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REVIEW

Supercritical CO_2 Extraction of Rice Bran Oil – the Technology, Manufacture, and Applications

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Abstract: Rice bran is a good source of nutrients that have large amounts of phytochemicals and antioxidants. Conventional rice bran oil production requires many processes that may deteriorate and degrade these valuable substances. Supercritical CO_2 extraction is a green alternative method for producing rice bran oil. This work reviews production of rice bran oil by supercritical carbon dioxide (SC-CO_2) extraction. In addition, the usefulness and advantages of SC-CO_2 extracted rice bran oil for edible oil and health purpose is also described.

Key words: rice, rice bran oil, supercritical carbon dioxide extraction, phytochemicals, antioxidants

1 Introduction

Globally, approximately 610 million metric tons of rice (*Oryza sativa*) are produced every year[1]. Rice bran is a by-product (about 8% of rice paddy) derived from the rice milling process in which the outer layers of the rice kernel are removed. These layers include the hull, the germ, and the bran, which is a combined layer of pericarp, seed coat, nucellus, aleurone layer, and sometimes embryo[2]. Up to 60 million metric tons of rice bran are generated annually[3]. Rice bran is a good source of protein, fatty acids, dietary fiber and some minerals including iron, phosphorus and magnesium[4]. Full fat rice bran contains, on average, 18–22% oil and its unsaponifiable lipids including phytosterols, tocopherols, tocotrienols, γ-oryzanols, squalene, polycosanols, and carotenoids[5, 6]. These lipids are known to have many health benefits. Polyphenols, vitamin E, tocotrienols and carotenoids from rice bran can help prevent oxidative damage to DNA and other body tissues[7]. Since the quantity of γ-oryzanols is up to 10 times greater than that of vitamin E in rice bran, γ-oryzanols would play a greater role as an antioxidant in the reduction of cholesterol oxidation than would vitamin E, which has been considered to be the major antioxidant in rice bran[8]. Physiological advantages of these functional compounds have been reported as anti-oxidative, neuroprotective, anti-hypercholesterolemic, and anti-angiogenic properties[9−12].

Despite their health benefits, it is difficult for consumers to obtain rice bran functional compounds on a daily basis because rice bran is largely removed during the process of producing white rice. Instead, about 90% of the rice bran produced annually in the world is used cheaply as animal feed for cattle and poultry. Using rice bran as animal feed undermines its economic value in the nutraceutical, pharmaceutical and cosmeceutical industries[13]. However, in recent decades, there are increasing applications of rice bran in the food industries for increasing the nutritional quality of processed foods. Typically, rice bran oil (RBO) is edible oil extracted from rice bran. RBO can be used as cooking oil or as a certain ingredient in some oriental dishes. Addition of rice bran in various foods contributes to the development of value-added foods or functional foods that now are of increasing interest to the public. Rice bran has been successfully supplemented in bread, cakes, noodles, pasta, and ice creams without any negative effect on their functional and textural properties. To the applications available, using rice bran oil as a cooking oil or as a supplement appears to be the easiest and straightforward way to obtain the functional nutrients of rice bran.

This review describes overviews of RBO, supercritical carbon dioxide technology, the production of RBO using supercritical carbon dioxide technology, and the usefulness, advantages, and applications of RBO supercritically extracted.

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2 Rice bran oil

Rice bran oil (RBO) is a hydrophobic liquid extracted from crude rice bran. RBO is composed of saponifiable lipids (96%) and unsaponifiable compounds (4%). Typically, the saponifiable lipids include triaclyglycerols (47% monounsaturated fats, 33% polyunsaturated fats, and 20% saturated fats), glycolipids and phospholipids. Unsaponifiable lipids in RBO are tocopherols, tocotrienols, γ-oryzanols, sterols and carotenoids[14], most of which have been reported for nutritional benefits such as antioxidative and chemopreventive properties[13]. RBO is the richest source of natural oryzanols. RBO phytochemicals have wide range of positive health properties, including hypolipidemic, anti-atherogenic, and anti-diabetic properties[15]. At present, RBO supplements are now being recommended as a good source of these bioactive compounds.

Regarding RBO preparation techniques, the most used method is solvent extraction, where hexane is most commonly used as the extraction solvent. Hexane is highly volatile and considered toxic to animals and humans. Typically, conventional methods usually involve high temperature, which is partly responsible for the degradation of valuable substances. Also, the use of hexane as a solvent can lead to product contamination with solvent residues[16]. Crude RBO obtained from the solvent extraction method contains the predominant triacylglycerols (more than 80%) along with various impurities such as waxes, gums, phosphatides, free fatty acids, and coloring substances. Nonetheless, oil refinements are unavoidable processes required for RBO manufacture. These processes include settling or filtration of bran fines, degumming, dewaxing, deacidification, bleaching and deodorization[17]. For instance, the high content of free fatty acid in crude RBO requires neutralization with alkali to eliminate the free fatty acid. This step causes a decrease in the oil yield and waste water pollution since a considerable amount of triaclyglycerols and soap are emulsified together in the alkali solution. Thus, due to a number of processes necessary for RBO production, some bioactive compounds either deteriorate or separate into unusable oil fractions. For instance, vitamin E (tocopherols and tocotrienols) is concentrated mainly in deodorized rice bran scum oil but not in commercial rice bran oil[18]. Degumming and dewaxing has been reported to remove 1.1% and 5.9% of γ-oryzanols from the original crude oil[19].

