

Enrichment of soybean oil with tocopherols and γ -oryzanol from rice bran through ohmic heating-assisted ultrasound extraction

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Abstract

Soybean oil enrichment with tocopherols and γ -oryzanol from rice bran using ohmic heating-assisted ultrasound extraction with soybean oil as a green solvent was investigated. Moisturized bran (30% wet basis) samples were ohmically-treated at 100 and 200 V/cm and then subjected to ultrasound extraction using soybean oil as a green solvent. Untreated rice bran and bran steamed with an autoclave before ultrasound extraction with soybean oil were used as controls (UB-UASO and CB-UASO). Ohmic heating applied to assist ultrasound soybean oil extraction (OH-UASO) gave higher yields of α - and γ -tocopherols and γ -oryzanol compared to UB-UASO and CB-UASO. Enriched oil obtained by OH-UASO also gave the highest antioxidant activities (DPPH, FRAP and total antioxidant capacity assays), with low concentrations of free fatty acids, thiobarbituric acid (TBA) and peroxide values (PV) during storage for 28 days. Production of edible vegetable oils as a green solvent containing high tocopherols and γ -oryzanol was achieved, with improved efficiency through ohmic heating.

Introduction

Rice bran is a valuable by-product generated during the rice milling process that contains high quantities of bioactive substances such as tocopherols, tocotrienols, γ -oryzanol and phenolic compounds (Loypimai et al., 2009; Loypimai et al., 2015). Tocopherols and γ -oryzanol are currently generating increasing interest because of their potential beneficial effects on human health and improvement of food storage stability.

Organic solvents including methanol, ethanol and hexane have been commonly used to recover tocopherols and γ -oryzanol from rice bran (Loypimai et al., 2009; Loypimai et al., 2016). One major concern about the use of these solvents is their toxicity to humans and environmental pollution. Extraction using edible vegetable oils as a solvent is a green process that produces natural products such as β -carotene from carrots using sunflower oil (da Silva et al., 2020), green absolute from thyme using sunflower oil (Mnayer et al., 2017), carotenoids from pomegranate wastes using vegetable oils (Goula et al., 2017) and γ -oryzanol from heat-treated rice bran using corn oil (Yi et al., 2020). Using vegetable oil as a solvent gives a protective barrier against attracting oxygen molecules that cause oxidation and degradation of desired compounds such as carotenoids (Pu et al., 2010). Vegetable oils are also biodegradable and nontoxic and perform similarly to organic solvents (Goula et al., 2017), while the product obtained can be directly used as a functional ingredient in food formulation (Saini et al., 2018). However, oil viscosity is a critical problem impacting its diffusivity and extractability. A novel extraction method combined with vegetable oil can improve mass transfer of the solvent and obtain higher yields of the desired lipophilic compounds such as tocopherols and γ -oryzanol.

Recently, ultrasound has become a popular technique for extracting bioactive substances from food materials with low process time, high penetration depth and improved product quality and extraction yields. During the ultrasound extraction process, mechanical and cavitation phenomena induce rupture of the cell walls and particle size reduction resulting in enhanced mass transfer across the cell membrane and, consequently, increased recovery yield of bioactive compounds (Wang et al., 2013; Goula et al., 2017;

Mnayer et al., 2017). However, ultrasound extraction efficiency depends on several aspects including the contained chemical compounds and physical structures of the food materials, types of solvent used, pretreatment techniques and extraction conditions. Here, ultrasound was combined with a new hybrid extraction technology to enhance product quality and increase recovery yield.

In ohmic heating (OH), the innate electrical resistance of foods generates heat when passing a direct electric current. Most food materials contain moisture and ionic components such as salts and conductive acids (Palaniappan and Sastry, 1991; Loypimai et al., 2009). In OH, food materials containing these compounds generate heat by converting electrical energy into heat energy. This causes a rapid homogeneous increase in temperature and avoids the loss of valuable nutrients and heat-sensitive compounds in the food (Palaniappan and Sastry, 1991; Loypimai et al., 2015). OH is the preferred choice for food processing methods such as blanching, evaporation, pasteurization, sterilization and extraction. In particular, extraction by applying an electric field under OH disrupts the cell membranes of plant materials through electroporation and increases the capability of solute diffusion and extraction. OH-assisted extraction increased extraction yields of polyphenols from red grapes (El-Darra et al. 2013), phenolic compounds from cornelian cherry (Kutlu et al., 2021), steviol glycosides and phytochemicals from *Stevia rebaudiana* leaves (Moongngarm et al., 2020) and anthocyanins, tocols and γ -oryzanol from black glutinous rice bran (Loypimai et al., 2015; Loypimai et al., 2019).

