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Comparison of the policosanol contents in commercial health foods and policosanol stability in enriched rice bran oil

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Abstract

Policosanols, found in relatively large amounts in rice bran and sugarcane wax, are of interest due to their cholesterol-lowering bioactivity. Many dietary supplements and functional foods containing policosanols are available globally. The amount and stability of policosanols affect the health benefits; however, while many products claim to contain policosanols, data on the amounts and stability, including in dietary supplements and rice bran oil, are limited. This study examined the policosanol contents of commercial rice bran cooking oil and commercial dietary supplements. The policosanol stability was investigated through a model of rice bran oil enriched with policosanols extracted from defatted rice bran. The highest and lowest policosanol content of commercial rice bran cooking oil were 73.99 ± 2.96 and 18.65 ± 2.21 mg/100 g, respectively, with the major policosanols being tetracosanol (C24) and hexacosanol (C26). Functional oil products containing rice bran oil had the highest policosanol levels (215.72 ± 2.49 mg/100 g), with the rice content of the rice bran oil affecting the policosanol content. Some dietary supplements contained lower amounts of policosanol than the claimed 5 and 20 mg/serving. Policosanol was stable to heat treatment at 150 and 180 °C, with heat treatment transiently increasing the policosanol level, and was stable during 6 months of storage.

Keywords Cane wax, Dietary supplement, Rice bran wax, Soft gel, Stability, Enrichment, Policosanol

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Graphical Abstract

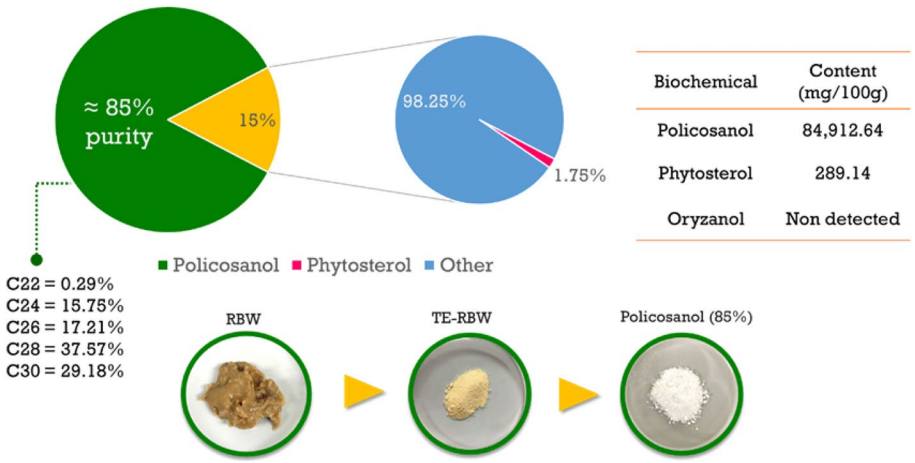
Introduction

Policosanols (PCs) are very-long-chain alcohol compounds, mostly a mixture of aliphatic primary alcohols and aldehydes (C20–C36), including docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), and triacontanol (C30) (Asikin et al., 2012; Cravotto et al., 2004). Major natural sources of PCs are sugarcane and rice bran, but also beeswax and wheat germ (Irmak et al., 2006). Several clinical reports have described serum lipid-lowering properties of PCs (Fernández-Travieso et al., 2021; Jenkins et al., 2024; Russo et al., 2024). For example, moderate decreases in plasma and cholesterol levels were measured in hypercholesterolemic patients taking 5–25 mg of PCs per day (Fernández-Travieso et al., 2021; Lekhaphanrat et al., 2014; Russo et al., 2024). Other effects include reduced platelet aggregation (Sharma et al., 2019), increased high-density lipoprotein (HDL) levels, and decreased low-density lipoprotein (LDL) levels (Cho et al., 2018; Fernndez et al., 2017; Kim et al., 2018). Studies of the acute and chronic toxicity, carcinogenicity, and mutagenicity of PCs have shown that these compounds are non-toxic and are safe for long term clinical applications (Alemán et al., 1994; Gámez et al., 2001; Swanson & Keithley, 2008). Their bioavailability after oral administration was reported to be 5–12%, with absorption ranging from 10 to 35% (Kabir & Kimura, 1993).

Consequently, many PC-containing commercial products are available on the global market, including rice bran oil (RBO) in soft gel, capsule, and other forms. The amount of PCs in these products, according to their manufacturers, is in the range of 5–20 mg. However, the level and stability of PCs in rice bran cooking oil, functional oils containing rice bran, and dietary supplements have not been independently investigated. This information is important because the amount and stability of PCs affect the health benefits of the products.

Food enrichment and restoration describe the addition back into a food product of nutrients that were partially or totally lost during processing (Doley, 2017). Additions can also be made to obtain functional food products. In our previous study, rice bran wax (RBW) discarded during RBO processing was subjected to transesterification and subcritical liquified dimethyl ether extraction to obtain PCs with a purity of 85% (Wongwaiwech et al., 2020). Docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), and triacontanol (C30) were present in amounts of 0.29%, 15.75%, 17.21%, 37.57%, and 29.18%, respectively (Fig. 1). Therefore, the re-addition of PCs lost during the refining of RBO results in the valorization of RBW and value-added RBO.

The present study consisted of a quantitative analysis of the PC contents of five commercial PC dietary supplements, nine rice bran cooking oil products, and four functional products containing RBO produced in different countries and available in Thailand and online in 2022. Since the stability of PCs is also an important issue but has not been adequately examined, we determined the amounts and heat stability of PCs in PC-enriched RBO (PRBO).