In order to achieve the best benefit of rice bran phytochemicals, the technology that can sustain the nutritive value of rice bran functional compounds needs to be considered.

3 Supercritical carbon dioxide extraction

Supercritical fluid extraction is a separation technology that uses supercritical fluid as the extraction solvent. The technique is regarded as a green technology for extracting sensitive or valuable natural compounds from complicated plant samples, and it has attracted considerable attention recently in the food, pharmaceutical, and cosmetic industries[20, 21]. Carbon dioxide (CO_2) is considered as a supercritical fluid when it is above its critical temperature of 31.1℃ and critical pressure of 7.38 MPa. This makes supercritical carbon dioxide (SC-CO_2) an ideal extraction solvent for thermally sensitive compounds. A number of studies evidence that CO_2 is a good solvent for extracting lipid-soluble compounds and it enables a high level of recovery[22]. The hallmark characteristics of SC-CO_2 are its density, viscosity, diffusivity, heat capacity and thermal conductivity[23]. The high density provides better solubility of compounds and its low viscosity facilitates penetration into sample materials with less friction.

For the working principle, CO_2 is introduced at specific pressure and temperature conditions. The conditions are adjusted to allow the CO_2 to attain supercritical solvent properties for extraction of lipids from a sample. When a sample is exposed to SC-CO_2 under controlled conditions of temperature, pressure and time, lipids from the sample are dissolved in the supercritical fluid. By regulating the system pressure, the dissolved lipids can then be separated from the supercritical solvent[24]. As a result, targeted lipids are successfully extracted from the sample without extraction solvent residue remaining.

SC-CO_2 extraction technology is now in focus within the food and health industries because of its advantages in terms of chemistry, environment and economy. Due to its supercritical state, SC-CO_2 is more effective in penetrating porous solid materials than are liquid solvents, which contributes to better mass transfer and leads to faster extraction. A study by Henning and co-workers[24] reported that the extraction time could be reduced from many hours using solvent extraction to a few minutes of using SC-CO_2 extraction. CO_2 itself is non-flammable and non-toxic, so it can be safely used in food and health related products. Since SC-CO_2 solvent can be easily removed from an extract, repetition of the extraction can be done rather quickly. By depressurizing SC-CO_2, extracted compounds are easily purified with minimal loss of volatile components. During extraction, a fluid continuously flows through the sample, resulting in complete extraction of targeted compounds[25]. The physical properties of extraction fluid, such as density, diffusability, and viscosity, can be manipulated by changing extraction temperature and pressure. Doing this enhances or depresses the ability of the SC-CO_2 to extract targeted compounds[26]. At optimum conditions, overall extraction efficiency and selectivity of the specific compounds can be improved. The extraction is employed at relatively low temperature, which would reduce loss of thermally labile compounds[27]. The process

of supercritical fluid extraction normally reduces the use of organic solvents[28]. In larger scale SC-CO_2 extraction, depressurized SC-CO_2 after sample extraction can be recycled and reused, thereby minimizing waste generation and reducing operating cost. The technology can be up-scaled relatively easily from an analytical scale to preparative, pilot plant, and industrial scales[29]. Despites its advantages, SC-CO_2 extraction has some limitations that need to be considered. To achieve the highest extraction efficiency, extraction time must be long enough to allow solvent to penetrate into solid particles and for solute (target compounds) to diffuse from inside to the solvent. Phase equilibrium of the solvent-solute system is rather complicated, making design of extraction conditions difficult[30]. Another disadvantage of SC-CO_2 extraction technology is the requirement for high pressures to maintain the solvent in supercritical state. This results in higher capital costs for plants and operating costs[31]. Typically, SC-CO_2 extraction technology is used where there are significant advantages (e.g., recovery of high value functional compounds, when conventional extractions are inappropriate, and need treatments of toxic wastes).

Recently, SC-CO_2 extraction has been employed to extract RBO for health purpose. A study by Kuk and Dowd[32] reported that the concentration of γ-oryzanols in SC-CO_2 extracted RBO was higher than in RBO derived from hexane-Soxhlet extraction. Another study showed that RBO extracted using SC-CO_2 contained high levels of tocopherols and tocotrienols, suggesting that the oil as a good source of natural vitamin E[33]. However, although SC-CO_2 has shown some potential for extraction of high quality RBO, understanding the influence of extraction parameters as well as material preparation are factors to be considered in achieving the best benefits from rice functional compounds.