To the best of our knowledge, there are no reports regarding the extraction of bioactive compounds from rice bran by ohmic heating-assisted ultrasound extraction using soybean oil as a green substitute for organic solvents. Therefore, this study investigated the enrichment of soybean oil with tocols and γ -oryzanol from rice bran using ohmic heating-assisted ultrasound extraction. The enriched oils obtained were characterized for physicochemical qualities and results were compared to conventional extraction methods and commercial soybean oil.

Materials And Methods

Materials

Fresh rice bran (*Oryza sativa* L.) with 8% degree of milling was obtained from a local rice processing factory in Nakhon Ratchasima Province, Thailand. The bran was immediately passed through a 750 μm (20 mesh) sieve to separate foreign materials, packed into a polyethylene bag and stored at -20°C in a freezer. The initial moisture content of the bran sample was determined by AOAC (2000).

Purified soybean oil as the solvent was purchased from a local supermarket (Bangkok, Thailand). The tocol and γ -oryzanol contents in the soybean oil and raw rice bran sample were determined before experimentation.

Chemicals and reagents

Standard tocopherols including α -tocopherol, δ -tocopherol, γ -tocopherol and γ -tocotrienol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Standard γ -oryzanol was purchased from Tsuno Food Industrial Co., Ltd. (Wakayama, Japan). Methanol (HPLC grade) and n-butanol were purchased from BHD (Poole, UK). All chemicals and reagents were of analytical grade.

Ohmic heating-assisted ultrasound extraction with soybean oil

Ohmic heating was used as a pretreatment to assist ultrasound extraction of tocopherols and γ -oryzanol from rice bran using soybean oil as a solvent. In previous studies by Loypimai et al. (2009 and 2015), deionized water was sprayed directly into the rice bran sample to increase the 30% (wet basis) moisture content. Each moisturized bran sample (180 g) was ohmically-treated at two levels of electric field strengths ($E=100$ and 200 V/cm). During ohmic heating of the rice bran, the voltage, current and temperature were continuously recorded using a data logger controller (Digicon, DP-74SD). When the coldest point of the bran reached 105°C , this temperature was held for 1 min before removing the bran from the chamber and cooling to room temperature.

Ohmically-treated bran samples were extracted for tocopherols and γ -oryzanol by ultrasound using soybean oil as a green solvent (OH-UASO), following the procedure of Loypimai et al. (2020) with slight modifications. Briefly, 40 g of the ohmically-treated bran sample were added to a beaker containing 200 mL of soybean oil. The mixture was then subjected to radiation using an ultrasound (VCX 500 Vibra-Cell™, Sonics & Materials Inc., USA) equipped with a titanium microprobe (diameter 13 mm, length 136 mm, weight 340 g) (model CV334, USA). Parameters including amplitude levels and solution temperature were set at 40% and 65°C , with power of 500 W at 20 kHz following our previous study (Loypimai et al., 2020). During the extraction process, the solution temperature in pulse mode was measured continuously using a probe controller. After ultrasonic extraction, the mixture was filtered twice through a vacuum pump (Buchi, Switzerland) using Whatman No. 4 filter paper and centrifuged at $6,000 \times g$ for 10 min to obtain the enriched oil sample. All experiments were performed in triplicate.

Untreated rice bran and bran steamed with an autoclave (ACV-3167 IWAKI) at 115°C were included as controls. The inside temperature of the sample was raised to 105°C , held for 1 min, then removed from the chamber and cooled to ambient temperature. Untreated bran and conventionally treated bran were then subjected to ultrasound soybean oil extraction and defined as UB-UASO and CB-UASO, following the same procedure used for tocopherols and γ -oryzanol.

Color measurement

Colors of the enriched oils obtained from the different extraction methods were determined using a Colorimeter (Minolta, model CR400, Japan) and calibrated before each analysis with white and black standard tiles. Color readings were expressed following the CIELAB system for L^* (darkness to lightness), a^* (green to red) and b^* (blue to yellow). The L^* , a^* and b^* values were also used to calculate total color change (ΔE).

HPLC analysis for tocols and γ -oryzanol

The solvent (soybean oil), raw rice bran and enriched oils obtained from the different methods were analyzed for tocol and γ -oryzanol contents using an HPLC-PDA apparatus following the method of Gimeno et al. (2001) with slight modifications. Each oil extract sample (0.5 g) was added to 5.0 mL of n-hexane and homogenized for 1 min using a vortex mixer. The mixture was then filtrated through a 0.45 μm nylon syringe filter (Whatman, USA) and separated on a C18 analytical column (Phenomenex 4 μm , C18, 150 \times 4.6 mm) with temperature controlled at 45°C, protected by a security guard column (Phenomenex 4 μm , C18, 50 \times 4.6 mm). The mobile phase solvent under gradient elution was methanol: water: 1-butanol (92: 4: 4 (%v/v)) at a flow rate of 1.0 mL/min for 12 min. The mobile phase was then changed to methanol: water: 1-butanol (92: 3: 5 (%v/v)) at a flow rate of 1.5 mL/min for 25 min. Total gradient run time was 25 min before returning to the initial condition. The eluent band was monitored using a UV absorbance detector set at 292 nm for α -tocopherol, δ -tocopherol and γ -tocopherol and 325 nm for γ -oryzanol. Tocols and γ -oryzanol in the test sample were verified by comparing retention times with the reference standards. The increases in tocols or γ -oryzanol contents in the enriched oils were calculated as follows:

