oryzanol from RB than hexane. These authors note that the hydroxyl groups on the benzene ring of ferulate esters might make these compounds more extractable in alcohol than in hexane. In addition, these solvents are water miscible, water on the rice bran surface can not retard the penetration of these solvents compared with the low polarity solvent. Although butanol is polar, the extraction efficiency of butanol was lower than ethanol and isopropanol for γ-oryzanol extracted from RB. When isopropanol is used as the extraction solvent, the amount of γ-oryzanol in RB is 3.74 mg/g (dry weight basis), which is very close to the Soxhlet extraction method (3.67 mg/g dry bran). Thus, isopropanol is suitable for extractive determination of γ-oryzanol in the RB at 30 °C. The amount of γ-oryzanol obtained was close to the widely accepted classical method.

Table 5
Comparison of the γ-oryzanol contents in rice bran (Kor Khor 6 variety) using the classical method and the partial extraction with different extraction solvents

Extraction method	γ-Oryzanol (mg/g)	K_d	Boiling point (°C)
1. Classical method			
(Petroleum ether)	3.67 ± 0.07	–	40–60
2. Partial extraction method			
Hexane	3.43 ± 0.14	1.76	69
Ethyl acetate	3.45 ± 0.39	1.77	77
Di-isopropyl ether	3.48 ± 0.17	2.06	68–69
Di-*n*-butyl ether	3.41 ± 0.35	1.87	142
Ethanol	3.79 ± 0.45	2.28	78
Isopropanol	3.74 ± 0.30	1.83	82
Butanol	3.29 ± 0.14	1.14	118

The proposed method can simultaneously determined the amount of γ-oryzanol and its K_d value using different extraction solvents. These results indicated that the K_d values of γ-oryzanol for ethers and alcohols are higher than that for hexane. The higher K_d value means that more solute is extracted from rice bran by the same solvent volume but it does not infer higher accuracy. The higher K_d value may result in an inaccurate determination of the sample because the difference in the amounts of solute being extracted by one and two volumes of solvents is minimal. A small error in determination of the solute in each extract would introduce large error in the final quantification. Similar error would also be speculated for the other end of the K_d value. Therefore, there would be a suitable K_d value and the suitable value for the determination of total lipids and γ -oryzanol content should range from 0.5 to 2.0. The extraction solvent capacity is about 71–91 % at a 5:1 (v/w) ratio of solvent to RB. On the other hand, solvent with a higher K_d value would be good for a single step extractive determination, that is most of the solute is extracted with a small volume of the solvent.

4. Conclusions

This study has shown that the partial extraction method can be used in the analytical laboratory for determination of total lipids and γ-oryzanol in rice bran, with accuracy comparable to that determined by classical solvent extraction methods. The method is simple, rapid and economical. In addition, the proposed method can simultaneously determine the K_d value of total lipids and γ-oryzanol in rice bran. The knowledge of K_d is important. It gives us a clear and quantitative view on how much a solute is extracted by a volume of the solvent. It was also found that moisture in the RB decreased the K_d values for both of the total lipids and γ-oryzanol with hexane. For the determination of γ-oryzanol in rice bran, isopropanol was recommended as the extraction solvent based on the accuracy in determination of γ-oryzanol.

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