Rice bran oil (RBO) has become a popular oil globally. However, the RBO extraction process leaves various residue products, which contain bioactive substances of varying potency which could be significant sources of functional ingredients for both food production and pharmaceutical manufacture. The objective of our study was to compare the bioactive substances in various by-products derived from the two rice bran oil processing methods; solvent extraction and cold pressing. The residues from solvent extraction processing contained up to 97.37 mg/100 g of \( \gamma \)-aminobutyric acid in defatted rice bran, and the rice acid oil contained high levels of vitamin E (tocopherols, tocotrienols), up to 120.59 mg/100 g, as well as \( \gamma \)-oryzanol (3829.65 mg/100 g), phytosterol (599.40 mg/100 g), and policosanol compounds (332.79 mg/100 g). All of these values are higher than in the residues derived from cold pressing. Importantly, high amounts of total nutraceuticals (8.3 kg/100 kg) were found in residues from both processing methods, indicating the commercial potential of these residues as a source of functional ingredients for food production, as dietary supplements, and in pharmaceutical manufacture.
Thailand has nine rice bran oil companies with fourteen of the extraction and refining factories and produce the crude rice bran oil about 140,000 ton or 4200 million baht each year. More than 640,000 ton of defatted rice bran are remained as low-value agro waste mainly used in the feed industries.

In Thailand, RBO produced from solvent (normally hexane) extraction is preferred as a cooking oil, after further refinement, while the oil produced by cold pressed extraction is considered to be a superior functional oil as a dietary supplement and medicinal purposes. After solvent extraction, further refining of the rice bran oil involves degumming, neutralization, bleaching, dewaxing and deodorizing to produce quality cooking oil, resulting in large amount of waste products left from the processes [4]. Degumming involves the removal of phospholipids and lipoproteins through hydration by water with citric or phosphoric acid [5]. Free fatty acids (FFAs) are removed by caustic soda (NaOH) in the neutralization process [6]. Rice acid oil (RAO) is the major waste product from these two steps. Then, the bleaching step is applied to remove the pigments (including chlorophylls and carotenoids) of the RBO by adsorption on activated carbon or bleaching earth [7]. A dewaxing and winterization steps follow by maintaining the oil at a low and very low temperature respectively to cause the solidified waxes and other high melting point substances as well as high melting point wax are separated as waste products [4,7]. The cold pressed RBO is produced by compressing the rice bran through a mechanical screw press under mild heating (<50°C), and the resulting oil is then filtered through filter paper, to produce the refined functional oil [8]. Defatted rice bran and filter cake are the main by-products from this process.

During both processes, a considerable amount of by-product is produced, some of which contains more or less quantities of potential ingredients for functional food, nutraceuticals and pharmaceutical manufacture [9]. These ingredients include mainly of γ-oryzanol, tocotrienol, tocopherol, phytosterol, lecithin, carotenoids and long-chain alcohols [10,11]. Rice bran wax (RBW) is also a rich source of aliphatic primary alcohols known as policosanols and many studies have shown that rice policosanols moderately decrease plasma cholesterol levels in hypercholesterolemic patients [12,13], reduce platelet aggregation [14].

Understanding the bioactive compounds from rice bran lost into the waste product in the RBO processes, and the importance of these by-products as the source of functional ingredients, is important to and of benefit not only to the rice bran oil industry, but also to the associated industries of functional food, nutraceutical and pharmaceutical manufacture. The objective of the present study was therefore, first, to analyze the bioactive substances, including γ-aminobutyric acid (GABA), tocopherol, tocotrienol, γ-oryzanol, phytosterols, and the policosanol content, in the various by-products from the RBO processes, then to quantify and compare the amounts of these bioactive compounds extractable from the by-products from both the solvent extraction and cold pressed extraction processes.

