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The influence of extraction methods on composition and antioxidant properties of rice bran oil

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Abstract

The current study was employed to assess the influence of the different extraction methods on total tocols, γ-oryzanol content, and antioxidant properties of Chiang Mai Black rice, Mali Red rice, and Suphanburi-1 Brown rice bran oil. Rice bran oil (RBO) was extracted by Hexane, Hot pressed, Cold pressed, and Supercritical Fluid Extraction (SFe) methods. High yield of RBO was extracted by hexane and SFe methods. Total and subgroups of tocols, and γ-oryzanol content were determined by HPLC. The hexane extracted sample accounts for high content of γ-oryzanol and tocols. Besides, all of RBO extracts contain a significantly high amount of γ-tocotrienol. *In vitro* antioxidant assay results indicated that superior quality of oil was recovered by hexane extraction. The temperature in the extraction process also affects the value of the oil. Superior quality of oil was recovered by hexane extraction, in terms of phytochemical contents and antioxidant properties compared to other tested extraction methods. Further, thorough study of factors compromising the quality and quantity of RBO recovery is required for the development of enhanced functional foods and other related products.

Keywords: rice bran oil; tocols; γ-oryzanol; antioxidants.

Practical Application: Influence of different extraction methods for the recovery of the principal compounds from RBO has been demonstrated.

1 Introduction

The seed of *Oryza* species is commonly known as rice, which are the foremost cereal food crop in most of the developing countries. Approximately 95% of the rice production is documented in Asian countries, and about half of the world population consumes rice as their primary source of carbohydrate (Muthayya et al., 2014). Rice grains, known as endosperm, are surrounded by nutrient rich (almost 60% of total nutritional value) outer layer called rice bran (RB) and it accounts for 8% of total weight of the rice. The color of the rice coat differs with respect to the degree of deposition of anthocyanin pigment. Consequently, the rice cultivars also varied with respect to the color of the rice, which can be black, brown, or red (Chaudhary, 2003).

Oryza sativa L. *indica*, commonly known as black rice (BlR), is routinely cultivated in Southeast Asian countries. BlR is reported to have an abundant amount of protein, vitamins, minerals and bioactive compounds with slight quantity variations which depends on land cultivation and diversity (Suzuki et al., 2004). BlR has been reported as one of the potential sources of antioxidants and health promoting compounds (Hu et al., 2003; Yawadio et al., 2007; Fardet et al., 2008; Goufo & Trindade, 2014; Pengkumsri et al., 2015). BlR has the ability to prevent and can be useful to treat oxidative stress related diseases and cancer (Salgado et al., 2010). The outer layer of BlR is enriched with phytochemicals and these bioactive were broadly divided into polar (phenolic acids, flavonoids, anthocyanins) and non-polar components (tocopherols, tocotrienols, γ-oryzanol, and sterols) (Patel & Naik. 2004; Zhou et al., 2004; Holtekjølen, et al., 2006). Studies have proven that the active components of RB improve the human health because of the high content of antioxidants (Laokuldilok et al., 2011). Jun et al. (2012) reported that black and red rice brans exhibited high content of phenolic compounds and antioxidant activity.

Rice bran oil (RBO) is one of the best sources of tocols and oryzanol. Tocols (tocopherols and tocotrienols), a family of vitamin E-active substances, are wildly used plant-based ingredients in the food, cosmetics and pharmaceutical industries (Bramley et al., 2000; Abidi, 2003). Studies suggested that tocotrienols are more efficient antioxidant, anti-cancer agent and inhibitor of cholesterol synthesis than the tocopherols (Singh et al., 2013). Gamma-oryzanol is one of the major components of RBO, and it is a mixture of several ferulate esters of triterpene alcohols and plant sterols (Friedman, 2013). Oryzanol is a well known

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antioxidant compound and is linked with decreasing serum and plasma cholesterol, decreasing platelet aggregation, and cholesterol absorption. Moreover oryzanol has been used in the treatment of hyperlipidemia, and disorders of menopause (Patel & Naik, 2004). The extraction and refining of RBO and the content of γ-oryzanol, along with its health benefits have been reviewed in detail (Patel & Naik, 2004; Lerma-García et al., 2009). The quality and concentration of the tocols and γ-oryzanol content depend on the extraction processes and refining steps. Therefore, the current study was conceived to compare the non-polar extracts of CBlR, SBrR and MRR bran oil of Thai rice variety with respect to its tocols content, oryzanol content as well as its antioxidant properties.