Tocols or γ – oryzanol ($\mu\text{/g}$) content

= content in enriched oil – content in the soybean oil (solvent)

Antioxidant activity analysis

The oil extract was prepared for antioxidant activity analysis following the method reported by Loypimai et al. (2015). Briefly, the sample (1.0 g) was dissolved in 5 mL of mixture solvents (methanol: hexane, 3:2) by placing the mixture on a sonicator (Vibra-Cell™, 130 W, 20 kHz) for 5 min, followed by evaporating in a rotary evaporator to remove the solvent. The residue was then dissolved in 2 mL of methanol and antioxidant activity was analyzed using three different chemical assays based on diverse food system mechanisms.

Diphenyl-2-picrylhydrazyl (DPPH)

DPPH radical-scavenging activity was evaluated using a UV-Vis Spectrophotometer (G10S UV-Vis model, Thermo Fisher Scientific, China). The values were calculated as the concentration of the sample providing 50% of inhibition activity (IC_{50}) (Dasgupta and De, 2004).

Ferric reducing antioxidant power (FRAP)

Reducing power of the extract was determined by the capacity to convert Fe^{3+} -TPTZ to a blue-colored Fe^{2+} -TPTZ (Benzie and Strain, 1996). Absorbance was then measured at 539 nm, with results expressed in $\mu\text{mol FeSO}_4$ equivalent/g enriched oil sample.

Total antioxidant capacity (TAC)

TAC assay was determined following the method of Dasgupta and De (2004). The result was expressed as the number of standard synthetic gallic acid equivalent/g enriched oil sample.

Chemical quality analysis

Changes in chemical quality of the enriched oil samples obtained from the different extraction methods during storage at room temperature were determined by AOCS (1997), and results were compared to commercial soybean oil.

Free fatty acids (FFA) were measured by titration of the sample (1.0 g) with alkali and calculated as oleic acid (AOCS Cd 3a-63).

The peroxide value (PV) was determined by titrating the oil sample (0.5 g) with sodium thiosulfate solution (AOCS Cd 8-53).

Thiobarbituric acid (TBA) was measured by heating a 5 mL aliquot of a solution of sample (50–200 mg) in 25 mL 1-butanol with 5 mL TBA reagent at 95°C for 120 min, and reading the absorbance at 530 nm (AOCS, 1997). All determinations were carried out in triplicate.

Statistical analysis

Data were analyzed using F-test (one-way ANOVA) with a statistical package program (SPSS trial version). Results were reported as mean values and standard deviations from triplicate samples of each treatment for all experiments. Duncan's multiple range test was performed to determine significant differences between treatments, with statistical significance declared at $p < 0.05$.

Results And Discussion

Characterization of soybean oil and raw rice bran

Before extraction of rice bran, the tocopherols and γ -oryzanol contents in the soybean oil as solvent were determined (data not shown). Three tocopherols including α -tocopherol ($44.35 \pm 7.34 \mu\text{g/g}$), γ -tocopherol ($35.22 \pm 7.25 \mu\text{g/g}$) and δ -tocopherol ($148.6 \pm 18.4 \mu\text{g/g}$) were present in the soybean oil solvent. These findings concurred with Loypimai et al. (2020) who reported that three isomers of tocopherol present in purified soybean oil were α -tocopherol, γ -tocopherol and δ -tocopherol. Grilo et al. (2014) reported that α -tocopherol and γ -tocopherol were observed in commercial soybean oils at average concentrations of $71.3 \pm 6.4 \mu\text{g/g}$ and $273.3 \pm 11.1 \mu\text{g/g}$, respectively. As expected, γ -oryzanol was not detectable in soybean oil as solvent, as this compound is a unique phytochemical which is distributed mostly in rice grains, especially in the rice bran layer. This result was similar to previous studies (Loypimai et al., 2009; Zhang et al., 2013; Loypimai et al., 2015), who reported rice bran and rice bran oil containing high concentration

of γ -oryzanol ranging from 115 to 4,200 $\mu\text{g/g}$. Here, the soybean oil was enriched with tocopherols and γ -oryzanol from rice bran through ohmic heating-assisted ultrasound extraction as a green process.