Biochemical	Content (mg/100g)
Policosanol	84,912.64
Phytosterol	289.14
Oryzanol	Non detected

Fig. 1 PC compounds isolated from rice bran wax (RBX) via transesterification (TE-RBW) and subcritical liquified dimethyl ether extraction (Wongwaiwech et al., 2020)

Materials and methods

Materials

The nine commercial RBO products were purchased from Tops Supermarket (Phitsanulok, Thailand) (Batch No. RBHSSL11A, 2005220, RBHSTB03A, 020620, B066B2, 290320, 250420, and RBO123032020. Two (production date of 06/06/2018 and 06/12/2017) of five commercial brands of PC-containing dietary supplements were purchased from Thailand and the rest (Lot number of 266251-02, 52762, and 478135-03) imported from U.S.A. Four functional products containing RBO were purchased onsite and online from Thailand. Refined RBO was obtained from Surin Bran Oil Co., Ltd (Buri Ram, Thailand) batch No. 01062020. All samples were kept at − 20 °C until used.

Reagents and standards

Analytical grade PC standards, including docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), and triacontanol (C30), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dotriacontanol (C32) was purchased from BOC Sciences (Shirley, NY, USA) and tetratriacontanol (C34) from TRC (Toronto, ON, Canada). The silylation reagent *N*, *O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), with 1% trimethylchlorosilane (TMCS), was purchased from Sigma-Aldrich. Hexane and methanol were purchased from RCI Labscan (Bangkok, Thailand), and KOH pellets from Merck Millipore (Darmstadt, Germany).

Preparation of PC-enriched RBO (PRBO)

The method developed by Tian and Acevedo (2018), with some modifications, was used to obtain PRBO. PC extracted from RBW (purity, 85%) according to the method of Wongwaiwech et al. (2020) was added to refined RBO (Surin Bran Oil Co., Ltd.) to obtain final concentrations of 300 ppm ($PRBO_{300}$) and 600 ppm ($PRBO_{600}$). The extracted PCs contained docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), and triacontanol (C30) concentrations of 0.29%, 15.75%, 17.21%, 37.57%, and 29.18%, respectively (Fig. 1). The oil samples were heated at 60 °C, stirred at 1,000 rpm for 10 min, cooled at room temperature (27 ± 1 °C), and then stored at − 20 °C until used in the chemical analysis. The amounts of PC used in the experiments, 10 and 20 mg/day, were based on the amounts recommended for consumption and corresponded to 3.3 and 6.6 mg/meal (i.e., 10 and 20 mg per three meals). The Ministry of Public Health of Thailand recommends that fat intake, including animal fats, plant fats, and sweets, should not exceed 65 g per day, equal to 21 g per meal (assuming 3 meals/day) (Food Division, 2012). In addition, fat intake from edible oil should not exceed 50% of the fat intake in a meal and should thus be limited to ≤ 10.5 g/meal (totaling 21 g over two meals in a day). Therefore, the optimum amount of PC from oil should be 300 and 600 ppm per meal. The PC concentrations needed to achieve 300 and 600 ppm in RBO ($PRBO_{300}$ and $PRBO_{600}$) were calculated as follows:

$$\frac{1,000 \times 3.3}{10.5} \equiv 317.46 \text{ mg} \approx 300 \text{ ppm}$$

$$\frac{1,000 \times 6.6}{10.5} \equiv 628.57 \text{ mg} \approx 600 \text{ ppm}$$

Since the native PC content in the refined RBO was 300 ppm, the addition of 300 and 600 ppm PC yielded RBO

containing maximum levels of 600 and 900 ppm PC, respectively.

PC stability in PRBO

Heat treatment of PRBO

The heat treatment procedure was adapted from Lira et al. (2017) with some modifications. Briefly, 30 mL of PRBO was transferred to a shallow pan (diameter: 19 cm) and heated to 150–180 °C for 10, 15, and 20 min (Majchrzak et al., 2017). The treated PRBOs were immediately cooled to room temperature and kept in an amber glass vessel at − 20 °C throughout the experiment.

Characterization of PRBO

The color values of PRBO were measured using a colorimeter (CR-400; Konica Minolta, Tokyo, Japan) and recorded as L* (lightness), a* (redness), and b* (yellowness). The C* value represents color saturation and hue angle (H* value) reflects the chromaticity or tone of color were also monitored (Long et al., 2024). The pH was measured using a pH meter (Ionix Instruments, Singapore), and the viscosity using a viscometer (DV-II; Brookfield Engineering, Middleboro, MA, USA) equipped with an RV spindle 501 (Brookfield Engineering). The acid value (AV) and peroxide value (PV) were determined following the official AOAC method (Association of Official Analytical Chemists (AOAC), 1995). All results presented in the tables are the arithmetic mean of triplicate experiments.

PC analysis

PC extraction procedure

The method of Wongwaiwech et al. (2019) was used in the PC extraction. Briefly, 1 g of sample was placed in a 15-mL polypropylene tube and hydrolyzed with 10 mL of 0.2 M NaOH (water: methanol 3:1 v/v) via sonication (Branson 8510; Branson Ultrasonics Co., Danbury, CT, USA) at 44 Hz and 250 W for 90 min at 60 °C. The hydrolyzed mixture was then extracted with toluene, cooled to 2 °C, and centrifuged at 4,000 rpm for 10 min. The upper layer was collected in a glass tube and filtered through a 0.45-µm filter.

