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Study of chemical composition and antioxidant properties of Sangyod and Tubtimchumpae rice bran oil

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Abstract: Sangyod rice bran oil is an oil extracted from a local rice strain grown in Phattalung Province,Thailand. Tubtimchumpae rice bran oil is another kind oil obtained from Tubtimchumpae rice grown in Kamphaengphet Province, Thailand. Both types of red rice containing high anthocyanin were prepared for the oils by cold-press extraction. Since they do not pass through heat processing, they are rich in nutritional values. The aim of this research was to study the chemical compositions and antioxidant properties of Sangyod and Tubtimchumpae rice bran oils. The results showed that the chemical compositions of in terms of free fatty acids, acid value, peroxide value, unsaponifiable matter and saponification of the two rice bran oils were significantly different ($p \leq 0.05$). Both rice bran oils consisted of high unsaturated fatty acid contents (80.46 and 80.35%, respectively), with oleic acid and linoleic acid as main components. However, Tubtimchumpae rice bran oil contained higher tocopherol than Sangyod rice bran oil (11.88 and 9.13 mg/100g, respectively). Total phenolic contents were found to be 1.48±0.05 and 1.54±0.03 mg gallic acid equipvalent/100 g oil, respectively. DPPH (IC_{50}) values were 13.90 and 13.97 µg/ml, ABTS (IC_{50}) were 15.99 and 16.09 µg/ml, respectively. In addition, the two types of rice bran oil were found to possess important nutritional values, such as unsaturated fatty acids, antioxidants and vitamin E (α-tocopherol). Therefore, it was suggested that these oils can be used to increase health benefits in food products.

Keywords: Sangyod rice bran oil, Tubtimchumpae rice bran oil, chemical composition, antioxidant properties.

Introduction

Rice bran oil (RBO) is the oil extracted from the germ and inner husk of rice. RBO contains significant unsaturated fatty acids, saturated fatty acids and micronutrients, such as tocopherol, tocotrienol and oryzanol. Several studies have shown that RBO has an effect on metabolic activities, causing the decrease of bad cholesterol LDL but without significant affecting the blood level of HDL cholesterol (Yoshino et al., 1989, Qureshi et al., 1991, Hegsted et al., 1990, Kahlon et al., 1992, Hegsted and Windhauser 1993). RBO containing high antioxidant activities is known to have health benefits and for improving stability of foods during the storage (Shahid et al., 2005). RBO also contains high concentration of tocopherols compared with other seed oils (Kao and Luh, 2010). Previous studies have also shown that the rice grains with dark red or purple color contain high amount of anthocynanins, a group of natural pigments found in deep purple or reddish fruits and vegetables (Yawadio et al., 2013). On the other hand, rice grains with red color consist high amount of polyphenols, phenolics and flavonoids (Gunaratne et al., 2013; Sompong et al., 2011). Sangyod rice is a traditional rice variety grown in Phatthalung province for more than a hundred years. Sangyod Muang Phatthalung rice is soft with approximately 14-15% of the amylose content. The analysis of its nutrient volume has been shown that Sangyod rice has high nutritional value, iron, vitamin B and niacin (Department of Health, 2004). For Tubtimchumphae rice, it is a new Thai rice varieties which is a hybrid between Jasmin rice and Sangyod rice. It contains high amount of phenolic, flavonoid, Vitamin E (α-tocopherol and γ-oryzanol) (Rice Department, 2016). The objective of this research work was to study the chemical composition and antioxidant properties of RBOs obtained from the two rice varieties, in order to provide information for their uses in food and supplementary products.

Materials and Methods

Materials

Sangyod rice bran oil (SRBO) was obtained from Phattalung Province's Community Enterprise and Tubtimchumpae rice bran oil (TRBO) from Kamphaengphet Province's Community Enterprise.

Physical parameters

The color of RBOs was measured by the CIE L*, a*, b* with standard illuminant D_{65} and standard observer 10° by Spectrophotometer (CM-3500d, Minolta, Japan)

Chemical parameters

Acid value (AV), free fatty acid (FFA), Peroxide value (PV), iodine value (IV), saponification value (SV) and unsaponifiable matter were determined according to the methods described in AOAC (2000).

Determination of fatty acid composition

The fatty acid compositions were determined by GC-FID according to the methods described by Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Thailand. Capillary GC (Agilent 6890 Serie) equipped with a capillary column (BPX 70 SGE; 70% Cyanopropyl Polysilphenylene-siloxane; 30 m×0.25 mm–0.25 µm film) was used. The GC conditions operated at the initial temperature of 160 °c for 0.5 min, then increased to 160-200 °c at 10°c /min and the final temperature of 200 °c for 4.6 min with a total run time of 9.1 min. The flow rate of gas (Nitrogen) was 1 ml/min. injection volume of 1 µl were used. The fatty acid composition was obtained by comparison of the peak retention times with the respective fatty acids standards.