Influence of extraction methods on content of tocopherols

Extraction yields of α - and γ -tocopherols of the enriched oils obtained from different extraction methods are shown in Figure 1. The enriched oils obtained from rice bran treated by OH ($E = 100$ and 200 V/cm) and extracted using ultrasound-assisted soybean oil (OH-UASO) yielded higher γ -tocopherol than extracted by CB-UASO and UB-UASO, respectively. Maximum yields of α -tocopherol ($123.4 \pm 8.7 \mu\text{g/g}$) and γ -tocopherol ($263.5 \pm 12.2 \mu\text{g/g}$) were observed in the enriched oils using OH-UASO at $E = 100 \text{ V/cm}$ with extraction time of 60 min, due to the combined effects of electroporation of OH as pre-treatment and cavitation of ultrasound extraction. Electroporation of the rice bran by OH broke down the rice bran cell membranes, resulting in increased release of intracellular compounds such as tocopherols into the solvent. This finding was similar to Nair et al. (2014) who reported that electric energy applied under OH was responsible for the breakdown of the rice bran cell membrane and increased extraction yield of rice bran oil. Pulsed OH treatment caused cell membrane damage resulting in enhanced extraction rate of polyphenols from red grape pomace (El-Darra et al., 2013). OH as a pretreatment increased the extraction of steviol glycosides and phytochemicals from *Stevia rebaudiana* leaves to prepare sweetening agents (Moongngarm et al., 2020), black rice bran anthocyanins as a functional food colorant (Loypimai et al., 2015) and rice bran oil and unsaponifiable matter to produce functional oil ingredients for food formulation (Moongngarm et al., 2019). The ultrasonic-assisted extraction process also generated cavitation bubble collapse on the surface of the material, resulting in swelling and hydration and causing expansion of the pores in the cell wall (Vinatoru, 2001; Ashokkumar, 2014; Wen et al., 2018), thereby increasing extraction yield of rice bran tocopherols. However, the number of cavitation bubbles varied with the frequency of the sound wave pulse (Wen et al., 2018). Ultrasound treatment with high amplitude created liquid agitation rather than cavitation (Chemat et al., 2017; Wen et al., 2018). Ultrasound extraction using soybean oil as a green solvent at 40% amplitude, 40 min and 45°C achieved high yields of rice bran tocopherols (Loypimai et al., 2020). Therefore, OH as a pre-treatment is an effective way to assist ultrasound soybean oil extraction by stabilizing heat-sensitive rice bran compounds and improving the extraction rate.

Influence of extraction methods on γ -oryzanol content

In addition to rice bran tocopherols, γ -oryzanol is one of the most important rice bran bioactive compounds with a significant positive effect on human health and food stability. The enriched oil recovered from ohmically-treated rice bran extracted by ultrasound-soybean oil extractions yielded higher concentration of γ -oryzanol than that obtained from conventionally-treated and untreated rice bran samples as shown in Figure 2. Maximum concentration of γ -oryzanol was observed in the enriched oil obtained from OH-UASO ($E = 100$ and 200 V/cm) followed by the oil extracted by CB-UASO and UB-UASO, respectively. Porous rice bran cells were formed by OH electroporation, shearing and crushing by cavitation under ultrasound waves that enhanced the contact surface area between target compounds and liquid

surfaces. This improved penetration of the oil solvent into the bran matrix resulting in increased extraction rate. This result concurred with Loypimai et al. (2021) who reported that the oil extract subjected to ultrasound extraction with soybean oil as solvent showed high yield of γ -oryzanol and reached the desired level in a short extraction time (40 min). Yi et al. (2020) reported on the extraction of γ -oryzanol from heated rice bran using corn oil. They found that enriched corn oils with high rice bran γ -oryzanol enhanced their oxidative stability. Ohmic heating before ultrasound extraction of phenolic compounds from cornelian cherry (*Cornus mas*) obtained high yield with reduced extraction time (Kutlu et al., 2021), while extraction yields of peanut oil and oxidation degree were associated with the cavitation effect (Zhang et al., 2017).

Influence of extraction methods on color

Color values in terms of L^* , a^* , b^* and ΔE of soybean oil and the enriched oils obtained from different extractions are displayed in Table 1. Soybean oils enriched with tocopherols and γ -oryzanol from rice bran showed significantly ($p < 0.005$) lower L^* and higher b^* values compared with soybean oil (as solvent). Moreover, highest ΔE was observed in enriched oils obtained by OH-UASO ($E = 100$ and 200 V/cm), while extraction methods had no significant effect ($p > 0.005$) on a^* value because the enriched oils contained higher yellow-colored pigments. Our results concurred with Kutlu et al. (2021), who reported that increase in L^* and a^* values of cornelian cherry extracts obtained by ohmic heating-assisted ultrasound correlated with an increase in total anthocyanins.