2. Materials and methods

2.1. Materials

Samples of the residue from processing RBO (cooking oil) using the solvent extraction were provided by Surin Bran Oil Co., Ltd (Buri Ram, Thailand), and other samples of by-products produced from the cold pressed extraction system were provided by at Lopburi Vegetable Oil Industries (Lopburi, Thailand). The samples from both organisations were collected directly from the processing line, immediately after

![Flow chart of rice bran oil (RBO) production](image)

Fig. 1 – Flow chart of rice bran oil (RBO) production (a) RBO from solvent extraction process (b) RBO from cold pressed extraction process.
each refining operation. The collected samples were kept in plastic containers at −20 °C until used.

The samples collected from the solvent extraction process were followed the scheme shown in Fig. 1a. Defatted rice bran (DFRB-S) was obtained by hexane extraction at 50 °C; rice acid oil (RAO) was obtained by the neutralization process; the dewaxing process at 15 °C produced wax (RBW) and high-melting point wax (HMW) was obtained in the winterization step at 5 °C. The samples from the cold pressed extraction process were collected as the chart shown in Fig. 1b, composed of defatted rice bran from a screw press (DRFB-C), and filtered cake (FC); filtered through a 0.25 μm filter paper. The various final by-products used in this study are summarized in Table 1. In all cases, two different lots of samples were analyzed in triplicate.

2.2. Reagents and standards

γ −aminobutyric acid (GABA) (PubChem CID: 119), 5z-cholestane (PubChem CID: 272895) and policosanols standards including docosanol (C22) (PubChem CID: 12620), tetracosanol (C24) (PubChem CID: 10472), hexacosanol (C26) (PubChem CID: 68171), octacosanol (C28) (PubChem CID: 68406) and triacontanol (C30) (PubChem CID: 68972) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Tocopherol and tocotrienol mixed solution standard were purchased from Chro- maDex (Irvine, CA, USA). Total γ-oryzanol (98.5%) (PubChem CID: 5282164) standard was purchased from Tsuno Rice Fine Chemical Co., Ltd (Wakayama, Japan).

Derivatizing reagents and norvaline (PubChem CID: 65098) was from the Phenomenex (Torrance, CA, USA) EZ: faast amino sample test kit for GC/MS profiling of protein hydrolysates by following the instructions offered by the company with slightly modification [16]. A 100 μL volume of extract or standard solution and 50 μL of internal standard (0.20 mM norvaline in 20 mM HCl) were pipetted into a glass vial and passed through a solid phase extraction tip (SPE) came with the “EZ:faast” kit. The amino compounds were eluted from the solid phase with alkaline eluting medium (200 μL) composing of sodium hydroxide solution n-propanol and p–picoline.

The obtained eluate was thoroughly mixed with an organic solution (50 μL) consisting of chloroform as the derivatizing reagent by vortexing for 30 s. The derivates were then extracted by mixture of chloroform and isooctane (100 μL) followed by vortexing for 30 s and he chemical reaction was then allowed to progress for 2 min. The derivates were then redissolved in a mixture of chloroform and isooctane (10/90, v/v). When the mixture was separated, the supernatant (150 μL) was put into a GC vial for analysis. The derivatization was freshly performed.

2.3. GABA GC–MS conditions

GABA analysis was modified from method of Shigematsu et al. [17] with modifications. GC–MS analysis was performed using a Zebcon ZB-AAA column (10 m × 0.25 mm i.d × film thickness 0.25 μm, provided with the EZ:faast amino acid test kit). The 2 μL of derivatized sample were injected into the GC–MS analysis (Agilent Technologies 5973, 6890N) using an ionization energy of 70 eV with a split ratio of 1:15. GABA were identified and quantified by a SIM (single ion monitoring) mode according to their retention times and MS spectra. Norvaline was used for the carrier gas was set at 1.1 mL/min up to 300 °C and held for 2 min. The injector and detector temperatures were set at 320 ºC. Helium as the internal standard. Norvaline (158, 72) and GABA (130, 144, 17) were identified and quantified according to their molecular target ion, m/z, retention time and EZ:faast mass spectral library (Phenomenex).