4 SC-CO_2 extraction of RBO

Considering extraction of edible RBO, the ideal extraction method should result in total recovery and high purity of the isolated lipids without harmful chemical residue. Due to the inherent variability in chemical and physical properties of rice bran components, many substances can be extracted by SC-CO_2. Manipulation of the extraction conditions, especially temperatures, pressures, extraction time, and co-solvents (so-called "modifiers") is necessary to obtain maximum extraction efficiency of RBO. Optimal temperature and pressure are very important since they influence the extraction yield and the extract composition[34]. Extractions using only SC-CO_2 normally yield good recoveries of non-polar lipids[35].

The solubility of RBO in SC-CO_2 as affected by temperature and pressure was studied by Tamita *et al.*[36]. They found that the influence of increasing pressure (2 to 4 MPa) on RBO solubility was greater than that of increasing temperature (60 to 80℃). The finding was explained by the effect of greater diffusion of SC-CO_2 in rice bran matrix and increased vapor pressure of oil with the increased temperature. At lower pressure, the density of SC-CO_2 is considerably decreased, which causes lower diffusion of SC-CO_2 into RBO[36]. Considering as an alternative to the conventional solvent extraction method of RBO, RBO derived from SC-CO_2 extraction (testing range of 40–80℃ and 15–25 MPa) contained fatty acid profile consisting of oleic acid (39–51%), linoleic acid (28–38%), and palmitic acid (20–22%), which was comparable to RBO derived from compressed liquefied petroleum gas extraction having oleic acid (35–48%), linoleic acid (30–44%), and palmitic acid (19–22%)[37]. The study by Kuk and Dowd[32] used SC-CO_2 at 62 MPa and 100℃ to obtain a maximal oil yield of 20.4%. This is comparable to hexane extraction at 69℃ and 0.101 MPa that yield 20.5% oil. Although the both extraction methods could provide RBO with not much difference in yield and antioxidant activity, CO_2 as solvent is considered as generally recognized as safe (GRAS), this being the main advantage of using it instead of other compressed gases[37]. In general, the amount of free fatty acids (palmitic acid, oleic acid, and linoleic acid) in SC-CO_2 extracted RBO is much less than that obtained from hexane extraction, indicating that SC-CO_2 has lower affinity to free fatty acid than hexane[32]. When rice bran is extracted with SC-CO_2, the constituents of the selective fractions obtained at different pressures differed[38]. Oil fractions obtained at higher pressure had less free fatty acid, waxes and unsaponifiable matters. The contents of iron and phosphorus were found very low in SC-CO_2 extracted oil. The color was also significantly lighter than that of hexane-extracted RBO[38].

Since RBO is known to have a rather high acid value, conventional RBO extraction requires a deacidification process for refinement of RBO. Regarding RBO manufacturing, free fatty acid in edible RBO is restricted not to exceed an acid value of 0.2, which is equivalent to 0.1% free fatty acid. In India, refined RBO can have a maximum acid value of 0.5, which is equivalent to 0.25% free fatty acid[39]. A study by Chen *et al.*[39] reported that SC-CO_2 under the conditions of the pressure of 20 to 30 MPa and temperature from 70 to 90℃ was able to allow deacidification or RBO up to 97.8%.

In addition to being used for total fat quantification, the extraction parameters (pressure, temperature, time, and modifier) in supercritical fluid extraction can be manipulated to selectively extract particular compounds from sample matrices into the oil fraction. The study by Sarmento's group[33] investigated the influence of temperature (25–60℃) and pressure (15–20 MPa) in extracting RBO to obtain fractions enriched with tocopheol and tocotrienol.

In the experiment, tocols from the extracted oil varied from 35 to 940 mg/100g oil, suggesting that the amount of tocols in RBO is sensitively affected by operational conditions and that optimal conditions for the highest tocol amounts were 20 MPa and 40℃. It has been evidenced that regular RBO that is commercially refined using conventional caustic refining contains only a small amount of γ-oryzanols, that high oryzanol RBO, commercially refined using special technique, contains about 0.6% γ-oryzanols, and that $SC\text{-}CO_2$ processed RBO contains 1.78% oryzanols. This difference suggests that $SC\text{-}CO_2$ as a technology for production of a phytosterol-enriched vegetable-oil extract[40]. A study by Wang *et al.*[41] employing $SC\text{-}CO_2$ extraction as a technology for preparing γ-oryzanols from powdered rice bran reported that total RBO yield was 18.1% and the extraction efficiencies of γ-oryzanols and triglycerides were 88.5 and 91.3% respectively. The concentration of γ-oryzanols and triglycerides in $SC\text{-}CO_2$-extracted RBO was higher than that in Soxhlet-hexane extracted oil[41].