Influence of extraction methods on antioxidant activities

Three established methods as DPPH radical scavenging (IC_{50}), ferric reducing/antioxidant power (FRAP) and total antioxidant capacity (TAC) were used to evaluate antioxidant activity of soybean oils as a solvent and the enriched oils obtained from different extraction methods (Table 2). Overall, the extraction method significantly affected antioxidant activity in all assays. The enriched oils obtained from OH-UASO ($E = 100$ and 200 V/cm) showed significantly ($p < 0.05$) lower IC_{50} values ranging 198.6 - 206.3 mg/g oil, better FRAP values ranging 28.6-29.3 $\mu\text{mol FeSO}_4/\text{g oil}$ and higher TAC ranging 8.87-9.12 mg GAE/g oil, respectively compared to enriched oils obtained from CB-USO and UB-USO. However, enriched oils obtained from all extraction methods gave higher antioxidant activities than using soybean oil as a solvent. The enriched oils contained higher contents of tocopherols and γ -oryzanol which had antioxidant properties. This finding agreed with Loypimai et al. (2020), who reported that the oil extract obtained by ultrasound using soybean oils as solvent showed significantly higher antioxidant activities (DPPH, FRAP and TAC assays) than oil extracted without ultrasound. Black rice bran colorant powder containing higher anthocyanin pigments, tocopherols, tocotrienols and γ -oryzanol had higher antioxidant activity (Loypimai et al., 2015). γ -Oryzanol has a phenol group as BHA and BHT and can act as an antioxidant by inhibiting lipid oxidation in corn oil (Yi et al., 2020), and canola oil (Tao et al. 2022). Moreover, rice bran is used mostly as a potential source of natural antioxidants especially oryzanols, which have 10 times higher antioxidant activity than tocopherols (Abdel-Aal and Hucl, 1999).

Chemical quality of enriched oils during storage

Extraction methods had a significant effect ($p < 0.05$) on FFA, PV and TBA of the enriched oils during storage, as shown in Figure 3. During 21 days of storage, FFA, PV and TBA of the enriched oils increased as storage time increased. Among the extraction processes, the enriched oils obtained from OH-UASO gave significantly lowest FFA, PV and TBA ($p < 0.05$) during storage, while the highest values were observed in the oil obtained from UB-USO. However, the enriched oils obtained from different extraction methods had higher values than using soybean oil as the solvent. In this study, concentrations of FFA (4.32-4.87%) in the enriched oils obtained from OH-UASO were still suitable for human consumption, as they were less than 5% FFA, as suggested by Taoet al. (1993). This occurred because the energy applied under OH inactivated the rice bran enzymes (both lipoxygenase and lipase) causing an increase in FFA and lipid peroxidation. These findings concurred with Loypimaiet al. (2015), who reported that oil obtained from rice bran stabilization using OH and then extracted using enzymes produced lower levels of FFA at 1.50% and 1.51%, respectively than oil obtained from unstabilized rice bran. FFA content of the rice bran oil extracted by OH stabilized and extraction increased more slowly than oil obtained from bran without OH (Lakkakulaet al., 2004). Enriched oils obtained from OH-UASO also contained higher concentrations of tocopherols and γ -oryzanol, as the main reason for reducing lipid oxidation reaction of the oil during storage.

Conclusions

Ohmic heating-assisted ultrasound extraction was successfully applied to produce soybean oil containing high rice bran tocopherols and γ -oryzanol using soybean oil as a green solvent. The enriched oil obtained by OH-UASO offered higher yields of α - and γ -tocopherols, and γ -oryzanol compared to conventional methods, while the enriched oil obtained contained the highest antioxidant activities. Moreover, the enriched oil obtained from OH-UASO showed improved chemical quality by prohibiting increase in FFA, TBA and PV during storage (28 days). Results suggested that OH-UASO was an effective alternative process to enrich soybean oil with rice bran bioactive substances as a functional oil ingredient in food formulation.

Declarations

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contribution P. Loypimai: Conceptualization, Methodology, Software, Writing- review & editing. A. Moongngarm: Conceptualization, Writing - review & editing. K. Sittisuanjik: Methodology, Software, Writing - review & editing. T. Wongsadee: Methodology, Software, Software, Writing - review & editing.

Conflict of interest The authors have not disclosed any competing interests.

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Tables

Table 1

Color values of soybean oil (as solvent) and the enriched oils obtained from different extraction methods

Treatment	L*	a*	b*	ΔE
Soybean oil	49.23 \pm 0.11 ^a	-1.45 \pm 0.05 ^a	0.65 \pm 0.15 ^d	-
UB-UASO	46.34 \pm 4.21 ^b	-1.41 \pm 0.02 ^a	2.27 \pm 0.08 ^c	2.21 \pm 0.42 ^c
CB-UASO	44.86 \pm 3.74 ^c	-1.40 \pm 0.01 ^a	2.95 \pm 0.25 ^b	3.19 \pm 0.63 ^b
OH-UASO (E100)	43.46 \pm 3.11 ^c	-1.41 \pm 0.02 ^a	3.27 \pm 0.21 ^a	3.76 \pm 0.19 ^a
OH-UASO (E200)	44.07 \pm 4.13 ^c	-1.39 \pm 0.04 ^{ab}	3.19 \pm 0.09 ^a	3.71 \pm 0.22 ^a
UB-UASO refers to untreated bran with ultrasound-assisted soybean oil extraction				
CB-UASO refers to conventionally treated bran with ultrasound-assisted soybean oil extraction				
OH-UASO refers to ohmically-treated bran with ultrasound-assisted soybean oil extraction				
E: Electric field strength (V/cm)				
Values are means \pm SD of triplicate samples (n = 3).				
Values with the same superscript in the same column are not significantly different ($p < 0.05$).				