PCs were extracted from the dietary supplements using the same method (Wongwaiwech et al., 2019). The defatted sample (1 g) was dissolved in 1 mL of toluene and subjected to 10 min of shaking. The supernatant was then filtered through a 0.45-µm syringe filter and transferred to a vial for further derivatization followed by gas chromatography–mass spectrometry (GC-MS) analysis.

PC derivatization

The method of Wongwaiwech et al. (2019) was used to prepare the PC-TMCS derivatives. A 200-µL sample of each PC was mixed with 100 µL of BSTFA containing 1% TMCS. The mixtures were heated at 50 °C for 30 min. An aliquot of each one was injected into a GC-MS column.

GC-MS conditions for PC analysis

PCs were extracted as described by Wongwaiwech et al. (2019) and subsequently identified and quantified using an Agilent 6890 GC fitted with an Agilent DB-5ms fused silica capillary column (0.25 mm × 30 m i.d., film thickness 0.25 µm; Agilent Technologies, Santa Clara, CA, USA). For each sample, 1 µL was injected using an Agilent 7683 auto sampler with a split ratio of 1:10. Selected ion monitoring mode was set for the identification and quantification of docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28) triacontanol (C30) dotriacontanol (C32), and tetratriacontanol (C34) according to their molecular target ions and retention times.

Statistical analysis

The results are expressed as the mean ± standard deviation based on the dry weight and determined in triplicate samples. Differences between the average values were determined via analysis of variance, with the subsequent application of Duncan's test. The analyses were performed using SPSS ver. 19 (IBM Corp., Armonk, NY, USA). Average values were considered significantly different at $P \leq 0.05$.

Results and discussion

PC contents of the commercial rice bran cooking oils and commercial functional RBO products

The large body of evidence supporting the health benefits of dietary phytochemicals has spurred the rapid development of nutraceuticals, dietary supplements, and functional foods (Renuka Devi & Arumughan, 2007). Rice bran is a good source of PC-containing waxes, mainly docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), and triacontanol (C30) (Cravotto et al., 2004; Harrabi et al., 2009; Irmak et al., 2006), with significant amounts retained in RBO. Clinical studies have shown the potential serum lipid-lowering properties of PCs, suggesting their use as cholesterol-lowering nutraceuticals (Chen et al., 2008; Francini-Pesenti et al., 2008; Janikula, 2002). In this study, PC concentrations were calculated based on the validation method in Wongwaiwech et al. (2019). Each component was identified based on the mass fragment pattern of its TMCS derivative as the target ion, in *m/z*. The target ions for docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28), triacontanol (C30), and dotriacontanol–tetratriacontanol (C32–34) were *m/z* 383 (qualifier ions, *m/z* 103, 384, 385), *m/z* 411 (qualifier ions, *m/z* 103,

412, 413), *m/z* 439 (qualifier ions, *m/z* 103, 440, 441), *m/z* 467 (qualifier ions, *m/z* 103, 468, 469), *m/z* 495 (qualifier ions, *m/z* 103, 496, 497), and *m/z* 523 (qualifier ions, *m/z* 103, 551, 553), respectively. The GC-MS chromatograms of the standards and sample extract are shown in more detail (Supplementary Fig. 1S).

Tables 1 and 2 present the PC contents of the nine commercial rice bran cooking oils and the four commercial functional RBOs (FT1, FT2, FT3, and FT4, all as soft gel capsules). In none of the 13 products was the PC concentration indicated on the label. The RBO contents of FT1 and FT2 were 30–50%, and those of FT3 and FT4 100%.

PCs comprise a mixture of very-long-chain saturated fatty alcohols (C24–C34) purified from the natural waxes extracted from plants (Duodu & Awika, 2018). The major form of PC in the commercial rice bran cooking oils was tetracosanol (C24), followed by dotriacontanol (C32), while the main form in the functional RBO was dotriacontanol (C32), followed by octacosanol (C28), and tetracosanol (C24). Tetratriacontanol (C34) was found in one of the rice bran cooking oils (CK5). In contrast to our results, in rice samples analyzed by Kim et al. (2012) and Ishaka et al. (2014), the predominant PCs were octacosanol (C28) and triacontanol (C30).

The PC contents of the commercial rice bran cooking oils ranged from 18.65 to 73.99 mg/100 g (average: 40.24 mg/100 g). In a previous study, the PC contents of crude RBO extracted using hexane and cold pressing

Table 1 Policosanol contents of the commercial rice bran cooking oil products (mg/100 g sample)