2.4. γ -oryzanol, tocopherol (Ts) and tocotrienol (T3s) analysis

2.4.1. Extraction of γ -oryzanol, Ts and T3s

γ -oryzanol, Ts and T3s were determined using a modified method of Azrina et al. [18]. One gram of samples were

| Table 1 – The samples collected from rice bran oil process from solvent and cold-pressed extraction from 2 different companies. |
|-----------------------------------------------|
| **RBO** extraction system | By-products | Abbreviation |
|-----------------------------------------------|
| Solvent extraction | Defatted rice bran | DFRB-S |
| | Rice acid oil | RAO |
| | Wax | RBW |
| | High-melting point wax | HMW |
| Cold-pressed extraction | Defatted rice bran | DFRB-C |
| | Filtered cake | FC |
| **RBO** = rice bran oil. |
extracted with 15 mL of chloroform and methanol mixture (3:2, v/v), then vortexed for 10 min, and the mixture allowed to react for 30 min following centrifugation (10 min, 1306 x g). The 500 μL of supernatant was filtered using a 0.45 μm syringe filter and the solution was added to 500 μL of acetonitrile, methanol and isopropanol mixture (25:70:5 v/v). An aliquot was injected onto the HPLC for Ts and T3s and LC-MS for γ-oryzanol.

2.4.2. γ-oryzanol LC-MS conditions
The identification and quantification of γ-oryzanol was performed by a LC-MS using a method of Sakunpak et al. [19]. HPLC analysis was performed using an Agilent Technologies 1100 with diode array detector (DAD) connected to an Agilent Technologies 1100 auto injector. The sample was separated at 40 °C on an Agilent Zorbax Eclipse XDB C18 column (4.6 x 150 mm, 5 μm) using mobile phase consisting of acetonitrile, methanol and isopropanol (25:70:5 v/v) with a flow rate of 1 mL/min (18 min). Injection volume was 20 μL and the γ-oryzanol was determined by UV detection at wavelengths of 298 and 325 nm. The mass spectrometer was an Agilent Technologies LC/MSD SL equipped with an electrospray ion source (ESI). The ESI-MS spectra were acquired in positive ionization mode with the follows: capillary voltage, 4000 V; nebuliser pressure, 50 psi; gas temperature, 350 °C; drying gas, 13.01/min and recorded on a mass range of m/z 200–800. The γ-oryzanol was identified by Agilent Mass Hunter software and based on retention time indicated in the standards.

2.4.3. Ts and T3s RP-HPLC conditions
The Ts and T3s components of rice bran lipid were separated by reverse-phase HPLC determined using method modified from Chen and Bergman [20]. The HPLC system consisted of an Agilent Technologies 1100 with diode array detector (DAD). The injection volume was 20 μL and the samples were separated at 40 °C on a Luna CN 100A column (4.6 x 250 mm, 5 μm). The initial mobile phase conditions of extraction were 94% acetonitrile, 7% MeOH, 5% ISoH, and 0.1% of aq acetic acid and held there for 8 min. The Ts and T3s (δ, β, γ and α forms) were determined by UV/UV-Vis detection at wavelengths of 298 and 328 nm. The Ts and T3 were identified based on retention time indicated in the standards.

2.5. Phytosterol analysis

2.5.1. Extraction of phytosterol
The extraction was conducted based on methods of Beveridge et al. [21] and Thanh et al. [22] with some modification. A 1 g sample was accurately weighed into a screw tube and spiked with 1 mL internal standard into the matrix, followed by 2 mL of KOH (60%), 2 mL of ethanol 95%, 2 mL of NaCl (10%) and 0.3 g of pyrogallol (antioxidant). The mixture was saponification under nitrogen (N2) and then incubated at 70 °C for 45 min (vortexed every 15 min) to ensure complete saponification. After cooling the solution in an ice bath, the saponified portion was extracted with 15 mL of hexane: ethyl acetate (9:1, v/v) twice. The upper layer (unsaponifiables) was collected into a glass tube and the solvent evaporated to dryness under a stream of N2 at 45 °C.