Table 2

Antioxidant activities of soybean oil (solvent) and the enriched oils obtained from different extraction methods

Treatment	DPPH (IC ₅₀ , mg/g)	FRAP (μ mol FeSO ₄ /g)	Total antioxidant capacity (mg GAE/g)
Soybean oil	387.8 \pm 22.5 ^a	19.2 \pm 5.6 ^c	0.24 \pm 0.09 ^d
UB-UASO	304.5 \pm 24.3 ^b	26.3 \pm 4.6 ^{ab}	3.45 \pm 0.77 ^c
CB-UASO	235.9 \pm 19.8 ^c	28.1 \pm 6.3 ^a	6.35 \pm 0.05 ^b
OH-UASO (E100)	198.6 \pm 16.7 ^d	29.3 \pm 5.6 ^a	9.12 \pm 0.38 ^a
OH-UASO (E200)	206.3 \pm 23.4 ^d	28.6 \pm 7.1 ^a	8.87 \pm 0.85 ^{ab}
UB-UASO refers to untreated bran with ultrasound soybean oil extraction			
CB-UASO refers to conventionally treated bran with ultrasound extraction soybean oil			
OH-UASO refers to ohmicallly-treated bran with ultrasound soybean oil extraction			
E: Electric field strength (V/cm)			
Values are means \pm SD of triplicate samples (n = 3) (dry weight).			
Values with the same superscript in the same column are not significantly different ($p < 0.05$)			
DPPH is expressed as IC ₅₀ , mg/g as the concentration of oil extract to inhibit 50% of stable free DPPH radical; FRAP: Ferric reducing antioxidant power; GAE: Gallic acid equivalent			