Samples	Policosanol (mg/100 g sample)							
	C22	C24	C26	C28	C30	C32	C34	Total
CK1	$0.54 \pm 0.03^{d, B}$	$14.16 \pm 2.04^{d, A}$	$1.07 \pm 0.15^{b, B}$	$0.92 \pm 0.07^{b, B}$	$1.96 \pm 0.08^{a, B}$	ND	ND	18.65 ± 2.21^{e}
CK2	$0.58 \pm 0.04^{d, B}$	$29.62 \pm 0.96^{c, A}$	$0.22 \pm 0.03^{f, B}$	$0.20 \pm 0.01^{f, B}$	$0.41 \pm 0.03^{g, B}$	ND	ND	31.04 ± 1.06^{ef}
CK3	$0.61 \pm 0.01^{cd, B}$	$39.67 \pm 1.88^{b, A}$	$0.74 \pm 0.01^{cd, B}$	$0.38 \pm 0.06^{e, B}$	$0.80 \pm 0.12^{f, B}$	ND	ND	42.19 ± 2.08^{c}
CK4	$0.58 \pm 0.02^{d, B}$	$31.19 \pm 1.37^{c, A}$	$0.49 \pm 0.06^{e, B}$	$0.51 \pm 0.05^{e, B}$	$1.26 \pm 0.07^{bc, B}$	ND	ND	34.03 ± 1.28^{d}
CK5	$0.84 \pm 0.03^{a, B}$	$2.16 \pm 0.05^{e, B}$	$0.68 \pm 0.04^{de, B}$	$0.77 \pm 0.05^{c, B}$	$0.98 \pm 0.14^{ef, B}$	$20.44 \pm 3.93^{b, A}$	3.01 ± 0.20^{B}	28.89 ± 4.06^{f}
CK6	$0.65 \pm 0.01^{cd, D}$	$36.13 \pm 0.34^{b, A}$	$1.10 \pm 0.04^{b, BC}$	$0.85 \pm 0.08^{bc, CD}$	$1.41 \pm 0.10^{b, B}$	ND	ND	40.13 ± 0.54^{c}
CK7	$0.71 \pm 0.12^{bc, C}$	$46.62 \pm 3.09^{a, A}$	$1.67 \pm 0.13^{a, C}$	$1.16 \pm 0.09^{a, C}$	$1.40 \pm 0.03^{b, C}$	$22.43 \pm 3.93^{a, B}$	ND	73.99 ± 2.96^{a}
CK8	$0.60 \pm 0.07^{cd, B}$	$38.04 \pm 1.36^{b, A}$	$1.03 \pm 0.17^{b, B}$	$0.82 \pm 0.02^{bc, B}$	$1.10 \pm 0.12^{cd, B}$	ND	ND	41.59 ± 1.50^{c}
CK9	$0.79 \pm 0.04^{ab, B}$	$48.67 \pm 1.13^{a, A}$	$0.93 \pm 0.03^{bc, B}$	$0.46 \pm 0.06^{e, B}$	$0.81 \pm 0.09^{f, B}$	ND	ND	51.66 ± 1.35^{b}

Each value represents the mean ± S.D

ND indicated amount of detectection lower than LOD

Values with different superscript lowercase letters in a column are significantly different ($P < 0.05$)

Values with different superscript uppercase letters in a row are significantly different ($P < 0.05$)

LOD = 1.08 ppm

Table 2 Policosanol contents of the commercial functional rice bran oil products (soft gel capsule) (mg/100 g sample)

Samples	Policosanol (mg/100 g sample)							
	C22	C24	C26	C28	C30	C32	C34	Total
FT1 (31% rice oil)	$1.03 \pm 0.02^{a, F}$	$19.12 \pm 0.50^{b, C}$	$9.09 \pm 0.29^{b, E}$	$39.46 \pm 1.57^{b, A}$	$26.57 \pm 0.75^{b, B}$	$26.16 \pm 1.12^{b, B}$	$15.79 \pm 0.33^{a, D}$	137.22 ± 4.01^{b}
FT2 (> 50% rice oil)	$0.85 \pm 0.06^{b, D}$	$23.26 \pm 0.95^{a, C}$	$14.44 \pm 0.57^{a, E}$	$46.47 \pm 1.54^{a, B}$	$44.22 \pm 1.86^{a, B}$	$70.56 \pm 7.44^{a, A}$	15.93 ± 0.02^{a}	215.72 ± 2.49^{a}
FT3 (100%rice)	$0.26 \pm 0.04^{c, D}$	$14.60 \pm 1.30^{c, A}$	$2.62 \pm 0.09^{c, E}$	$2.12 \pm 0.08^{c, E}$	$7.88 \pm 0.01^{c, C}$	$9.70 \pm 0.18^{c, B}$	$7.13 \pm 0.28^{b, C}$	44.32 ± 1.96^{c}
FT4 (100% rice)	$0.35 \pm 0.03^{c, E}$	$18.52 \pm 0.45^{b, A}$	$2.50 \pm 0.30^{c, D}$	$2.34 \pm 0.09^{c, D}$	$5.92 \pm 0.51^{c, C}$	$6.89 \pm 0.06^{c, B}$	$4.64 \pm 0.07^{c, E}$	41.16 ± 1.31^{c}

Each value represents the mean ± S.D

FT1-FT4 are the commercial functional products containing rice bran oil

ND indicated amount of detectection lower than LOD

Values with different superscript lowercase letters in a column are significantly different ($P < 0.05$)

Values with different superscript uppercase letters in a row are significantly different ($P < 0.05$)

LOD = 1.08 ppm

methods were 230–246 and 205–219 mg/100 g, respectively (Wongwaiwech et al., 2023). Several studies have shown that RBW is a very good source of PC (Asikin et al., 2012; Ishaka et al., 2014; Pandolsook & Kupongsak, 2020). Wongwaiwech et al. (2019) measured the PC content in by-products from the refining of crude RBO, including defatted rice bran (36.79 mg/100 g), RBW (332.79 mg/100 g), high-melting point wax (HMW; 169.44 mg/100 g), and rice acid oil (RAO; 99.33 mg/100 g). C28 and C30 compounds were the major compounds found in discarded RBW and in the HMW compounds from the refining of crude RBO via solvent extraction. Higher PC contents were determined in the by-products RBW, HMW, and RAO than in RBO subjected to cold-pressed extraction (Wongwaiwech et al., 2019).

The above studies suggest that, since PC is a component of wax, it is lost from RBO during the refining process, especially during the removal of by-products. PC losses probably exceed 50%, with particularly large losses of C28. Wongweiwech et al. (2023) found that C28 and C30 were the major forms of PC in crude RBO extracted via solvent-based and cold pressing methods. Our results suggest that the refining process leads to the loss of large amounts of C28.