2 Materials and methods

2.1 Collection of rice bran and extraction

Chiang Mai black rice (CBlR), Suphanburi-1 brown rice (SBrR) and Mali red rice (MRR) were collected from the farm at Maerim district, Chiang Mai, Thailand and dried at 60 °C for 48 h. Then it was milled and the rice bran was separated through 60-mesh strainer. Lipase in RB was destroyed through 100 °C for 5 min thereafter RB was then stored at –20 °C until the time of processing. RBO was extracted with hexane (1:10 ratios of RB and hexane was incubated at 40 °C with shaking at 150 rpm for 30 min and thereafter filtrated through 0.45 µm membrane and the solvent was evaporated to obtain crude RBO), hot press (screw pressing at 80-100 °C), cold press (screw pressing at 40-60 °C), and supercritical fluid (40 °C, 200 bar, CO_2 rate 15 g/min, 1 h) extraction. The collected oil samples were membrane filtered (0.45 µm) and the percentage of yield was calculated (Equation 1).

$$\text{Percentage of Yield} = (\text{RBO (g)} / \text{Initial weight of RB (g)}) \times 100 \quad (1)$$

Filtered RBOs were stored at –20 °C under nitrogen gas (N_2) to prevent the degradation of active compounds until analysis.

2.2 Determination of γ-oryzanol content

γ-oryzanol content of RBO samples were determined by reversed-phase HPLC (Ajilent 1100, USA) (Chalermpong et al., 2012). The ACE® C18 column (250 mm × 4.6 mm; 5 µm) was used. Solvent mixture of methanol (50%), acetonitrile (44%), acetic acid (3%), and dichloromethane (3%) was used as mobile phase under isocratic condition. UV detector at 330 nm was equipped for the sample detection, and the flow rate was set at 1 mL/min. All the samples were measured in triplicate.

2.3 Determination of tocols content

The tocols content (both tocotrienols and tocopherols) of RBO samples were evaluated by reversed-phase HPLC which is equipped with LC-10AV *VP* pumps, SPD-10AV *VP* (Shimadzu, Japan) and fluorescent detector (Bruscatto et al., 2009). The KINETEX PFP column (150 mm × 4.6 mm; 100 A), was used (Phenomenex, USA) and 90% methanol in deionized water acts as mobile phase. The flow rate was set at 0.5 mL/min. The excitation and emission wavelength was set at 296 and 325 nm, respectively. All the samples were measured in triplicate.

2.4 Determination of antioxidant capacity

Antioxidant capacity of RBO extracts were assessed by ABTS (2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid), DPPH (1, 1-diphenyl-2-picryl-hydrazil), FRAP (Ferric reducing antioxidant power), Nitric oxide ($NO^{\bullet}$), Superoxide ($O_2^{\bullet-}$) radical scavenging assay and inhibition of lipid peroxidation (LPO) assay as described in our previous publications. $ABTS^{\bullet+}$ radical scavenging activity was measured by ABTS assay according to Chalermpong et al. (2012). $DPPH^{\bullet}$ radical scavenging activity was assessed by DPPH assay according to Rattanachitthawat et al. (2010) and Wahid et al. (2014). FRAP assay was performed according to the method of Suwannalert et al. (2010) and inhibition of lipid peroxidation was assessed according to Chalermpong et al. (2012). Nitric oxide ($NO^{\bullet}$) radical scavenging activity was measured according to Francis & Andrew (2010) and Pengkumsri et al. (2015). Superoxide ($O_2^{\bullet-}$) radical scavenging activity was assessed according to Kusirisin et al. (2009).

2.5 Statistical analysis

The quantification of γ-oryzanol, tocols (tocotrienols and tocopherols) content and determination of antioxidant activity of RBO were performed in triplicates to confirm the reproducibility of the results. The report of the data was given as mean ± SD. Analysis of variance (ANOVA) was performed using statistical SPSS software version 17 (Chicago, SPSS Inc, U.S.A). The Least Significant Difference (LSD) post hoc test was performed in order to analyze the significant differences in antioxidant activities and $p < 0.05$ was considered to be significant.

3 Results and discussion

The yield of RBO during different extraction methods has been tabulated (Table 1). The hexane extraction (He) of CBlR, MRR, and SBrR yielded about 11.61 ± 0.55, 10.92 ± 0.64, and 12.89 ± 0.58% of RBO, respectively. The supercritical fluid extraction (SFe) of CBlR, MRR, and SBrR yielded about 9.60 ± 1.06, 8.12 ± 0.97, and 7.06 ± 0.78% of RBO, respectively. The yield of RBO suggested that hexane extraction (He) produced high quantity of oil followed by SFe (Table 1).

The γ-oryzanol content of RBO has been assessed by HPLC with respect to different extraction methods. About 17.54 ± 0.75, 17.54 ± 0.88, and 18.49 ± 1.52 mg/g of γ-oryzanol content in CBlR, MRR, and SBrR bran oil, respectively were recorded in hexane extracts, whereas a very low amount (1.75 ± 0.09 and 2.71 ± 0.14 mg/g in CBlR and SBrR, respectively) of γ-oryzanol were observed in RBO obtained by SFe. Hot press extraction yielded 5.62 ± 0.28, 5.66 ± 0.25, and 6.23 ± 0.31 mg/g of γ-oryzanol content in CBlR, MRR, and SBrR bran oil extract, respectively. Cold press extraction represented 6.08 ± 0.34, 6.75 ± 0.3, and 6.48 ± 0.37 mg/g of γ-oryzanol content in CBlR, MRR, and SBrR bran oil, respectively (Figure 1). Previous studies have been reported on the recovery of γ-oryzanol from different RB varieties by methanol, hexane, and ethyl acetate mediated extraction techniques (Iqbal et al., 2005; Chotimarkorn et al., 2008; Lin & Lai, 2011). Varying quantity of γ-oryzanol have been reported based on the extraction methods and rice cultivars. In the present study, highest γ-oryzanol yield was obtained by He followed by

Table 1. Recovery of Rice bran oil from different rice cultivars with different extraction methods and sample codes used in the current study.