In general, higher concentrations of the targeted compounds were obtained using longer extraction times. However, when the solubility of any targeted compounds in a sample reached phrase equilibrium with $SC\text{-}CO_2$, the increase in extraction efficiency slowed down. This suggests that the optimum extraction time needs to be considered in regard to extraction efficiency and operational cost performance. There was a study on solubility of γ-oryzanols in $SC\text{-}CO_2$ taking advantage of this principle[42]. The study demonstrated that at the beginning of $SC\text{-}CO_2$ extraction, both the oil and the γ-oryzanol extraction rates were constant. However, later the oil extraction rate sharply declined, though γ-oryzanol extraction rate was almost constant, resulting the last extraction fractions to contain higher concentrations of γ-oryzanols than the very first fractions. The findings suggest the possibility of producing a γ-oryzanol-rich fraction through a process wherein the γ-oryzanols is not extracted together with oil, but is extracted in later steps after most of the oil fraction is excluded[42]. In addition, $SC\text{-}CO_2$ extraction can be used with other preparation techniques to obtain maximum amount of targeted compounds. For instance, the work by Chen *et al.*[14] studied the use of $SC\text{-}CO_2$ as a tool for extracting γ-oryzanols from the raw material powdered rice bran. $SC\text{-}CO_2$ extraction at 35 MPa and 40℃ for 4 h yielded 17.5% oil with 85% extraction efficiency of γ-oryzanols. With combination of a normal-phase medium-pressure column partition fractionation, γ-oryzanols were successfully concentrated up to 37% wt of RBO[14].

Since $SC\text{-}CO_2$ is relatively highly non-polar, species of polar lipids may remain unextracted. Therefore, rice bran samples containing antioxidants with functional polar groups may be difficult to extract. Rice bran antioxidants normally include vitamin E (tocopherols and tocotrienols), phytosterols (mostly γ-oryzanols), and vitamin A. The polarity of $SC\text{-}CO_2$ can be improved by adding co-solvents with polarity, including methanol, ethanol, isopropanol or even water. Among available co-solvents, ethanol is the preferable choice for food applications since ethanol is generally recognized as safe (GRAS). A study by Ramsay and co-workers[43] demonstrated that the addition of 5 wt% ethanol to the $SC\text{-}CO_2$ solvent stream could improve oil yield from 17.98 to 18.23%, implying that more polar compounds, including antioxidants, are better extracted by increased polarity of the solvent than by carbon dioxide alone. A previous study by Sookwong *et al.*[44] also evidenced that using 10 wt% ethanol as the co-solvent could improve overall extraction of rice bran bioactive compounds including tocopherols, tocotrienols, γ-oryzanols, and xanthophylls. The study was aimed to optimize operational conditions for the highest antioxidants, and the optimized conditions were 60 min, 43℃, and 37.4 MPa with 10% ethanol as a modifier[44]. However, there was a report indicating that excessive use of modifier, such as 10% to 30%, could negate the recovery of oil extract[45].

As mentioned earlier, by controlling extraction variables, physical and chemical properties of $SC\text{-}CO_2$ can be altered and differ solubility of targeted compounds. Experimental designs using response surface methodology (RSM) based on the central composite design (CCD) have been employed to evaluate desired variables related to RBO extraction (e.g., extraction efficiency, yield, and concentration of targeted compounds). RSM is an effective combination of mathematical and statistical technique useful for investigating complex processes where numerous factors and their interaction affect the desired response[46]. CCD is a factorial or fractional factorial design with center points augmented with a group of axial points that is commonly used in response surface designed experiments. For applications, a number of researchers have employed RSM and CCD methodology on $SC\text{-}CO_2$ extraction of RBO. It has been used for supercritical extraction of RBO with high γ-oryzanol content[14, 41]. Operation parameters were optimized for the highest oil recovery[47], for oil with highest antioxidants[44], and for the best operation processes of RBO deacidfication[39].

Together with the process optimization, sample preparation is another critical factor for extraction of high quality RBO. Generally, particle size of sample affects lipid recovery due to sample surface area directly exposed to $SC\text{-}CO_2$[48]. A sample with high moisture content tends to minimize its contact with $SC\text{-}CO_2$, and moisture acts as a barrier to the diffusion of $SC\text{-}CO_2$ inside the sample and the diffusion of lipids outside the sample[49]. Different varieties of rice bran also affect the quality of RBO. Sookwong *et al.*[44] extracted RBOs from various rice bran samples, including pigmented and non-pigmented glutinous and non-glutinous varieties, using $SC\text{-}CO_2$, and found that the

amount of bio-functional compounds in the extracted RBOs was rather variety dependant and that pigmented rice bran tended to provide RBO with greater amounts of vitamin E and xanthophylls. Considering using pigmented rice as a raw material, Nakornriab and co-workers[50] could quantify trans-β-carotene (33–41 ppm), quercetin (1.08–2.85 ppm) and isorhamnetin (0.05–0.83 ppm) as characteristic phytochemicals found in SC-CO_2 extracted oils from the black rice bran, as determined using high-performance liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS). These findings are congruent with other studies that showed that pigmented rice has superior nutrition value compared to ordinary white rice[51], suggesting pigmented rice bran to be a good source of RBO with the finest functional constituents.