Among the commercial functional RBOs, FT2 had the highest PC content (215.72 mg/100 g), followed by FT1 (137.22 mg/100 g); the lowest amount was in FT4 (41.16 mg/100 g). The PC concentration was not stated on the label of any of the commercial functional RBOs. However, since FT1 contained less RBO (around 31%), as indicated on the label, than FT2 (> 50%), its PC content would have been lower. Surprisingly, both FT3 and FT4 claimed to be 100% RBO products but they contained less PC than FT1 and FT2.

PC contents of dietary supplements

The PC contents of the commercial dietary supplements are shown in Table 3. Only two PC products (PDS3 and PDS5) contained the amount expected based on the level claimed by the manufacturer; in the others, the determined amounts were lower. Several studies have reported that the daily consumption of 5–25 mg of PC is effective in lowering total blood cholesterol and LDL levels, while increasing HDL levels (Cho et al., 2018; Fernndez et al., 2017; Kim et al., 2018; Serna-Saldivar et al., 2016), with octacosanol (C28) as the PC component with the strongest activity. Gouni-Berthold and Berthold (2002) reported that 10–20 mg of PC per day lowered total cholesterol levels by 17–21% and LDL cholesterol by 21–29%, while raising HDL cholesterol by 8–15%. They also found that PC in amounts of up to 20 mg per day were safe and well tolerated (Gouni-Berthold & Berthold, 2002). The claimed per-serving amount of PC in the commercial dietary supplements tested in this study ranged from 5 to 20 mg (Table 3).

In PDS1 and PDS3, the PC source was stated to be rice wax, and in PDS2, PDS4, and PDS5, sugarcane wax. Octacosanol (C28) (1,669.89 mg/100 g) was the main PC identified in PDS1, PDS2 (3,143.75 mg/100 g), and PDS5 (5,342.47 mg/100 g), whereas C30 (839.85 mg/100 g) and C26 (593.36 mg/100 g) compounds were the major PCs in PDS3. Octacosanol (C28), C24 (478.18 mg/100 g), and C30 (454.48 mg/100 g) compounds were the major PCs in PDS4.

Studies of the PCs in cane wax have identified octacosanol (C28) and triacontanol (C30) (Asikin et al., 2008;

Table 3 Policosanol contents (mg/100 g) of the commercial policosanol dietary supplement (tablet and soft gel capsule)

Samples	Material source of extraction	Claimed content (mg/capsule)	Policosanol (mg/100 g sample)						PC (mg/capsule)
			C22	C24	C26	C28	C30	Total PC	
PDS 1	RBW	5	ND	ND	$79.96 \pm 6.31^{c, C}$	$1,669.89 \pm 15.77^{c, A}$	$241.60 \pm 20.84^{d, B}$	$1,988.40 \pm 20.84^{c}$	3.56 ± 0.08
PDS 2	SCW	20	ND	$112.12 \pm 11.19^{c, D}$	$786.03 \pm 25.77^{a, C}$	$3,143.75 \pm 215.92^{b, A}$	$1,721.10 \pm 46.30^{a, B}$	$5,763.00 \pm 46.30^{b}$	13.30 ± 0.69
PDS 3	RBW	5	ND	$152.14 \pm 6.65^{b, D}$	$593.36 \pm 6.09^{b, B}$	$439.23 \pm 8.37^{d, C}$	$839.85 \pm 26.19^{c, A}$	$2,024.59 \pm 26.19^{c}$	4.87 ± 0.08
PDS 4	SCW	20	$6.87 \pm 1.06^{a, C}$	$478.18 \pm 10.85^{a, A}$	$126.16 \pm 16.97^{c, B}$	$159.02 \pm 37.27^{d, B}$	$454.48 \pm 59.20^{d, A}$	$1,164.89 \pm 59.20^{c}$	5.92 ± 0.61
PDS 5	SCW	20	ND	$72.47\ 5.93^{d, D}$	$751.37 \pm 42.69^{a, C}$	$5,342.47 \pm 342.81^{a, A}$	$1,380.40 \pm 201.68^{b, B}$	$7,474.13 \pm 201.68^{a}$	19.70 ± 1.55

PDS 1-5 are the commercial products of policosanol containing dietary supplements

Each value represents the mean ± S.D

ND indicated amount of detectection lower than LOD

RBW Rice bran wax, *SCW* Sugarcane wax, *ND* Non detection

Values with different superscript lowercase letters in a column are significantly different ($P < 0.05$)

Values with different superscript uppercase letters in a row are significantly different ($P < 0.05$)

LOD = 1.08 ppm

Meerod et al., 2019, 2020). Vali et al. (2005) identified tetracosanol (C24) and triacontanol (C30), and Ishaka et al. (2014) octacosanol (C28) and hexacosanol (C26) as the predominant PCs of rice bran wax. Consistent with the findings of Cravotto et al. (2004) and Kim et al. (2014) reported octacosanol (C28) to be the predominant component (46.4%) in rice bran, followed by triacontanol (C30) (31.6%) and tetracosanol (C24) (24.3%). In a study by Weerawatanakorn et al. (2017), the major PC in rice wax was triacontanol (C30) (51.6%), followed by hexacosanol (C26) (18.5%) and octacosanol (C28) (15.9%), whereas in crude RBO obtained via cold pressing extraction, octacosanol (C28) (33.6%) and tetracosanol (C24) (32.2%) predominated. The claimed sources of the PCs in the products were consistent with the analytical data. The variations in the amounts and predominant types can be attributed to differences in the rice and cane cultivars, the area of origin, and the chemical extraction and purification methods used to isolate PCs.