Determination of total phenolic content

The total phenolic content (TPC) of the crude RBO extracts was determined using Folin–Ciocalteu'sreagent method as described by Singleton et al., (1999) Oil extracts (300 µL) were mixed with 1.5 ml of Folin-Ciocalteu's reagent, left for 6 min and subsequently 1.25 ml of 7% sodium carbonate solution was added. The mixture was diluted to 3 ml with deionized water. The absorbance of the resulting mixture was read at 725 nm using a UV visible spectrophotometer (UV 1601, Shimadzu, Japan) after leaving for 30 min at the room temperature (25 ℃). The measurement was compared to a standard curve of gallic acid concentrations, which were expressed mg gallic acid equivalents/ g sample.

Determination of α-tocopherol

The α-tocopherol content in RBO was determined by Institute of Food Research and Product Development, Thailand, using an in-house method based on BS EN 12823-1:2000

Determination of DPPH radical scavenging capacity

DPPH radical-scavenging effects were determined by using the method modified from that reported previously by Brand-Williams et al., (1995). A solution of DPPH was prepared in methanol, and the solution of RBO was dissolved in DMSO to obtain the concentration between 5 and 30 µg/mL. Then, RBO and 1 ml of 0.1 mM DPPH solution were mixed in test tube. The mixture was shaken and incubated for 30 min in the dark at room temperature. The absorbance was determined at 517 nm by UV-VIS spectrophotometer (Shimadzu 160A, Japan). The radical scavenging activity was expressed as IC50 value that denotes the concentration of the sample required to scavenge 50% of DPPH radicals.

Determination of ABTS radical scavenging capacity

ABTS assay was determined according to the method described by Re et al., (1999) ABTS radical was generated by reacting 7 mM ABTS and 2.45 mM potassium persulfate at room temperature in dark for 16 h. The ABTS solution was diluted with 80 % ethanol to an absorbance around 0.700 at 734 nm. 2 ml of ABTS solution was added to 0.1 ml of the extracts and mixed thoroughly. The reaction mixture was kept at room temperature for 6 min, absorbance was measured at 734 nm by UV-VIS spectrophotometer (Shimadzu 160A, Japan).

Result & Discussion

Physical parameters: The CIE Lab color measurement method was determined by its color coordinates: L* represent the difference between light (L*= 100) and dark (L* = 0), a* represent the difference between green (–a*) and red (+a*) and b* represent the difference between blue (-b*) and yellow (+b*). It was found that the color of SRBO (L* = 56.48, a* = 15.60 and b* = 93.43) was darker, more green and yellow than the color of TRBO (L* = 43.64, a* = 13.97 and b* = 74.11). Therefore, the color of two varieties rice bran oil were significantly different ($p \leq 0.05$). based on the mentioned physical parameters.

Chemical parameters: The AV and FFA values in the samples of oil or fat were determined after the hydrolysis of triglycerides by the enzyme lipase in which its activity was increased rapidly after the milling process. In terms of lipid quality, the value of PV content is used as a parameter, and the values of IV is used to indicate the amount of unsaturation in RBO. The results showed that the values of AV, FFA, PV and IV of SRBO were 13.36 mg KOH/g, 6.71%, 1.78 mg eq/kg oil and 96.86 g Iodine/100 g oil, respectively. While the values of TRBO were 7.12 mg KOH/g oil, 3.58 %, 1.91 mg eq/kg oil and 100.74 g Iodine/100 g oil, respectively. CODEX standard (1999) has suggested that the maximum levels of AV for Crude rice bran oil is 4 mg KOH/g, the maximum level of PV is 10 mg eq/kg oil, and the recommended IV value is between 90–105 g Iodine/100 g oil. According to Tao et al.(1993), the level of FFA value for RBO should be not over 5%. Our results showed that the RBO samples had the AV and FFA contents higher than the recommendations. This could be affected by the raw material quality, as the bran did not stabilize before being used. The bran quality deterioration may be due to the presence lipase activity. For the PV and IV contents, the values of both rice bran oils were lower than those of the CODEX standard. Saponification value is the reaction that free hydroxide breaks the ester bonds between the fatty acids and glycerol of a triglyceride, resulting in free fatty acids and glycerol. Unsaponification value is the measure of non-lipids

constituents (sterols, pigments, and hydrocarbons) in the oil (Bodger et al. 1982). In this study, the saponification value and unsaponifiable matter of SRBO were determined to be 196.48 mg KOH/g oil and 2.29%, and of TRBO were 189.49 mg KOH/g oil and 3.49%, respectively. Statistical analysis showed that there were significant differences ($p \leq 0.05$) in the saponification values and unsaponifiable matter of two varieties oils.