Figures

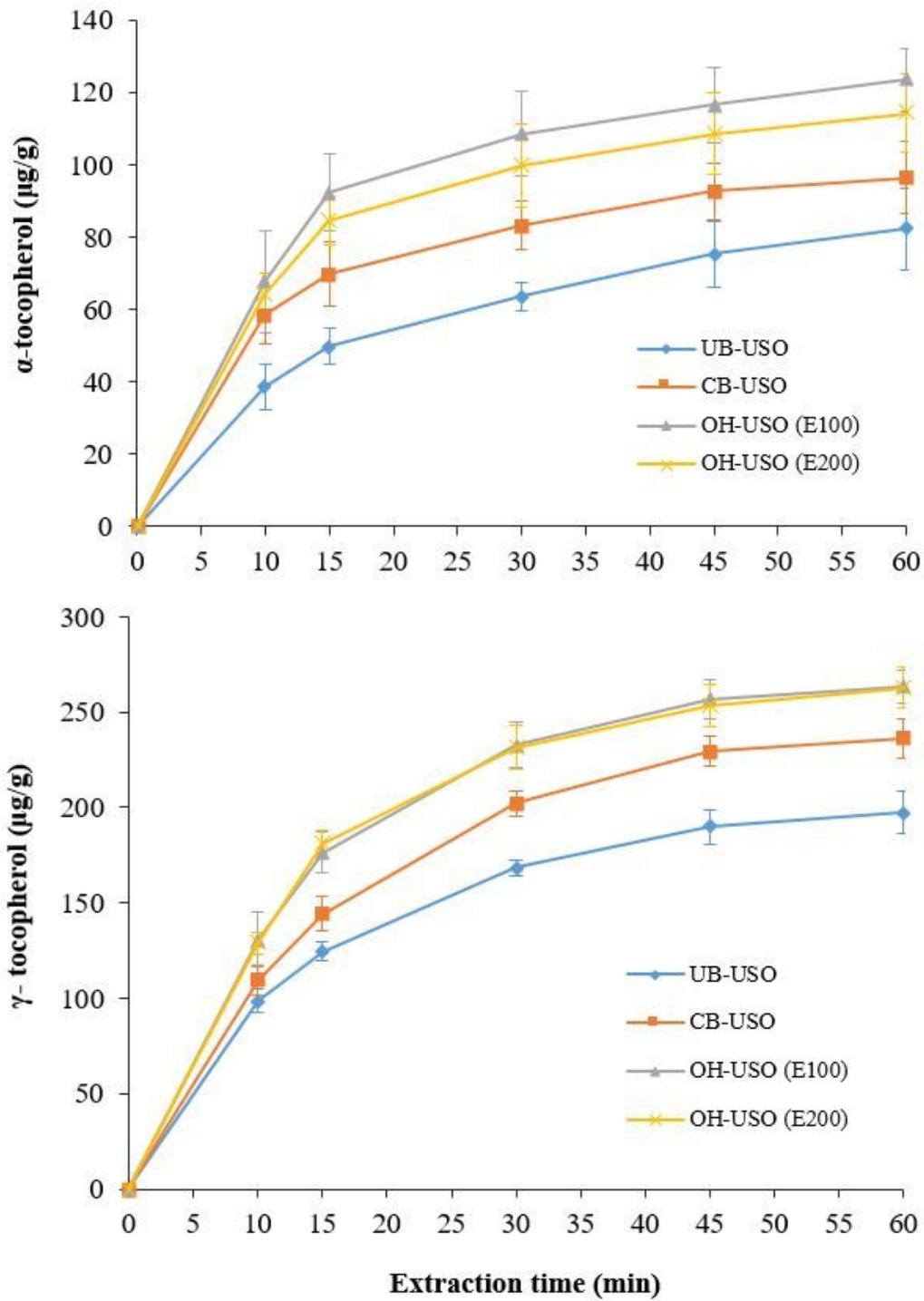


Figure 1

Extraction yields of tocopherols in enriched oils obtained from different extraction methods: UB-USO refers to untreated bran with ultrasound soybean oil extraction; CB-USO refers to conventionally treated bran with ultrasound soybean oil extraction. OH-USO refers to ohmically-treated bran at electric field strengths (E) of 100 and 200 V/cm with ultrasound soybean oil extraction.

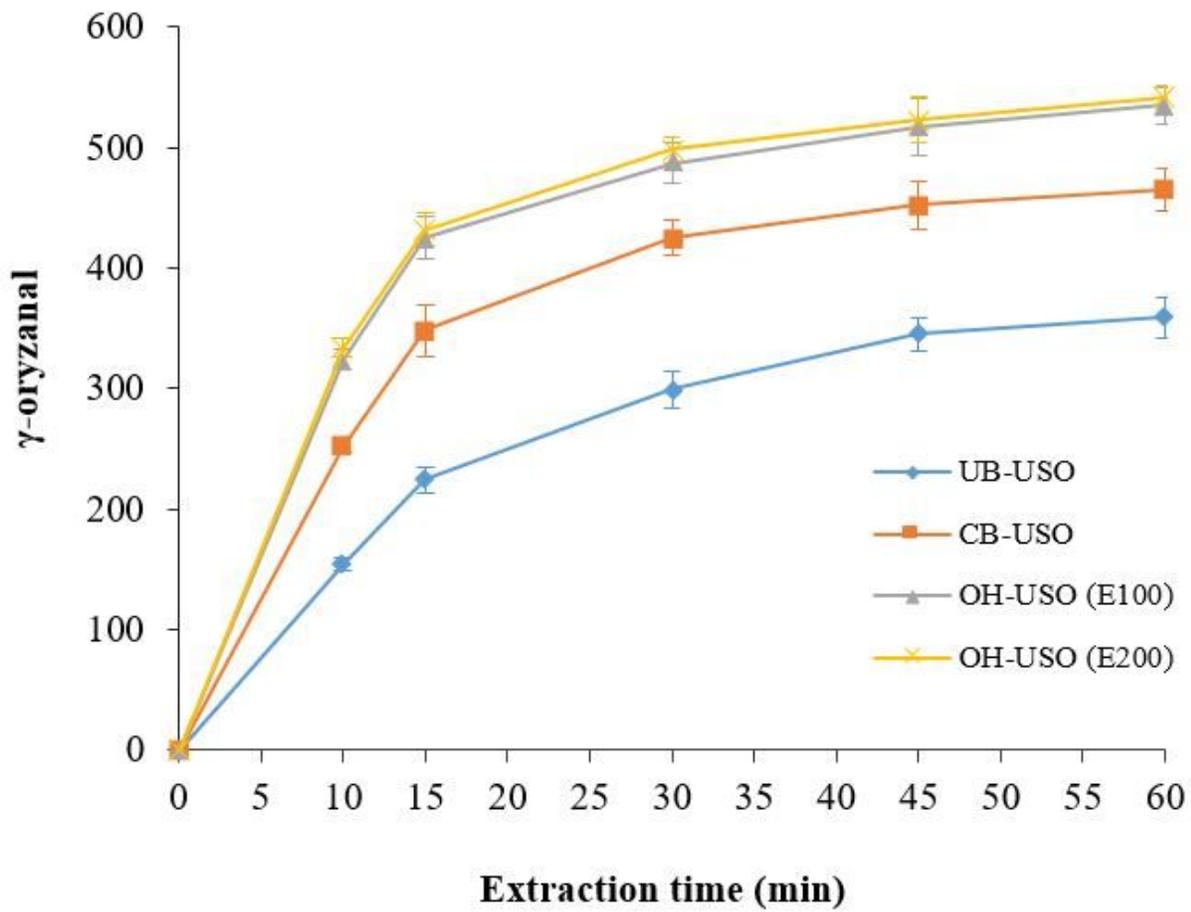


Figure 2

Extraction yields of γ -oryzanol in enriched oils obtained from different extraction methods: UB-USO refers to untreated bran with ultrasound soybean oil extraction; CB-USO refers to conventionally treated bran with ultrasound soybean oil extraction. OH-USO refers to ohmically-treated bran at electric field strengths (E) of 100 and 200 V/cm with ultrasound soybean oil extraction.