5 Usefulness, advantages and applications of SC-CO_2 extracted RBO

This part of the review describes some usefulness as well as advantages of RBO extracted with SC-CO_2. It was reported by Balachandran *et al.*[52] that RBO extracted with SC-CO_2 had an oil yield comparable to that extracted with hexane (22.5%). The SC-CO_2 oil contained little amount of phosphatides, wax and pro-oxidant metals (Fe and Cu), and the color quality of the oil was far superior to that of hexane-extracted oil. The RBO phytochemical contents under the optimum conditions of 50 MPa, 60℃ for 90 min were up to 1,800 ppm of tocols, 19,000 ppm of sterols, and 11,000 ppm of oryzanols[52]. On the other hand, the stabilities of RBOs obtained by SC-CO_2 extraction and hexane Soxhlet method were studied during accelerated shelf-life storage[53]. Compositionally, unsaponifiable matters and γ-oryzanols of SC-CO_2 samples were higher than from hexane Soxhlet extraction, but the amount of tocopherol was lower. After storage for 42 days at 70℃, the peroxide value, free fatty acid percentage, conjugated diene and *p*-anisidine values of the Soxhlet extracted RBO were higher than those of SC-CO_2 extraction. Thus, the SC-CO_2 RBO samples showed better stability than those RBO samples obtained from Soxhlet extraction[53]. Besides its stability in terms of edible oil, the physiological safety of SC-CO_2 extracted RBO has also been evaluated. Choi *et al.*[54] assessed the safety of SC-CO_2 extracted RBO by 3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay in mouse leukemic monocyte macrophage cell line (RAW264.7) as well as the single-oral-dose toxicity in Sprague-Dawley rats. The cytotoxicity assay revealed no inhibitory effects on cell viability and no significant changes were observed in any clinical symptoms, even at the estimated average lethal dose (ALD) at more than 10,000 mg/kg, implying that oral administration of SC-CO_2-RBO was considered as deemed safe[54]. Another safety study by Choi *et al.*[55] was conducted using three short-term mutagenicity assays, including bacterial reverse mutation assay, *in vitro* mammalian chromosomal aberration test, and *in vivo* micronucleus assay. The results demonstrated that SC-CO_2-RBO can be considered as a non-genotoxic material based on the *in vivo* and *in vitro* results.

Regarding physiological benefits of SC-CO_2-RBO, only a few studies have been conducted and reported. A study by Ismail and co-workers[56] investigated whether a γ-oryzanol-rich fraction prepared by SC-CO_2 extraction could regulate the expression of antioxidant and oxidative stress related genes in stressed rat's liver. The results showed that the SC-CO_2-RBO could up-regulate some antioxidant genes, such as Hao1, Apo E gene, SOD1 and CAT, and down-regulate some oxidative stress genes, such as Ubb, Stip1, Nfkbib, and Oxsr1, which was possibly due to the presence of antioxidants including γ-oryzanols and tocols[56]. The findings suggest physiological potency of SC-CO_2 extracted RBO as dietary antioxidant supplement. There was a study on the safety and efficacy of SC-CO_2 extracted RBO in the treatment of androgenic alopecia[57]. The results showed that the SC-CO_2-RBO significantly increased hair density and hair diameter in male subjects with no adverse effects, suggesting a promising use of SC-CO_2-RBO in the functional cosmetics and pharmaceutical industries[57]. Another study by Posuwan *et al.*[58] evaluated the influence of long-term supplementation of SC-CO_2 extracted RBO from pigmented rice bran (Riceberry) into streptozotocin-induced diabetic rats. The results showed that the Riceberry bran oil could decrease malondialdehyde and restored superoxide dismutase, catalase, glutathione peroxidase, coenzyme Q and ORAC levels in the rats, as regenerative changes of the pancreas, kidneys, heart and liver were observed[58]. These findings suggest physiological benefits of pigmented SC-CO_2-RBO, on diabetes by decreasing oxidative stress and recovering organ histology.

6 Perspective and conclusions

We have reviewed the importance of RBO as a good edible oil for cooking and as a good source of rice bran phytochemicals. The phytochemicals have been reported for their health benefits such as anti-oxidative, neuroprotective, anti-hypercholesterolemic, anti-angiogenic properties, and so forth. However, conventional RBO manufacture mostly relies on solvent extraction as the method for extracting lipids from the rice bran material. This results in a complex mixture of lipid classes that requires processes of oil refinement. The refinement processes generally employ chemicals and high temperature that can separate, deteriorate and degrade functional substances, thereby diminishing the nutritive value of RBO. To overcome these prob-

lems, together with the possible hazard of chemical residues, $SC\text{-}CO_2$ extraction is introduced as a green alternative production method of RBO. $SC\text{-}CO_2$ extraction takes advantages of $SC\text{-}CO_2$ properties being oil-soluble, nontoxic, cost-effective, and easily removed from the extract following decompression for extracting RBO. This review describes the influence of operation parameters and related factors in order to achieve high quality RBO. Using $SC\text{-}CO_2$, RBO can be separated from the rice bran, and the extracted oil can have a superior appearance, a higher amount of antioxidants, and little undesired matrixes, such as phosphatides, wax and metals. The usefulness, advantages and applications of $SC\text{-}CO_2$ extracted RBO as edible oil and for health purpose are also explained.