Extraction method	Sample	Sample code	% of yield
Hexane extraction (He)	Chiang Mai Black rice (CBlR)	He-CBlR	11.61 ± 0.55*
	Mali Red rice (MRR)	He-MRR	10.92 ± 0.64*
	Suphanburi Brown rice (SBrR)	He-SBrR	12.89 ± 0.58*
Hot press extraction (HPe)	Chiang Mai Black rice (CBlR)	HPe-CBlR	5.81 ± 0.87
	Mali Red rice (MRR)	HPe-MRR	7.01 ± 1.05
	Suphanburi Brown rice (SBrR)	HPe-SBrR	5.59 ± 0.84
Cold press extraction (CPe)	Chiang Mai Black rice (CBlR)	CPe-CBlR	4.04 ± 0.81
	Mali Red rice (MRR)	CPe-MRR	5.80 ± 1.16
	Suphanburi Brown rice (SBrR)	CPe-SBrR	4.85 ± 0.97
Supercritical fluid extraction (SFe)	Chiang Mai Black rice (CBlR)	SFe-CBlR	9.60 ± 1.06
	Mali Red rice (MRR)	SFe-MRR	8.12 ± 0.97
	Suphanburi Brown rice (SBrR)	SFe-SBrR	7.06 ± 0.78

*% of yield is significant (p<0.05) compared to respective RBO extract of other extraction methods (HPe, CPe and SFe).

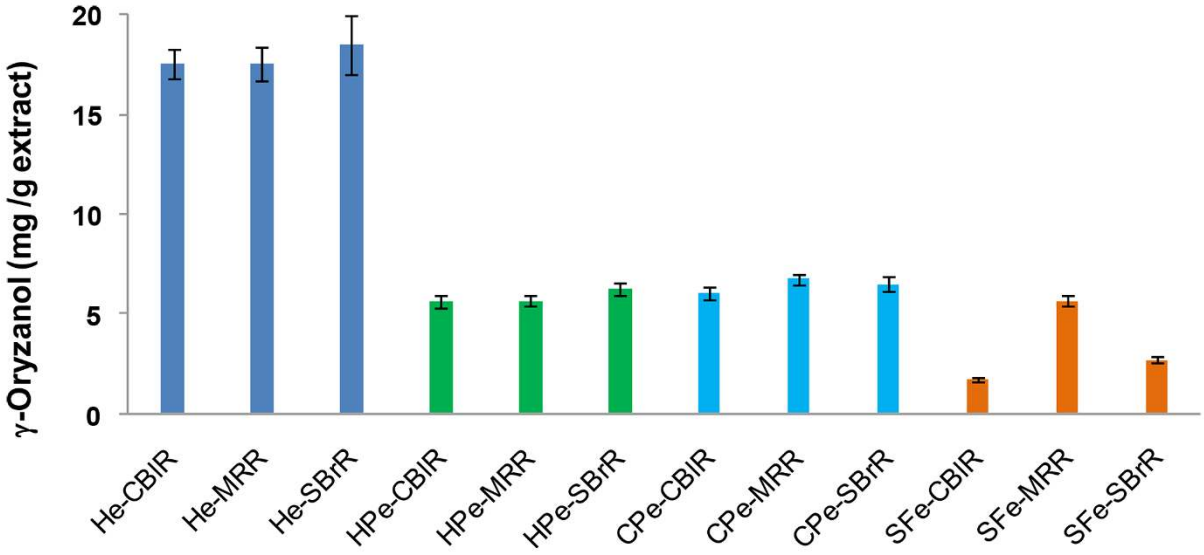

Figure 1. γ-oryzanol content in CBlR, SBrR, and MRR bran oil extracted by He, HPe, CPe, and SFe. The values were represented as mean ± SD.

CPe, HPe and SFe methods (Figure 1). This data suggested that variation in the amount of γ-oryzanol in RBO depends on the extraction methods.