2.5.2. Phytosterol derivatization
The sterols derivatization were prepared following a method modified from Beveridge et al. [21]. The residue was mixed with 200 μL of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) and 100 μL of pyridine, and heated at 60 °C for 30 min, then evaporated to dryness under a stream of N2 at 45 °C. The residues were dissolved with 1 mL hexane and the solution was filtered using a 0.45 μm syringe filter and then transferred into a vial for GC–MS analysis.

2.5.3. Phytosterol GC–MS conditions
The quantification and identification of sterols was performed by a Gas chromatography–mass spectrometry (GC–MS) using a modified method of Thanh et al. [22]. The fused silica capillary GC column was a DB-S5ms (30 m x 0.25 mm i.d. x film thickness 0.25 μm). Samples (1 μL) were injected using an Agilent Technologies 7890A Sampler and a split injector with a split ratio of 1:50. The sterols were separated using programmed oven temperatures originally set at 100 °C (1 min), then raised to 300 °C (14 min) at a rate of 10 °C/min. Helium was used as the carrier gas, at a flow rate of 1.5 mL/min. The ionization energy was set to 70 eV. The injector, MS quad temperatures, MS source and transfer line were 270, 150, 230, and 280 °C, respectively. TMS-phytosterols were identified and quantified by a SIM (single ion monitoring) mode according to their retention times and MS spectra. Cholestane was used for the internal standard. The m/z ratios of the ions used for the quantitative analysis of the TMS; Cholestane (374), TMS; Campesterol (382), TMS; Stigmasterol (484), TMS; β-sitosterol (486) and TMS; Sitostanol (488).

2.6. Policosanols (PCs) analysis

2.6.1. Extraction of PCs
PCs were extracted from full-fatted samples (FC, RBW, RAO and HMW) following the method of Ishaka et al. [23] with some modification. A 1 g sample was placed into a polyethylene tube and hydrolyzed with 0.2 M NaOH (10 mL) prepared in methanol aqueous solution (1:3, v/v) following sonication (8510 Branson Ultrasonics Co., Danbury, CT, USA) 44 Hz (250 W) at 60 °C (90 min). The hydrolyzed sample was then extracted with toluene, cooled down to 2 °C. After centrifugation at 3945 x g, the upper layer was collected into a glass tube and filtered using a 0.45 μm filter.

PCs were extracted from the defatted samples (DFRB-C and DFRB-S) according to the method of Asikin et al. [24]. The defatted sample (5 g) was extracted by Soxhlet method in cellulose thimbles (Whatman 33 mm x 100 mm) with the solvent mixture of hexane and methanol (20:1 v/v) for 18 h. The solvent mixture were then removed using a rotary vacuum evaporator at 40 °C. The obtained extracts were then diluted with toluene and filtered by syringe filter (0.45 μm) for further derivatization.

2.6.2. PCs derivatization
Samples of each of the PCs derivatives were prepared using a method modified from Asikin et al. [25]. A 200 μL
Each value represents the mean ± S.D.
ND indicated amount of detection lower than LOD, the LOD of GABA is 1.08 ppm (LOD and LOQ values of all samples are indicated in Table 2S (supplementary data).
Values with different superscript letters in the same column are significantly different (P < 0.05).
GABA = γ–aminobutyric acid; DFRB–C = defatted rice bran from cold pressed extraction process; FC = filtered cake; DFRB–S = defatted rice bran from solvent extraction process; RBW = rice bran wax; RAO = rice acid oil; HMW = high-melting point wax.