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References

1) Sereewatthanawut, I.; Prapintip, S.; Watchiraruji, K.; Goto, M.; Sasaki, M.; Shotipruk, A. Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. *Bioresour. Technol.* 99, 555-561 (2008).
2) Gul, K.; Yousuf, B.; Singh, A.K.; Singh, P.; Wani, A.A. Rice bran: nutritional values and its emerging potential for development of functional food – a review. *Bioact. Carbohydr. Dietary Fibre* 6, 24-30 (2015).
3) Pourali, O.; Asghari, F.S.; Yoshida, H. Sub-critical water treatment of rice bran to produce valuable materials. *Food Chem.* 115, 1-7 (2009).
4) Oliveira, M.S.; Feddern, V.; Kupsk, L.; Cipolatti, E.P.; Furlong, E.B.; Soares, L.A.S. Changes in lipid, fatty acids and phospholipids composition of whole rice bran after solid-state fungal fermentation. *Bioresour. Technol.* 102, 8335-8338 (2011).
5) Sookwong, P.; Nakagawa, K.; Murata, K.; Kojima, Y.; Miyazawa, T. Quantitation of tocotrienol and tocopherol in various rice brans. *J. Agric. Food Chem.* 55, 461-466 (2007).
6) Zhang, M.W.; Zhang, R.F.; Zhang, F.X.; Liu, R.H. Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. *J. Agric. Food Chem.* 58, 7580-7587 (2010).
7) Jariwalla, R.J. Rice-bran products: phytonutrients with potential applications in preventive and clinical medicine. *Drugs Exp. Clin. Res.* 27, 17-26 (2001).
8) Xu, Z.; Hua, N.; Godber, J.S. Antioxidant activity of tocopherols, tocotrienols, and gamma-oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-azobis (2-methylpropionamidine) dihydrochloride. *J. Agric. Food Chem.* 49, 2077-2081 (2001).
9) Khanna, S.; Roy, S.; Slivka, A.; Craft, T.K.; Chaki, S.; Rink, C.; Notestine, M.A.; DeVries, A.C.; Parinandi, N.L.; Sen, C.K. Neuroprotective properties of the natural vitamin E α-tocotrienol. *Stroke* 36, 2258-2264 (2005).
10) Miyazawa, T.; Shibata, A.; Sookwong, P.; Kawakami, Y.; Eitsuka, T.; Asai, A.; Oikawa, S.; Nakagawa, K. Antiangiogenic and anticancer potential of unsaturated vitamin E (tocotrienol). *J. Nutr. Biochem.* 20, 79-86 (2009).
11) Qureshi, A.A.; Sami, S.A.; Salser, W.A.; Khan, F.A. Dose-dependent suppression of serum cholesterol by tocotrienol-rich fraction (TRF25) of rice bran in hypercholesterolemic human. *Atherosclerosis* 161, 199-207 (2002).
12) Serbinova, E.; Kagan, V.; Han, D.; Packe, L. Free radical recycling and intramembrane mobility in the antioxidant properties of alpha-tocopherol and alpha-tocotrienol. *Free Radical Bio. Med.* 10, 263-275 (1991).
13) Patel, M.; Naik, S. Gamma-oryzanol from rice bran oil: a review. *J. Sci. Ind. Res. India* 63, 569-578 (2004).
14) Chen, C.-R.; Wang, L.-Y.; Wang, C.-H.; Ho, W.-J.; Chang, C.-M.J. Supercritical carbon dioxide extraction of rice bran oil and column partition fractionation of γ-oryzanols. *Sep. Purif. Technol.* 61, 358-365 (2008).
15) Chen, C.W.; Cheng, H.H. A rice bran oil diet increases LDL-receptor and HMG-CoA reductase mRNA expressions and insulin sensitivity in rats with streptozotin/nicotinamide-induced type 2 diabetes. *J. Nutr.* 136, 1472-1476 (2006).
16) Sparks, D.; Hernandez, R.; Zappi, M.; Blackwell, D.; Fleming, T. Extraction of rice brain oil using supercritical carbon dioxide and propane. *J. Am. Oil Chem. Soc.* 83, 885-891 (2006).
17) Ghosh, M. Review on recent trends in rice bran oil processing. *J. Am. Oil Chem. Soc.* 84, 315-324 (2007).
18) Bruscatto, M.H.; Zambiazi, R.C.; Sganzerla, M.; Pestana, V.R.; Otero, D.; Lima, R.; Paiva, F. Degradation of tocopherols in rice bran oil submitted to heating at different temperatures. *J. Chromatogr. Sci.* 47, 762-765 (2009).
19) Zullaikah, S.; Melwita, E.; Ju, Y. Isolation of oryzanol from crude rice bran oil. *Food Chem.* 100, 299-302 (2009).
20) Chen, Y.T.; Ling, Y.H. An overview of supercritical fluid

extraction in Chinese herbal medicine: from preparation to analysis. *J. Food Drug Anal.* 8, 235-247 (2000).