Total tocols and subgroups of tocopherol and tocotrienol were measured. The total tocopherol (included α, β, γ, and δ) content of about 0.40 ± 0.02, 0.15 ± 0.01, 0.16 ± 0.01, and 0.08 ± 0.00 mg/g in CBlR bran oil samples were obtained from He, HPe, CPe, and SFe methods, respectively. The total tocopherol content of about 0.22 ± 0.01, 0.19 ± 0.01, 0.21 ± 0.01, and 0.05 ± 0.00 mg/g in MRR bran oil samples were obtained from He, HPe, CPe, and SFe methods, respectively. The total tocopherol content of about 0.43 ± 0.02, 0.12 ± 0.01, 0.13 ± 0.01, and 0.06 ± 0.00 mg/g in SBrR bran oil samples were obtained from He, HPe, CPe, and SFe methods, respectively. In all the preparations, γ- tocopherol was found to be a major subgroup of tocopherol followed by α, δ and β types that were recorded for their abundance, respectively. The total tocopherol concentration also suggested that hexane extraction is significantly (p<0.05) better than the other mining methods employed in the present study. The total tocotrienol (included α, β, γ, and δ) content of about 0.67 ± 0.03, 0.28 ± 0.01, 0.34 ± 0.02, and 0.18 ± 0.01 mg/g in CBlR bran oil samples were obtained from He, HPe, CPe, and SFe methods, respectively. The total tocotrienol content of about 0.84 ± 0.04, 0.62 ± 0.03, 0.74 ± 0.04, and 0.26 ± 0.01 mg/g in MRR bran oil were obtained from He, HPe, CPe, and SFe methods, respectively. The total tocotrienol content of about 1.11 ± 0.06, 0.52 ± 0.03, 0.62 ± 0.03, and 0.31 ± 0.02 mg/g in SBrR bran oil samples were obtained from He, HPe, CPe, and SFe methods, respectively. The quantification of sub-groups revealed that the abundance of γ- tocotrienol was higher and followed by α- tocotrienol, δ- tocotrienol, and β- tocotrienol (Figure 2). The quantity of δ and β type of tocopherol and tocotrienol have no significant variation in both cold and hot press extraction. The quantity of δ and β type tocopherol and tocotrienol were not significantly affected by the cold press and hot press extraction when compared to the yield of hexane extracts. Similar to tocopherol, the abundance of tocotrienol was also recorded in hexane extracted oil (Figure 2). Chotimarkorn et al. (2008)