### 2.6.3. GC–MS conditions
Separation, identification and quantification of the PCs were determined using the method modified from Asakin et al. [25]. The PCs were identified by their trimethylsilyl derivates. Analysis was performed with an Agilent Technologies 6890 fitted with an Agilent DB-5ms fused silica capillary column (0.25 mm × 30 m × film thickness 0.25 μm). The GC injector was set at 350 °C. The sample volume of 1 μL was injected using an Agilent Technologies 7893 Auto Sampler with a split ratio of 1:10. The PCs were separated using programmed oven temperatures initially started at 150 °C and increased to 320 °C (15 min) at the rate of 4 °C/min. For MS detection, the electron impact (EI) ion source temperature was set to 200 °C and the transfer line temperature set to 280 °C. Mass spectra were taken at 70 eV; a SIM mode (single ion monitoring) was set for identified and quantified TMS-PCs. TMS; docosanol (C22), TMS; tetracosanol (C24), TMS; hexacosanol (C26), TMS; octacosanol (C28) and TMS; triacanbol (C30) were identified and quantified according to their retention times and molecular target ion, m/z at 383, 411, 439, 467 and 495 respectively.

### 2.7. Statistical analysis
All data obtained in this study were analyzed statistically. The results are expressed as means ± standard deviation (SD) based on dry weight for the triplicate analyses on the same sample. Differences among average values were estimated by analysis of variance (ANOVA) with the application of Duncan's
results found by Pestana et al. [4]. In general, \( \gamma \)-oryzanol is mainly composed of esters of trans-ferulic acid (hydroxycinnamic acid) with phytoesterols predominantly including cycloartenol, \( \beta \)-sitosterol, 24-methylene cycloartenol and campesterol [10].

A significant amount of \( \gamma \)-oryzanol was found in RAO (3901.59 mg/100 g) followed by FC (1058.28 mg/100 g) and RBW (862.80 mg/100 g) (Table 3) and the major oryzanols found in RAO were cycloartenyl ferulate and 24-methylene cycloartenyl ferulate (Fig. 2b). The data shows that the neutralization step in the extract refining process of the RBO from the solvent extraction system caused significant loss of \( \gamma \)-oryzanol into the RAO. The result found is in agreement with the results reported by Pestana et al. [4], thus strongly confirming that nearly all the \( \gamma \)-oryzanol was precipitated during the neutralization step. The strength of the alkali condition used during the refining process for the neutralization of the crude oil promoted carrying over of \( \gamma \)-oryzanol to the RAO. The loss of \( \gamma \)-oryzanol when using caustic soda or solvents and steam in the refining step of the high free fatty acid crude RBO was also reported by Yoon and Kim [31]. They found that 12—59% of the \( \gamma \)-oryzanol was lost during the refining process, using caustic substances [31]. This study strongly confirms that the gamma oryzanol content in acid oil from refining process of RBO is high at 3.9% and it is worth turning acid oil to high value of gamma oryzanol source for medicinal and pharmaceutical manufacture.

The tocotrienol (T3s) and tocopherol (Ts) contents are shown in Table 3. The chromatograms found (Fig. 1S) (supplementary data) were in accordance with the data of Pestana-Bauer et al. [5]. The maximum amount of total vitamin E (T3s + Ts) in the by-product from RBO process was 120.59 mg/100 g (RAO) whereas the minimum was 6.47 mg/100 g (DFRB-S). Compared with tocopherol (Ts), the tocotrienol (T3s) form of vitamin E is the major form of vitamin E found in all by-products. The greater amount of T3s was found in RAO (106 mg/100 g), followed by FC (97.38 mg/100 g) and the highest amount of Ts was also found in the RAO (60 mg/100 g). This was higher than the total concentration of Ts in crude RBO (53 mg/100 g) [7]. For this reason, it is of interest to recover both T3s and Ts from the RBO. The \( \beta \) form of both T3s and Ts were present in minor concentrations in the residues from RBO process. This data agreed with Pestana et al. [32] and other authors confirming that \( \beta \)-tocopherol was present in minor concentrations in RBO. The most abundant of the T3s was \( \gamma \)-T3s that found in RAO (86.01 mg/100 g), followed by \( \alpha \)-T3s found in RBW (18.45 mg/100 g) and \( \delta \)-T3s found in RAO (6.19 mg/100 g) as indicated in Table 3.