21) Ferreira, S.R.S.; Nikolov, Z.L.; Doraiswamy, L.K.; Meireles, M.A.A.; Petenatee, A.J. Supercritical fluid extraction of black pepper (*Piper nigrun* L.) essential oil. *J. Supercrit. Fluids* 14, 235-245 (1999).

22) Awasthi, A.; Trivedi, R.K. A review of supercritical carbon dioxide extraction of natural products. *Chem. Eng. World* 32, 65-71 (1997).

23) Xu, L.; Zhan, X.; Zeng, Z.; Chen, R.; Li, H.; Xie, T.; Wang, S. Recent advances on supercritical fluid extraction of essential oils. *Afr. J. Pharm. Pharmacol.* 5, 1196-1211 (2011).

24) Henning, J.A.; Core, R.J.; Gardea-Torresdey, J.L. Extracting volatile compounds from single plants using supercritical fluid extraction. *Crop Sci.* 34, 1120-1122 (1994).

25) Stashenko, E.E.; Puertas, M.A.; Combariza, M.Y. Volatile secondary metabolites from *Spilanthes americana* obtained by simultaneous steam distillation–solvent extraction and supercritical fluid extraction. *J. Chromatogr. A* 752, 223-232 (1996).

26) Dunford, N.T.; Teel, J.A.; King, J.W. A continuous counter current supercritical fluid deacidification process for phytosterol ester fortification in rice bran oil. *Food Res. Int.* 36, 175-181 (2003).

27) Dron, A.; Guyer, D.E.; Gage, D.A.; Lira, C.T. Yield and quality of onion flavor oil obtained by supercritical fluid extraction and other methods. *J. Food Process Eng.* 20, 107-123 (1997).

28) Otterbach, A.; Wenclawiak, B.W. Supercritical fluid extraction kinetics of pyrethrins from flowers and allethrin from paper strips. *J. Anal. Chem.* 365, 472-474 (1999).

29) Herrero, M.; Mendiola, J.A.; Cifuentes, A.; Ibánez, E. Supercritical fluid extraction: recent advances and applications. *J. Chromatogr. A* 1217, 2495-2511 (2010).

30) Beckman, E.J. Supercritical and near-critical CO_2 in green chemical synthesis and processing. *J. Supercrit. Fluids* 28, 121-191 (2004).

31) Perrut, M. Supercritical fluid applications: Industrial developments and economic issues. *Ind. Eng. Chem. Res.* 39, 4531-4535 (2000).

32) Kuk, M.S.; Dowd, M.K. Supercritical CO_2 extraction of rice bran. J. Am. Oil Chem. Soc. 75, 623-628 (1998).

33) Sarmento, C.M.P.; Ferreira, S.R.S.; Hense, H. Supercritical fluid extraction (SFE) of rice bran oil to obtain fractions enriched with tocopherols and tocotrienols. *Braz. J. Chem. Eng.* 23, 243-249 (2006).

34) Reverchon, E.; De Marco, I. Supercritical fluid extraction and fractionation of natural matter. *J. Supercrit. Fluids* 38, 146-166 (2006).

35) Temelli, F. Perspectives on supercritical fluid processing of fats and oils. *J. Supercrit. Fluids* 47, 583-590 (2009).

36) Tomita, K.; Machmudah, S.; Wahyudiono; Fukuzato, R.; Kanda, H.; Quitain, A.T.; Sasaki, M.; Goto, M. Extraction of rice bran oil by supercritical carbon dioxide and solubility consideration. *Sep. Purif. Technol.* 125, 319-325 (2014).

37) Soares, J.F.; Pra, V.D.; de Souza, M.; Lunelli, F.C.; Abaide, E.; da Silva, J.R.F.; Kuhn, R.C.; Martínez, J.; Mazutti M.A. Extraction of rice bran oil using supercritical CO_2 and compressed liquefied petroleum gas. *J. Food Eng.* 170, 58-63 (2016).

38) Zhao, W.; Shishikura, A.; Fujimoto, K.; Arai, K.; Saito, S. Fractional extraction of rice bran oil with supercritical carbon dioxide. *Agric. Biol. Chem.* 51, 1773-1777 (1987).

39) Chen, C.-R.; Wang, C.-H.; Wang, L.-Y.; Hong, Z.-H.; Chen, S.-H.; Ho, W.-J.; Chang, C.-M.J. Supercritical carbon dioxide extraction and deacidification of rice bran oil. *J. Supercrit. Fluids* 45, 322-331 (2008).

40) Dunford, N.T.; King, J.W. Phytosterol enrichment of rice bran oil by a supercritical carbon dioxide fractionation technique. *J. Food Sci.* 65, 1395-1399 (2000).