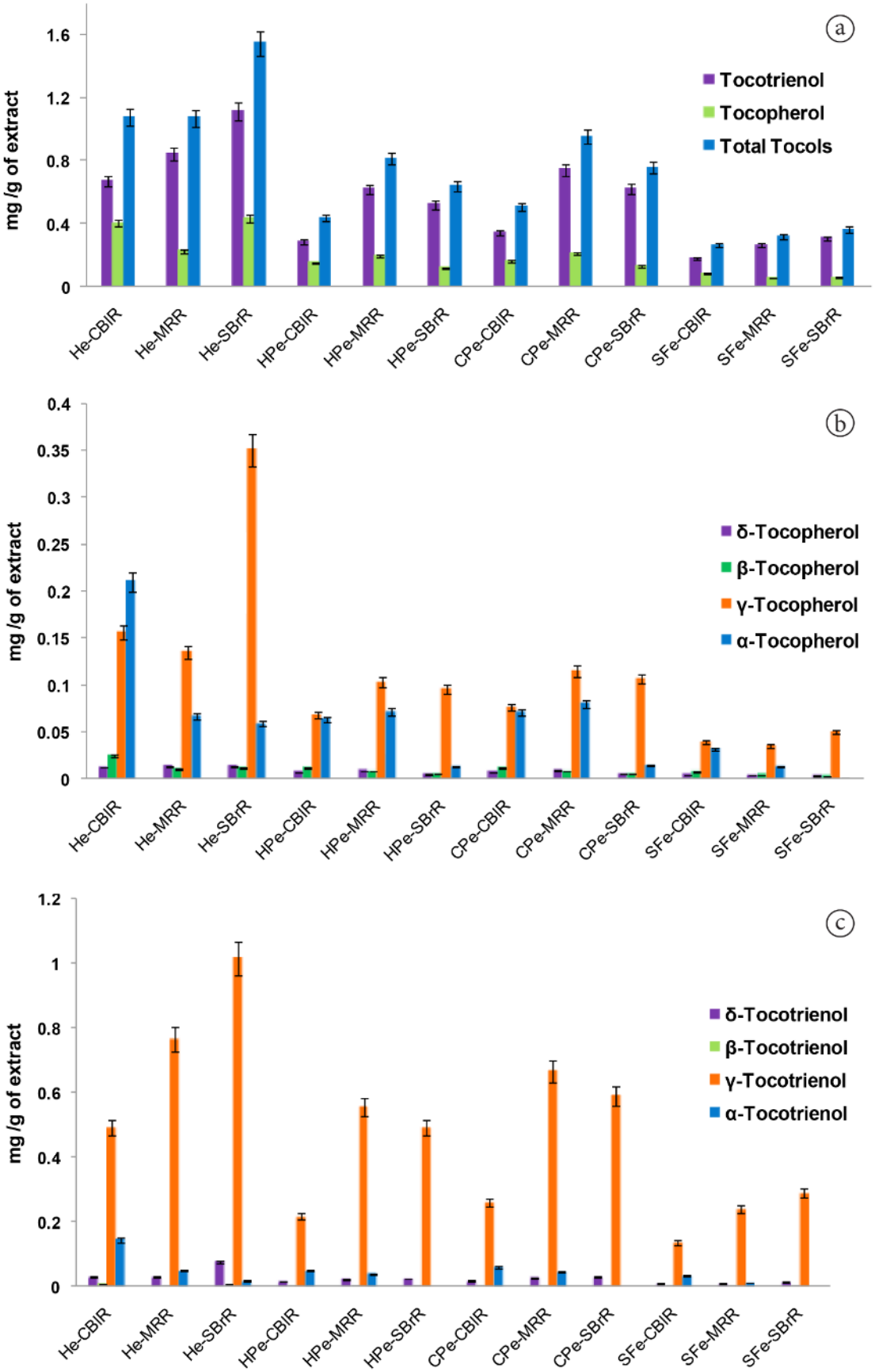

Figure 2. Total (a) and subgroups of tocopherol (b) and tocotrienol (c) content in CBlR, SBrR and MRR bran oil extracted by He, HPe, CPe, and SFe. The values were represented as mean ± SD.

has reported the richness of α-tocopherol and the absence of γ -tocotrienol in the methanolic extraction of RB than in those of the other types. Basically, concentration of total tocopherol and tocotrienol content variation depends on the cultivars and the extraction method used. Moreover, few studies have reported that the extractions of all the subgroups of tocols are not entirely succeeded in an efficient manner (Iqbal et al., 2005; Aguilar-Garcia et al., 2007). In the present study, all subgroups of tocols were reported in all the preparations with varying concentration.

Anti-oxidant properties of RBO have been studied through different *in vitro* assays such as ABTS, DPPH, FRAP, Inhibition of lipid peroxidation, superoxide anion, and nitric oxide radical scavenging assay. Hexane extracted samples showed the highest TEAC (mg of trolox equivalent antioxidant capacity per gram of extracts) (13.16 ± 0.66, 13.05 ± 0.65, and 17.17 ± 0.86 mg TEAC/g of CBlR, MRR, and SBrR bran oil extract, respectively) in ABTS assay compared to the samples extracted by HPe, CPe, and SFe methods (Figure 3a). Hexane extracted SBrR bran oil showed the highest $ABTS^{\bullet+}$ radical scavenging activity (IC_{50} = 29.31 ± 0.17 µg/mL) compared to the other samples (Table 2). DPPH assay also suggested that hexane extracted samples exhibit significant anti-oxidant activity (12.42 ± 0.58, 12.19 ± 0.56, and 18.80 ± 0.57 mg TEAC/g of CBlR, MRR, and SBrR bran oil extract, respectively) compared to the samples extracted by other methods (Figure 3b). Similarly, hexane extracted SBrR bran oil showed the highest DPPH radical scavenging activity (IC_{50} = 0.70 ± 0.04 mg/mL) compared to other samples (Table 2). Inhibition of Lipid peroxidation assay results indicated that hexane extracted sample has the maximum inhibition activity (27.76 ± 0.25, 27.51 ± 0.23, and 30.49 ± 0.51 mg TEAC/g (Figure 3c); IC_{50} = 0.67 ± 0.03, 0.67 ± 0.03, 0.61 ± 0.03 mg/mL (Table 2) of CBlR, MRR, and SBrR bran oil extract, respectively) compared to the samples extracted by other methods. The reducing power of hexane extracted samples (24.53 ± 1.23, 33.41 ± 1.67, and 35.03 ± 1.75 mg equivalent $FeSO_4$ per gram of CBlR, MRR, and SBrR bran oil extract, respectively) were observed to be higher compared to the samples extracted by HPe, CPe, and SFe methods (Figure 4). Superoxide ($O_2^{\bullet-}$) radical scavenging assay results showed that hexane extracted samples exhibited higher $O_2^{\bullet-}$ radical scavenging activity (7.34 ± 0.10, 7.01 ± 0.12, and 7.00 ± 0.12 µg ascorbic acid equivalent/µg (Figure 5a); IC_{50} = 12.49 ± 0.62, 13.07 ± 0.65, 13.09 ± 0.65 µg/mL (Table 2) of CBlR, MRR, and SBrR bran oil extract, respectively) compared to the samples extracted by HPe, CPe, and SFe methods. Nitric oxide ($NO^{\bullet}$) radical scavenging assay results also showed that hexane extracted samples exhibited higher $NO^{\bullet}$ radical scavenging activity (0.23 ± 0.01, 0.23 ± 0.01, and 0.20 ± 0.01 mg curcumin equivalent/mg (Figure 5b); IC_{50} = 0.18 ± 0.00, 0.18 ± 0.00, 0.21 ± 0.01 mg/mL (Table 2) of CBlR, MRR, and SBrR bran oil extract, respectively) compared to the samples extracted by HPe, CPe, and SFe methods.