Compared with from solvent extraction, the cold pressed extraction process left high amounts of the remaining \( \gamma \)-oryzanol (229.76 mg/100 g) and total vitamin E content (28.25 mg/100 g) in the defatted rice bran (Table 3). The results are in agreement with previous reports on \( \gamma \)-oryzanol content (249—348.2 mg/100 g) found in commercial rice bran of IR-64 rice variety and on total vitamin E content (27.3 mg/100 g) found in enzymatic extract rice bran [33,34]. Generally, crude RBO contains \( \gamma \)-oryzanol 1599—1666 mg/100 g [35] and vitamin E in terms of tocopherol and tocotrienol 53 mg/100 g and 82.5 mg/100 g, respectively [7]. Therefore, solvent extraction appears to offer higher extraction efficiency, but the refining
Fig. 2 – LC-MS chromatograms of γ-oryzanol standard compounds (a) RAO γ-oryzanol extract (b). The numbers on the peaks (a) correspond to the cycloartenyl ferulate (1), 24-methylene cycloartenyl ferulate (2), campesterol ferulate (3), β-sitosteryl ferulate (4).
process of the solvent extraction system may cause a great loss of these bioactive compounds into the other waste, such as RAO from the neutralization step and wax from the winterization step. Finally, the defatted rice bran as a by-product from cold pressed extraction method should receive much greater attention in terms of being a source of γ-oryzanol and vitamin E.

The phytosterol contents of the by-products from the RBO process are also listed in Table 3 and the chromatograms of the phytosterol components of the standard and by-product RAO,
with the comparison with the mass spectra fragmentation pattern, are shown in Fig. 3 (a) & (b). The five characteristic peaks were identified as cholestanol (internal standard) (peak 1 $t_R$ 20.38 min), campesterol (peak 2, $t_R$ 23.43 min), stigmasterol (peak 3, $t_R$ 23.68 min), $\beta$-sitosterol (peak 4, $t_R$ 24.35 min) and sitosterol (peak 5, $t_R$ 24.51 min) and mass fragmentation pattern of individual phytosterols are indicated in Table 1S (supplementary data).

The highest amount of total phytosterol was found in RAO (599.40 mg/100 g) following by FC (243.96 mg/100 g) and the most abundant phytosterol found was $\beta$-sitosterol, followed by stigmasterol and campesterol. The results are in agreement with other reports [35–37]. The phytosterol contents of RAO were in the same range as those reportedly found in RAO (457–564 mg/100 g) from the RBO refining process [35]. These results indicate that RAO produced from the solvent extraction system and FC produced from the cold pressed extraction system are both promising source of phytosterol as functional ingredients.

### 3.3. Policosanol contents of the by-products from rice bran oil processing

RB is a good source of waxes consisting of aliphatic primary alcohols, mainly docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28) and triacontanol (C30), known as policosanols (PCs) [38,39]. Consequently, both RBO and the by-products from the RBO process retain significant amount of PCs. Many studies have reported the serum lipid-lowering properties of PCs which can be used as cholesterol lowering nutraceuticals [40–42]. In this study, the data shown in Table 4 indicates the varying but significant amounts of PCs from various by-products of the RBO process. The PCs concentrations were calculated based on the peak area of each standard curve. Each PC component was identified by the mass fragment pattern of its derivative as the target ion in m/z. including 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), and m/z 495 (qualifier ions, m/z 103, 496, 497) for docosanol (C22-OH), tetracosanol (C24-OH), hexacosanol (C26-OH), octacosanol (C28-OH), triacontanol (C30-OH), respectively [24] as indicated by the GC–MS chromatograms (Fig. 4).