Studies have demonstrated that octacosanol suppresses platelet aggregation (Arruzazabala et al., 1994; Sharma et al., 2019), lowers blood cholesterol (Keller et al., 2008; Singh et al., 2006), reduces inflammation (Guo et al., 2017) and fatigue (Zhou et al., 2021), and relieves constipation (Jiang et al., 2020). These results suggest the use of octacosanol as a drug or food supplement for treating metabolic diseases without any side effects.

Stability of PRBO

Physicochemical properties of PRBO

The color and physicochemical properties of $PRBO_{300}$ and $PRBO_{600}$ were compared with those of the RBO control; the results are shown in Table 4. In terms of color, RBO had higher L* and b* values than both PRBOs, although according to their color values both PRBOs were more reddish (Supplementary Fig. 2S). Adding PC to RBO increased the pH only slightly (4.93 versus 4.36) but the viscosity increased significantly ($p < 0.05$) as increasing amounts were added.

Table 4 Physicochemical properties of rice bran oil (RBO) and PC-enriched RBO (PRBO)

Physical properties	RBO		
	Control oil	$PRBO_{300}$	$PRBO_{600}$
Color value			
- L*	42.99 ± 0.16^{a}	42.38 ± 0.43^{b}	40.61 ± 0.36^{c}
- a*	-2.16 ± 0.04^{c}	-2.01 ± 0.05^{b}	-1.68 ± 0.16^{a}
- b*	20.66 ± 0.21^{a}	20.33 ± 0.31^{a}	18.72 ± 0.76^{b}
- C*	20.78 ± 0.21^{a}	20.43 ± 0.31^{a}	18.80 ± 0.77^{b}
- H*	95.98 ± 0.11^{a}	95.65 ± 0.17^{b}	95.13 ± 0.33^{c}
pH value	4.36 ± 0.06^{b}	4.93 ± 0.04^{a}	4.93 ± 0.12^{a}
Viscosity (mPa.s)	88.53 ± 0.15^{b}	89.83 ± 0.6^{a}	90.40 ± 0.89^{a}

Each value represents the mean ± S.D. Values with different superscript letters in the same row are significantly different ($P < 0.05$)

PRBO300 $PRBO_{600}$ = rice bran oil enriched with 300 and 600 ppm of policosanol, respectively

Heat stability of PCs in PRBO

The heat stability of PCs was investigated by heating the PRBOs to temperatures of 150 and 180 °C for 10, 15, and 20 min and then measuring the remaining PC contents. The PC contents in the RBO control, $PRBO_{300}$, and $PRBO_{600}$ before and after heating are shown in Fig. 2 (A and B).

The PC contents of RBO, $PRBO_{300}$, and $PRBO_{600}$ were in the ranges of 30.91–49.13, 60.38–70.64, and 97.59–232.45 mg/100 g, respectively (Fig. 2). Heating RBO and the PRBOs at 150 and 180 °C increased their PC contents, with maximum values reached at 15 and 10 min, respectively (Fig. 2A, B). Heating at 180 °C yielded the highest PC contents: 30.91–59.87, 60.38–162.03, and 97.59–310.13 mg/100 g for RBO, $PRBO_{300}$, and $PRBO_{600}$, respectively (Fig. 2B). However, the PC contents of $PRBO_{300}$ and $PRBO_{600}$ were more stable at 150 °C than at 180 °C. Further heating led to oxidation and a 17.22–22.00% decrease in PC contents. Kim et al. (2014) reported that the PC level in rice bran increased after autoclaving (121 °C). Meanwhile, Bryngelsson et al. (2002) found an increase in lipophilic antioxidants after oats were heated. As proposed in that study, the increased levels determined in our sample might have reflected the liberation of bound forms of PCs (Bryngelsson et al., 2002), given that most of the PCs in rice bran are bound to other compounds, such as proteins and fatty acids (Kim et al., 2014).

PC stability in RBO and PRBOs during 6 months of storage

The stability of RBO and PRBOs over a 6-month period was evaluated. All three samples were kept in amber glass bottles that had been flushed with a nitrogen stream, sealed, and then stored at ambient temperature (27 ± 5 °C). The PC contents were then monitored once a month for 6 months. As shown in Table 5, the PC contents of the three oil samples during the 6-month storage differed significant ($P < 0.05$), with significant increases ($P < 0.05$) that reached a maximum and then decreases. The increased PC contents may have been due to the hydrolysis of triglycerides, which occur at the ester bond between a fatty acid and an alcohol group. Caradec et al. (2004) reported PC formation via the degradation of fatty acids. However, after 6 months of storage there was no significant difference in the PC levels of RBO and $PRBO_{300}$ compared with the initial PC amounts. Possible causes of PC variation during storage include chemical