Therefore, all *in vitro* antioxidant assay results indicated that He based RBO preparations are enriched with antioxidant properties significantly (p<0.05) than other tested HPe, CPe, and SFe based RBO preparations. Even though, hexane extraction yields efficient and enriched RBO, the presence of hexane residues that are not completely eliminated is the disadvantage of the solvent based extraction process. The present study also investigated the efficiency of RBO yielded by the solvent independent extraction process. Overall results indicated that the efficiency of HPe, CPe, and SFe which are solvent independent extraction process is less compared to the He of RBO. The second high yield of RBO was obtained by SFe (Table 1), but the quality of the oil was slightly compromised in its antioxidant property compared to HPe and CPe samples (Figures 3 and 5). The oil extraction temperature greatly influenced the yield and quality of the oil. Hot pressed method produced a slightly enhanced yield of RBO compared to cold pressed, but the quality of cold pressed oil in terms of antioxidant properties was superior to the hot pressed one (Figures 3 and 5).

Considerable differences in antioxidant capacity of RBO extracts of different cultivars suggested that the content of the phytochemicals had a vast impact on their antioxidant properties (Iqbal et al., 2005; Chotimarkorn et al., 2008). The chemical composition (tocols, γ-oryzanol), and antioxidant

Table 2. Antioxidant activity (IC_{50}) of different Rice bran oil extracts with different evaluation method.

RB oil extract sample code	ABTS assay (µg/mL)	DPPH assay (mg/mL)	$NO^{\bullet}$ radical scavenging assay (µg /mL)	$O_2^{\bullet-}$ radical scavenging assay (µg/mL)	Inhibition of Lipid Peroxidation (mg/mL)
He-CBlR	37.32 ± 0.28*	1.06 ± 0.05*	0.18 ± 0.00*	12.49 ± 0.62*	0.67 ± 0.03*
He-MRR	38.44 ± 0.30*	1.08 ± 0.05*	0.18 ± 0.00*	13.07 ± 0.65*	0.67 ± 0.03
He-SBrR	29.31 ± 0.17*	0.70 ± 0.04*	0.21 ± 0.01*	13.09 ± 0.65*	0.61 ± 0.03*
HPe-CBlR	96.83 ± 0.22	1.81 ± 0.09	0.29 ± 0.01	20.61 ± 1.03	1.07 ± 0.05
HPe-MRR	84.07 ± 0.25	1.92 ± 0.09	0.31 ± 0.01	20.01 ± 1.00	0.72 ± 0.04
HPe-SBrR	87.93 ± 0.34	2.22 ± 0.10	0.32 ± 0.01	21.33 ± 1.06	0.99 ± 0.05
CPe-CBlR	68.92 ± 0.32	1.52 ± 0.08	0.24 ± 0.01	14.48 ± 0.72	0.82 ± 0.04
CPe-MRR	76.50 ± 0.16	1.65 ± 0.08	0.23 ± 0.01	15.78 ± 0.78	0.70 ± 0.04
CPe-SBrR	69.55 ± 0.42	1.64 ± 0.08	0.25 ± 0.01	16.90 ± 0.85	0.81 ± 0.04
SFe-CBlR	170. 01 ± 0.33	2.46 ± 0.12	0.29 ± 0.01	19.17 ± 0.95	1.48 ± 0.07
SFe-MRR	129.67 ± 0.43	2.03 ± 0.10	0.29 ± 0.01	18.85 ± 0.94	1.41 ± 0.07
SFe-SBrR	119.53 ± 0.55	1.90 ± 0.09	0.32 ± 0.01	19.54 ± 0.97	1.23 ± 0.06

All the values are represented as mean ± SD of triplicate experiments. *IC_{50} value is significant (p<0.05) compared to respective RB oil extract of other extraction methods (HPe, CPe and SFe).