In comparison with other residues, RBW from the solvent extraction system presented the highest amount of PCs (332.79 mg/100 g) followed by HMW (169.44 mg/100 g) and RAO (99.33 mg/100 g). These data was confirmed by the report of Cravotto et al. [38] and Ishaka et al. [23], showing that rice bran is a good source of PCs. The data given in Table 4 also agrees with the study by Kim et al. [43] and Ishaka et al. [23] who indicated that the predominant PCs components in rice were octacosanol (C28-OH) and triacontanol (C30-OH). The PCs content of the by-products RBW, HMW, RAO and FC, is even higher than the PCs contents of RBO from the cold pressed extraction system (74.9 mg/100 g) (data not shown).

Defatted rice bran (DFRB) is the predominant by-product of rice bran oil (RBO) both by solvent and cold pressed extraction system [26]. In the current study, we identified only a low amount of PCs in the DFRB contained as compared with other by-products that we tested. However, our previous data showed that extraction of the defatted rice bran from the cold-pressed rice bran oil process by saponification and purification gave higher amounts of PCs at the level of 6005.28 mg/100 g [44]. In this current follow-up study we showed that the further processing of the extracts by separation and purification increased the level of PCs and other nutraceuticals in the by-products. The result demonstrated that RBW and HMW from RBO process from solvent extraction system are a significant source of PCs.

### 3.4. Comparative amount of nutraceuticals of the by-products from the solvent extraction and cold pressed extraction systems

The total of bioactive compounds remaining in the by-products from the whole RBO process, both the solvent extraction and cold pressed extraction systems, are shown in Table 5. Compared with the cold pressed extraction system, the solvent extraction system with its various refining steps generated a variety of by-products with a huge amount of the bioactive compounds remaining. These included GABA, $\gamma$-oryzanol, phytosterol, vitamin E, and policosanols in 6.6 kg/100 kg waste products remained. $\gamma$-oryzanol is the major nutraceutical found in the by-products from both the solvent extraction (4839.16 mg/100 g) and the cold pressed extraction (1288.04 mg/100 g) system. The results also demonstrate that most of the nutraceuticals, including $\gamma$-oryzanol, phytosterol, PCs, and vitamin E, found in the by-products (Tables 3 and 4), exhibit health benefits especially in terms of reducing the risk of cardiovascular disease [45,46]. In Thailand, defatted rice

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**Table 4 – Policosanol contents in residues form two different RBO process, obtained using GC–MS.**

| PCs (mg/100 g) | Types of sample | DFRB-C | FC | DFRB-S | RAO | RBW | HMW |
|---------------|-----------------|--------|----|--------|-----|-----|-----|
| Docosanol (C22) | 5.08 ± 0.02⁠a | 3.75 ± 0.11⁠d | 3.35 ± 0.01⁠d | 7.20 ± 0.42⁠b | 7.99 ± 0.21⁠b | 7.50 ± 0.03⁠b |
| Tetracosanol (C24) | 21.56 ± 0.55⁠c | 24.24 ± 2.02⁠c | 13.94 ± 0.94⁠b | 52.36 ± 2.38⁠c | 53.80 ± 0.10⁠a | 45.06 ± 1.50⁠b |
| Hexacosanol (C26) | 1.83 ± 0.03⁠d | 11.56 ± 0.16⁠c | 1.72 ± 0.50⁠c | 10.63 ± 0.11⁠c | 42.75 ± 1.94⁠c | 13.82 ± 0.73⁠b |
| Octacosanol (C28) | 10.15 ± 0.45⁠c | 12.13 ± 0.27⁠c | 11.12 ± 0.31⁠c | 10.16 ± 0.01⁠c | 71.83 ± 2.65⁠b | 26.10 ± 0.08⁠b |
| Triacontanol (C30) | 6.91 ± 0.17⁠c | 24.30 ± 0.70⁠c | 6.66 ± 0.13⁠c | 18.96 ± 0.32⁠c | 156.41 ± 2.99⁠a | 76.92 ± 3.08⁠b |
| **Total policosanol** | **45.55 ± 0.14⁠c** | **76.00 ± 3.29⁠d** | **36.79 ± 0.48⁠d** | **99.33 ± 3.24⁠b** | **332.79 ± 7.27⁠c** | **169.44 ± 0.91⁠b** |