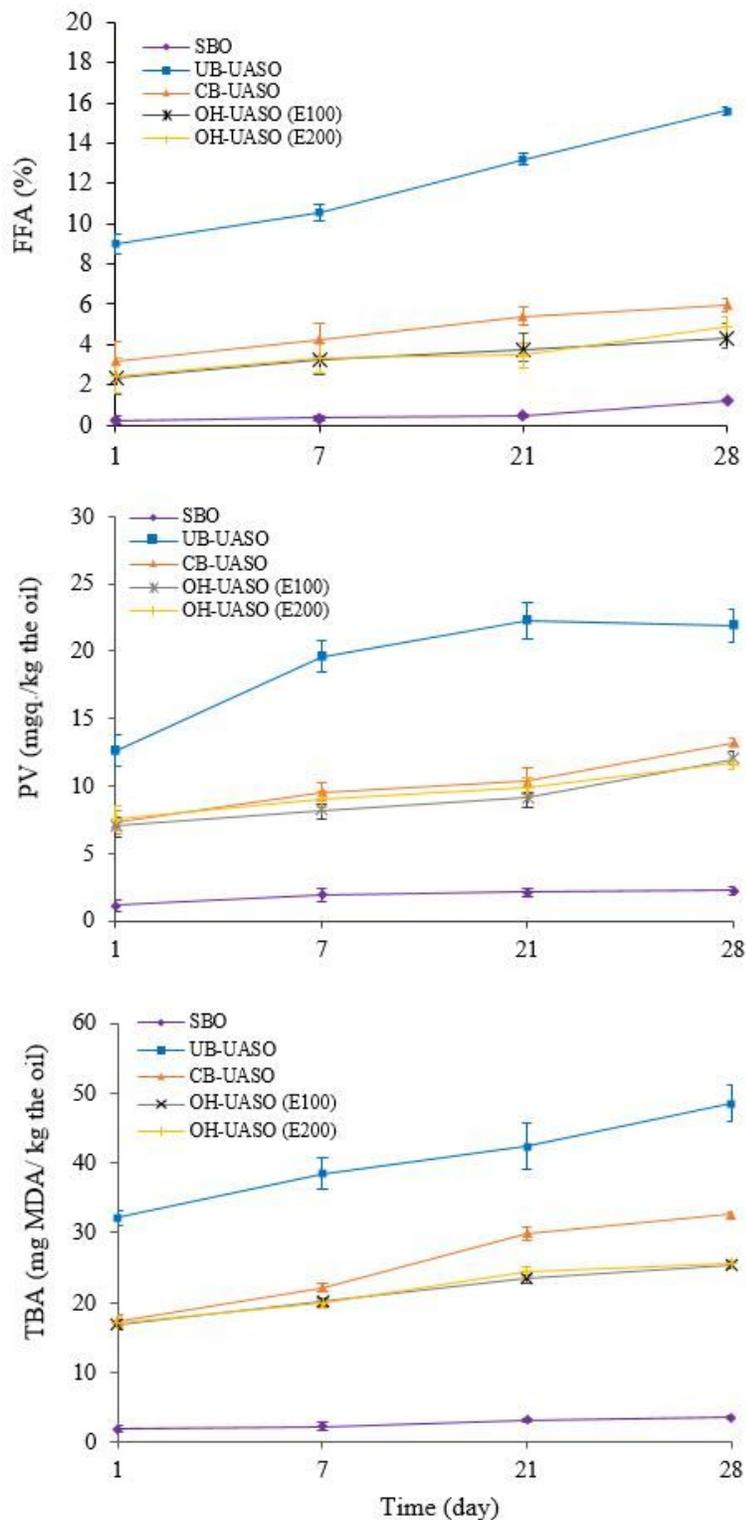


Figure 3

Changes in free fatty acid (FFA), peroxide value (PV) and thiobarbituric acid (TBA) in enriched oils obtained from different extraction methods: UB-USO refers to untreated bran with ultrasound soybean oil extraction; CB-UASO refers to conventionally treated bran with ultrasound soybean oil extraction. OH-UASO refers to ohmically-treated bran at electric field strengths (E) of 100 and 200 V/cm with ultrasound soybean oil extraction during storage at room temperature for 28 days.

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