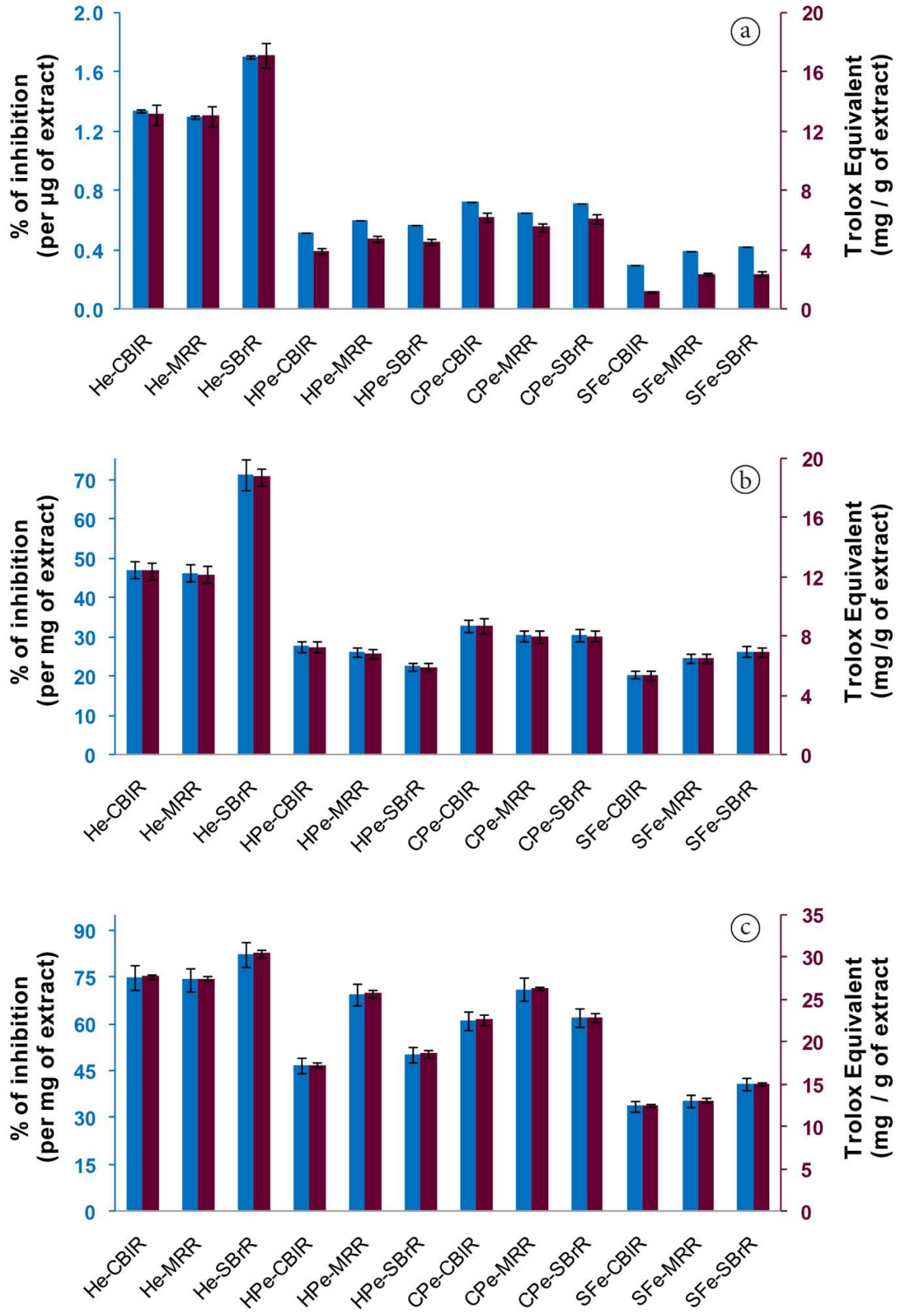

Figure 3. ABTS (a), DPPH (b) and Lipid peroxidation (c) inhibition activity of RBO extracts in comparison with its standard. The values were represented as mean ± SD.

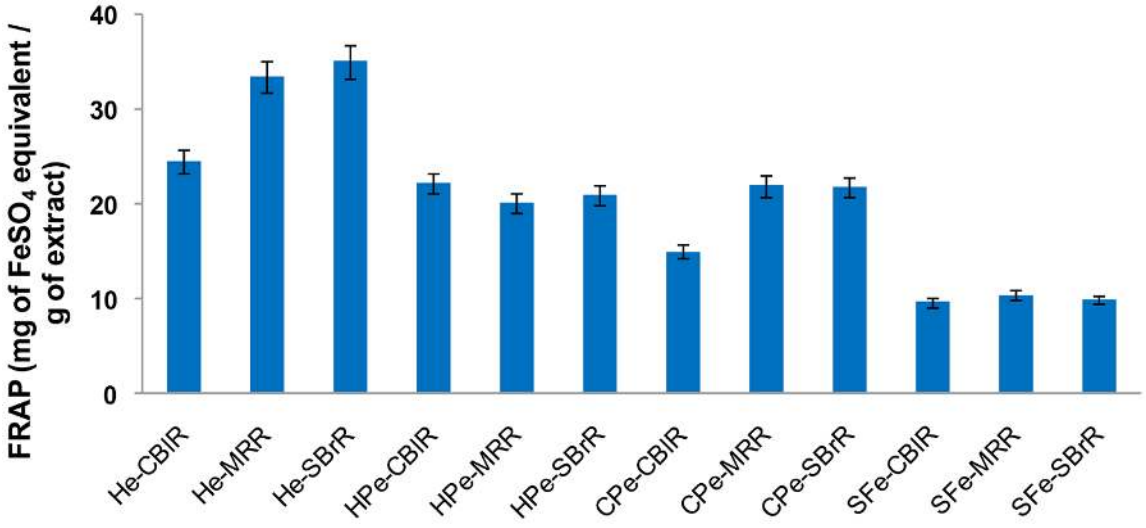

Figure 4. Reducing power of RBO extracts assessed by FRAP assay. The values were represented as mean ± SD.