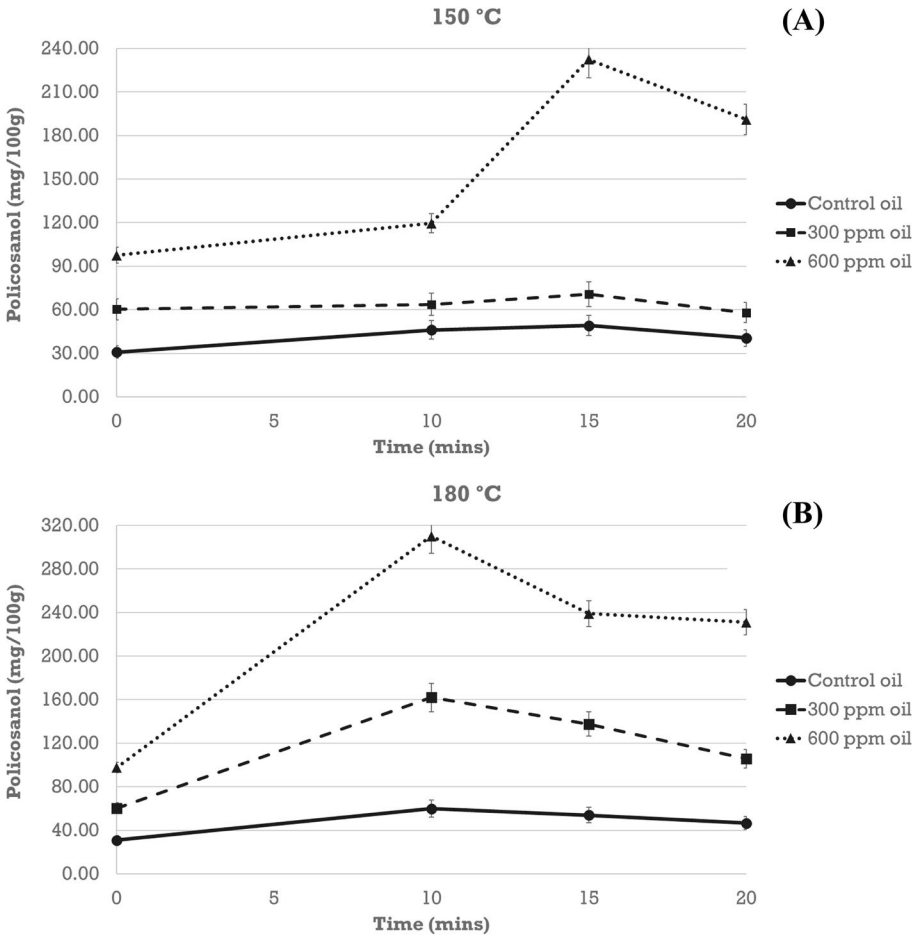


Fig. 2 Heat stability of policosanol content in policosanol enriched rice bran oil (PRBOs) at 300 and 600 ppm at different temperatures of 150°C (**A**) and 180°C (**B**)

Table 5 Changes in the PC contents of control rice bran oil and rice bran oil enriched with policosanol (PRBOs) during 6 months of storage

Time (month)	Policosanol content (mg /100 mg)		
	Control oil	$PRBO_{300}$	$PRBO_{600}$
0	30.91 ± 0.19^{c}	60.38 ± 0.31^{d}	97.59 ± 0.62^{b}
1	33.23 ± 0.11^{a}	67.30 ± 0.05^{a}	113.51 ± 3.21^{a}
2	32.93 ± 0.95^{ab}	65.39 ± 1.40^{b}	113.83 ± 3.32^{a}
3	32.83 ± 0.14^{ab}	63.35 ± 0.06^{c}	110.33 ± 1.92^{a}
4	32.15 ± 0.22^{abc}	63.27 ± 1.23^{c}	108.29 ± 3.44^{a}
5	31.60 ± 0.22^{bc}	61.99 ± 0.21^{cd}	106.78 ± 3.52^{a}
6	31.62 ± 0.21^{bc}	60.89 ± 0.61^{d}	106.59 ± 6.30^{a}

Each value represents the mean ± S.D

Values with different superscript letters in the same column are significantly different ($P < 0.05$)

PRBO300 $PRBO_{600}$ = rice bran oil enriched with 300 and 600 ppm of policosanol, respectively

transformations. For instance, PC levels could decrease as a result of their oxidation into fatty aldehydes and fatty acids (Lukić et al., 2015) or transformation alcohols to form fatty acid esters, as occurs in the transformation of octacosanol (C28) to octacosanoyl ester (Cabrera et al., 2003). Together, these results suggest that the PC contents of RBO and PRBO are stable during storage.

The quality of RBO and PRBOs in terms of the AV and PV was also examined (Figs. 3 and 4). The AV is a widely used indicator of oil deterioration (Sakaino et al., 2022). A lower AV indicates a higher quality and greater stability against hydrolytic rancidity (Arawande & Amoo, 2009). The changes in the AV of the RBO and PRBOs after storage are shown in Fig. 4. Increasing the storage time caused a steady increase in the AV of the oil samples, ranging from 0.13 to 0.18 mg KOH/g. The increase in the AV was higher in the RBO control than in either $PRBO_{300}$ or $PRBO_{600}$. The AV (0.16 mg KOH/g and 0.16 mg KOH/g) of PRBO300 and PRBO600 were similar after 105 days. Oils with higher mono- and polyunsaturated

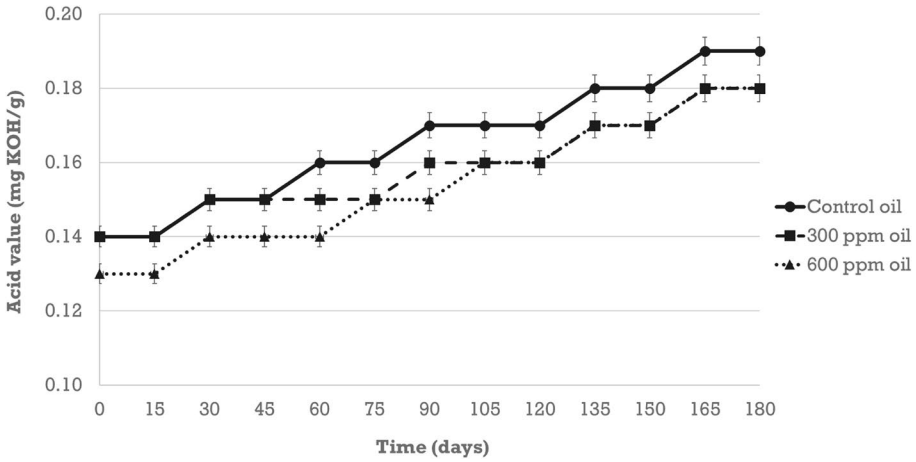


Fig. 3 Acid value (AV) of control rice bran oil and policosanol enriched rice bran oil (PRBOs) at 300 and 600 ppm during storage studies of 6 months