41) Wang, C.-H.; Chen, C.-R.; Wu, J.-J.; Wang, L.-Y.; Chang, C. -M. J.; Ho, W.-J. Designing supercritical carbon dioxide extraction of rice bran oil that contain oryzanols using response surface methodology. *J. Sep. Sci.* 31, 1399-1407 (2008).

42) Bitencourt, R.G.; Filho, W.A.R.; Paula, J.T.; Garmus, T.T.; Cabral, F.A. Solubility of γ-oryzanol in supercritical carbon dioxide and extraction from rice bran. *J. Supercrit. Fluids* 107, 196-200 (2016).

43) Ramsay, M.E.; Hsu, J.T.; Novak, R.A.; Reightler, W.J. Processing rice bran by supercritical fluid extraction. *Food Technol.* 45, 98-104 (1991).

44) Sookwong, P.; Suttiarporn, P.; Boontakham, P.; Seekhow, P.; Wangtueai, S.; Mahatheeranont, S. Simultaneous quantification of vitamin E, γ-oryzanols and xanthophylls from rice bran essences extracted by supercritical CO_2. *Food Chem.* 211, 140-147 (2016).

45) Seo, J.S.; Burri, B.J.; Quan, Z.; Neidlinger, T.R. Extraction and chromatography of carotenoids from pumpkin. *J. Chromatogr. A* 1073, 371-375 (2005).

46) Badwaik, L.; Prasad, K.; Deka, S. Optimization of extraction conditions by response surface methodology for preparing partially defatted peanut. *Int. Food Res. J.* 19, 341-346 (2012).

47) Ahmad, S.W.; Javed, F.; Ahmad, S.; Akram, M; Rehman, A. Parametric optimization of rice bran oil extraction using response surface methodology. *Pol. J. Chem. Technol.* 18, 103-109 (2016).

48) Eller, F.J.; King, J.W. Determination of fat content in foods by analytical SFE. *Semin. Food Anal.* 1, 145-165 (1996).

49) Dunford, N.T.; Temelli, F. Extraction conditions and moisture content of canola flakes as related to lipid composition of supercritical CO_2 extracts. *J. Food Sci.* 62, 155-159 (1997).
50) Nakornriab, M.; Sriseadka, T.; Wongpornchai, S. Quantification of carotenoid and flavonoid components in brans of some Thai black rice cultivars using supercritical fluid extraction and high-performance liquid chromatography-mass spectrometry. *J. Food Lipids* 15, 488-503 (2008).
51) Pitija, K.; Nakornriab, M.: Sriseadka, T.; Vanavichit, A.; Wongpornchai, S. Anthocyanin content and antioxidant capacity in bran extracts of some Thai black rice varieties. *Int. J. Food Sci. Tech.* 48, 300-308 (2013).
52) Balachandran, C.; Mayamol, P.N.; Thomas, S.; Sukumar, D.; Sundaresan, A.; Arumughan, C. An ecofriendly approach to process rice bran for high quality rice bran oil using supercritical carbon dioxide for nutraceutical applications. *Bioresour. Technol.* 99, 2905-2912 (2008).
53) Mariod, A.; Ismail, M.; Abd Rahman, N.F.; Matthaus, B. Stability of rice bran oil extracted by SFE and soxhlet methods during accelerated shelf-life storage. *Grasas Aceites* 65, 1-10 (2014).
54) Choi, J.-S.; Moon, W.-S.; Moon, J.-N.; Cheon, E.J.; Son, S.W.; Kim, J.-W.; Kim, M.-R. Cytotoxicity and single-dose oral toxicity testing for rice bran supercritical CO_2 extract. *Toxicol. Environ. Health Sci.* 5, 215-220 (2013).
55) Choi, J.-S.; Cheon, E.J.; Kim, T.-U.; Moon, W.-S.; Kim, J.-W.; Kim, M.-R. Genotoxicity of rice bran oil extracted by supercritical CO_2 extraction. *Biol. Pharm. Bull.* 37, 1963-1970 (2014).
56) Ismail, M.; Al-Naqeeb, G.; Mamat, W.A.A.; Ahmad, Z. Gamma-oryzanol rich fraction regulates the expression of antioxidant and oxidative stress related genes in stressed rat's liver. *Nutr. Metab.* 7: 23 (2010).
57) Choi, J.-S.; Park, J. B.; Moon, W.-S.; Moon, J.-N.; Son, S.W.; Kim, M.-R. Safety and efficacy of rice bran supercritical CO_2 extract for hair growth in androgenic alopecia: a 16-week double-blind randomized controlled trial. *Biol. Pharm. Bull.* 38, 1856-1863 (2015).
58) Posuwan, J.; Prangthip, P.; Leardkamolkarn, V.; Yamborisut, U.; Surasiang, R.; Charoensiri, R.; Kongkachuichai, R. Long-term supplementation of high pigmented rice bran oil (*Oryza sativa* L.) on amelioration of oxidative stress and histological changes in streptozotocin-induced diabetic rats fed a high fat diet; Riceberry bran oil. *Food Chem.* 138, 501-508 (2013).