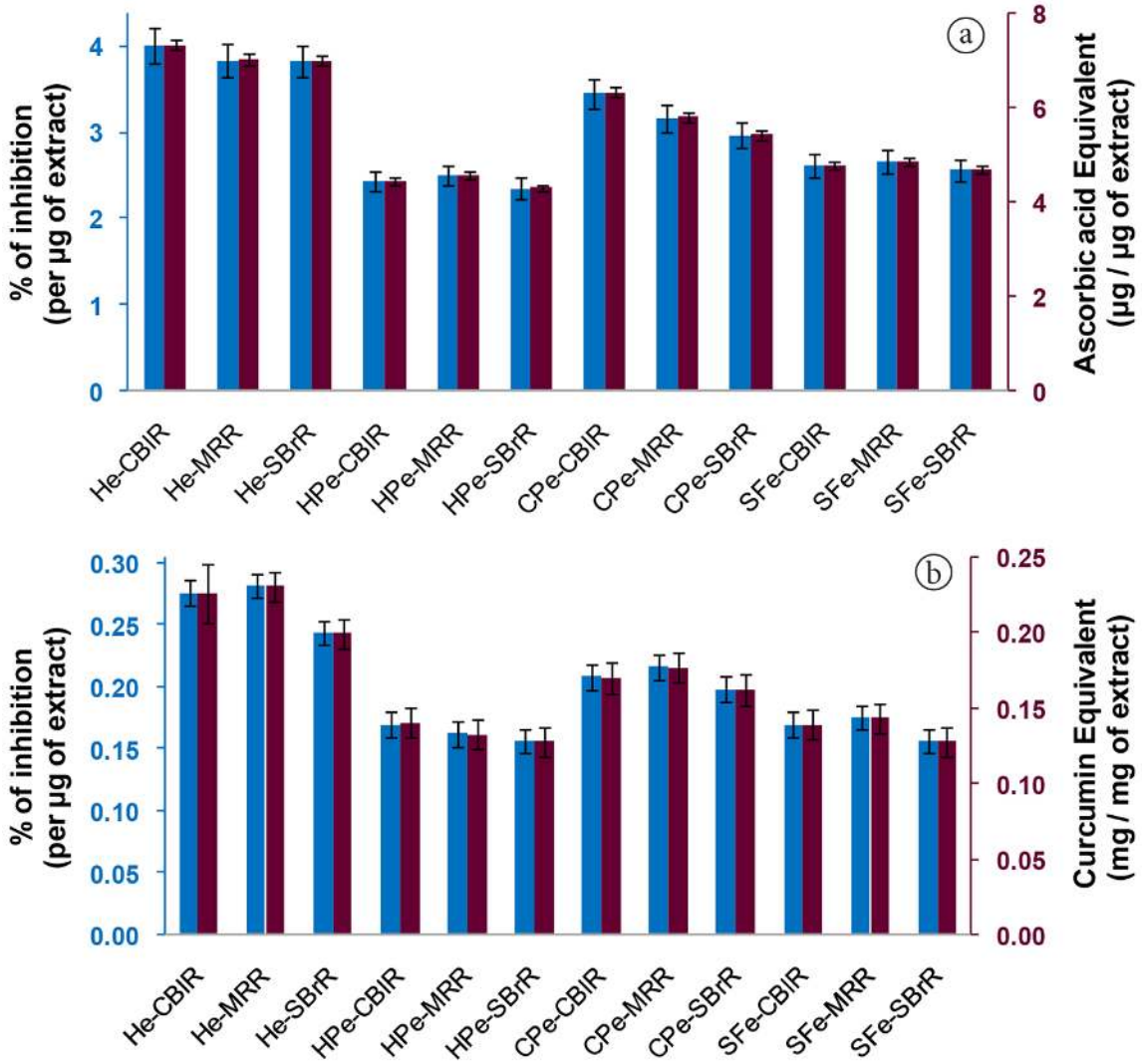

Figure 5. Superoxide ($O_2^{\bullet-}$) radical (a) and nitric oxide ($NO^{\bullet}$) radical (b) scavenging activity of RBO extracts in comparison with its standard. The values were represented as mean ± SD.

properties of white, red, and brown rice bran were compared with the unique extraction method (Aguilar-Garcia et al., 2007; Finocchiaro et al., 2007). However, all previous studies have shown that the phytochemical composition and the ability of the antioxidant of rice varieties depend on many factors, majorly, cultivars, and extraction methods. The results of the current investigation suggested that hexane extraction yielded prosperous RBO with respect to the content of active phytochemicals and antioxidants. Development of new or modified extraction method independent of solvent usage that provides an efficient yield of RBO will eliminate the health hazards that might occur when used in food, cosmetic and pharmaceutical industries.

4 Conclusion

The influence of the different extraction methods on the recovery of the principal compounds like total tocols and γ-oryzanol from RBO has been demonstrated. The effect of extraction methods on antioxidant activities of the same cultivar was also studied. This study revealed that hexane extraction yielded precious RBO, with respect to both quantity and quality wise. The SFE affects the desired qualities of RBO though it gives better yield in terms of quantity. The role of the temperature in RBO recovery and property was also explained. To the best of our knowledge, this is the primary study about the influence of extraction methods on phytochemical content and antioxidant properties of CBlR, MRR, and SBrR bran oil. Further, in-depth study on factors affecting the recovery of phytonutrients and its properties is required for the development of enhanced functional foods and other related products.

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