Total phenolic content, DPPH and ABTS radical scavenging capacity: The result of total phenolic content (TPC) was expressed as gallic acid equivalent, showing that the phenolic compounds in RBO contributes to their antioxidant properties as summarized in Table 2. The total phenolic contents of SRBO (1.48 mg GAE/100g) and TRBO (1.54 mg GAE/100g) were not significantly different ($p > 0.05$). For DPPH and ABTS radical scavenging activities of the RBO extracts, their values were expressed as IC_{50} which is inversely proportional to the antioxidant activity. Our results showed that the DPPH and ABTS radical scavenging activities of the two varieties RBO extracts were not significantly different ($p > 0.05$). On the other hand, the α-tocopherol contents were found to be higher in TRBO (9.13 mg/100g oil) than in SRBO (11.88 mg/100g oil), which were significantly different in both varieties ($p \leq 0.05$). It is well known that tocopherols possesses high antioxidant properties and the greatest vitamin E potency (Chen and Bergman, 2015). Therefore, DPPH, ABTS radical scavenging activity and Total phenolic content of TRBO were found to be higher than those values in SRBO.

Fatty Acid Composition: The fatty acid compositions of SRBO and TRBO are showed in Table 3. There were eight fatty acids identified including oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0), stearic acid (C18:0), linolenic acid (C18:3), arachidonic acid (C20:0), myristic acid (C14:0) and palmitoleic (C16:1). The saturated fatty acids were found in both rice bran oils such as myristic, palmitic, stearic and arachidonic acids with the concentrations of 0.34–0.45%, 15.80–16.35%, 2.23–2.40% and 0.97-0.99%, respectively. The unsaturated fatty acids were found to be palmitoleic, oleic, linoleic and linolenic acids with the concentration of 0–0.14%, 41.74–42.04%, 36.22–36.89% and 0.97-0.99%, respectively. The main components of the fatty acids were oleic acid, linoleic acid and palmitic acid. However, the fatty acid contents of SRBO and TRBO were not significantly different ($p \geq 0.05$). According to Mingyail. et al., (2017) the major fatty acids in the RBO samples are oleic acid, linoleic acid and palmitic acid. The content of the unsaturated fatty acids was apparently higher than the saturated fatty acids.

Conclusion

The results of this study showed that the fatty acid profiles of SRBO and TRBO contain a high percentage of the unsaturated fatty acids (approximately 80%), with the main components of oleic acid and linoleic acid. The results also suggested that, the two rice bran oils can be used as a raw material in the production of functional foods. They provided different physicochemical and antioxidant properties, which are potentially beneficial for the nutrition and health of human.

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Table 1. Physical-Chemical properties of SRBO and TRBO.

Properties	SRBO	TRBO
L*	56.48±0.15	43.64±0.14
a*	15.60±0.03	13.97±0.05
b*	93.43±0.29	74.11±0.27
Acid value*	13.36±0.32	7.12±0.40
Free fatty acid (as % oleic)*	6.71±0.16	3.58±0.20
Peroxide value (PV)*	1.48±0.24	1.91±0.10
Iodine value (IV)*	96.86±1.64	100.74±1.05
Saponification value*	196.48±3.19	189.49±2.23
Unsaponifiable matter*	2.29±0.85	3.49±0.29

*mean significantly different ($p \leq 0.05$)

Table 2. Total phenolic content, α-tocopherol, DPPH and ABTS radical scavenging of SRBO and TRBO.

Properties	SRBO	TRBO
Total phenolic content (mg GAE/100 g)ns	1.48±0.05	1.54±0.03
DPPH assay (IC_{50}, µg/ml)ns	13.97±1.07	13.90±1.64
ABTS assay (IC_{50}, µg/ml)ns	15.99±2.19	16.09±1.98
α-Tocopherol (mg/100 g)*	9.13±1.52	11.88±1.71

*mean significantly different ($p \leq 0.05$), ns mean significantly different ($p > 0.05$)

Table 3. The fatty acid compositions of Sangyod rice bran oil and Tubtimchumpae rice bran oil.

Fatty Acid Composition (%)	SRBO	TRBO
myristic acid (C14:0)ns	0.34±0.03	0.45±0.03
palmitic acid (C16:0) ns	16.35±0.12	15.80±0.09
palmitoleic (C16:1) ns	0.14±0.12	ND
stearic acid (C18:0) ns	2.23±0.06	2.40±0.02
oleic acid (C18:1) ns	42.04±0.24	41.74±0.09
linoleic acid (C18:2) ns	36.22±0.29	36.89±0.08
linolenic acid (C18: 3) ns	1.70±0.08	1.72±0.01
arachidonic acid (C20: 0) ns	0.97±0.05	0.99±0.04

ns mean significantly different ($p > 0.05$)
ND : Not detected.