| Each value represents the mean ± S.D. |
| Values in the table are expressed on a dry basis. |
| Values with different superscript letters in the same row are significantly different (P < 0.05). |

DFRB-C = defatted rice bran from cold pressed extraction process; FC = filtered cake; DFRB-S = defatted rice bran from solvent extraction process; RBW = rice bran wax; RAO = rice acid oil; HMW = high-melting point wax.
bran, the most abundant by-products, was discarded from manufacture about 640,000 tons each year (Report of International Conference on Rice Bran Oil 2017). This finding here showed that total amount of bioactive compound found in defatted rice bran from cold pressing method was 321.03 mg/100 g and from solvent extraction process was 181.45 mg/100 g (Tables 2–5) and when compare with rice bran from different origin found that defatted rice bran from Thailand had a fair

Fig. 4 – Chromatogram for PCs standards (a) and HPW PCs extract (b). Docosanol (C22-OH) (1), Tetracosanol (C24-OH) (2), Hexacosanol (C26-OH) (3), Octacosanol (C28-OH) (4), Triacontanol (C30-OH) (5).
amount of bioactive compound (Table 5) (supplementary data). Consequently, total amount of nutraceuticals remained in defatted rice bran, one type of many types of by products from rice bran oil manufactures, is estimated around 3200 tons per year (based on 640,000 tons of defatted rice bran). Making use of these disposed materials is not only smart economics, but also good for environment. With some extra processes including extraction and purification, these by-products can move from low-value agro waste to high-value bioactive ingredients that could benefit the food, cosmetics and drug industries.

4. Conclusions

Defatted rice bran from the solvent extraction process showed the highest amount of γ-aminobutyric acid (GABA) (97.37 mg/100 g). The highest amounts of γ-oryzanol (3829.65 mg/100 g), tocopherol (106.94 mg/100 g), tocotrienol (60.72 mg/100 g), and total phytosterol (599.40 mg/100 g) were found in RAO from solvent extraction process. The RBW derived from the dewaxing step of the solvent extraction system contained the highest amount of Pcs (332.79 mg/100 g). The predominant Pcs of rice bran oil waste product were triacontanol (C30) followed by octacosanol (C28). Total nutraceuticals of the by-products from the solvent extraction system (6.6 kg/100 g) was higher than that from the cold pressed extraction system (1.7 kg/100 g).

Conflicts of interest

All authors have no conflicts of interest to declare.

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Table 5 – The total comparative amount of bioactive compounds of the by-products from the solvent extraction and cold pressed extraction systems.

| Nutraceuticals | Solvent extraction system (mg/100 g) | Cold pressed extraction system (mg/100 g) |
|----------------|--------------------------------------|------------------------------------------|
| GABA           | 97.37                                | 29.22                                    |
| γ-Oryzanol     | 4839.16                              | 1288.04                                  |
| Phytosterol    | 787.12                               | 249.41                                   |
| Vitamin E      | 204.73                               | 140.67                                   |
| Policosanol    | 647.11                               | 121.55                                   |
| Total amount of nutraceuticals (kg/100 kg) | 6.6 | 1.7 |

Values in the table are expressed on a dry basis.

GABA = γ-aminobutyric acid.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jfda.2018.06.006.

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