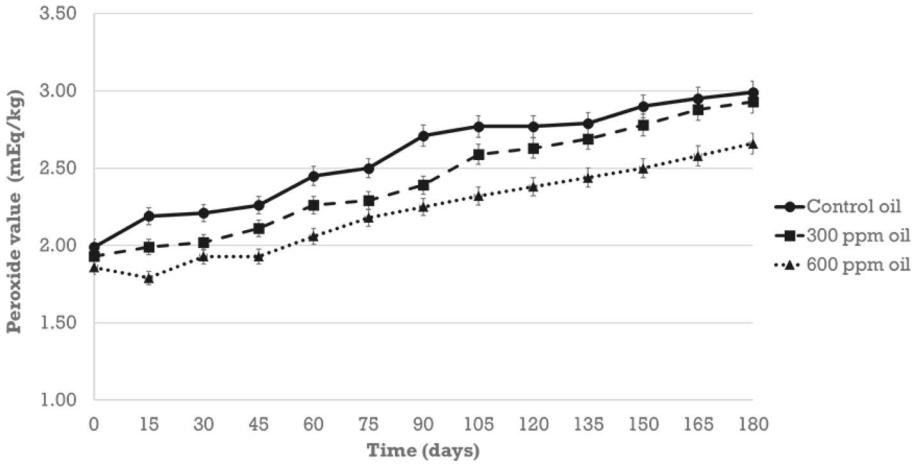


Fig. 4 Peroxide value (PV) of control rice bran oil and policosanol enriched rice bran oil (PRBOs) at 300 and 600 ppm during storage studies of 6 months

fatty acid values might be more prone to oxidation than saturated fatty acids due to the availability of double bonds (Selani et al., 2016). The increase in acid value of rice bran oil during storage is attributed to several chemical mechanisms, primarily related to the hydrolysis of triglycerides by either water or enzyme, oxidative degradation by oxygen and light exposure, and decompose of peroxide (Loypimai et al., 2015; Wu et al., 2020). These higher AV suggest that triglycerides are transformed into fatty acid. PV is a quality measurement for fats and oils that indicates the formation of peroxides and hydroperoxides during the initial stages of lipid oxidation (Zhang et al., 2010). The PV of all samples during storage ranged from 1.99 to 2.99 meq/kg oil and increased linearly with storage time (Fig. 4). Consistent with the AV, after 6 months of storage, RBO had the highest PV (2.99 meq/kg oil) and $PRBO_{600}$ the lowest PV (2.66 meq/kg oil).

The smaller changes in the AV and PV of the PRBOs than the control RBO were likely due to the ability of PCs to inhibit or slow the oxidation of RBO. In addition to their cholesterol-lowering effects, long-chain (C20–C36) aliphatic primary alcohols such as PCs exhibit antioxidant activity and other functional benefits (Harrabi et al., 2018; Kim et al., 2017; Liao et al., 2018; Montserrat-De La Paz et al., 2014). Factors such as temperature, light, moisture, metals, and oxygen affect the rate of oxidation, especially in oils containing unsaturated fatty acids as their main constituents, as is the case in RBO (Adeyanju et al., 2011; Hussain et al., 2021). The results suggest that the antioxidant properties of PC slow the oxidative degradation of rice bran oil (RBO) and work synergistically with other antioxidant compounds present in RBO, including oryzanol, tocopherol, phytosterols, and various phenolic compounds. However, after 180 days of

storage, the AV and PV of all samples were lower than the CODEX standard of fats and oils, according to which the maximum AV and PV of RBO are 0.5 mg KOH/g oil and 10 mg eq/kg oil, respectively.

Conclusions

The major PCs in the tested commercial rice bran cooking oil were tetracosanol (C24) and dotriacontanol (C32); those in commercial functional RBOs were dotriacontanol (C32), octacosanol (C28), and tetracosanol (C24). The PC content of commercial rice bran cooking oil available in markets was around 400 ppm. In commercial functional oils containing RBO as the major ingredient, the PC content ranged from 1,372 to 2,157 ppm. In both rice bran cooking oil and RBO-containing functional oils, the PC content differed from that claimed by the respective manufacturer. The sources of PC dietary supplement were claimed to be sugarcane and rice wax. The main PCs in the tested commercial dietary supplements made from sugarcane wax were octacosanol (C28), triacontanol (C30), and tetracosanol (C24). The claimed level of PC in dietary supplement products (as soft gels or tablets) ranged from 5 to 20 mg per serving. In some of those products, the PC levels were lower than stated on the label. PC was stable under heat treatment and during 6 months of storage.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
M.W conceptualized the study, was involved in supervision, project administration and writing – reviewing and editing manuscript. D.W. collected, analyzed, interpreted data, writing – reviewing and editing manuscript. N.M and S.K involved in chemical analysis and data curation; and interpretation, C.T.H. involved in validation, investigation, and editing manuscript.

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Data availability
The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
Dr. Chi-Tang Ho is a member of Editorial Board of *Food Production, Processing and Nutrition* and he was not involved in the journal's review of, or decisions related to this manuscript.

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