Enzyme-assisted Aqueous Extraction of Oil from Rice Germ and its Physicochemical Properties and Antioxidant Activity

Hong Wang\(^1\), Haoyuan Geng\(^1\), Honglin Tang\(^1\), Liqi Wang\(^3\), Dianyu Yu\(^1\)*, Junguo Wang\(^2\)*, and Yuqing Song\(^2\)*

\(^1\) School of Food Science, Northeast Agricultural University, Harbin, 150030, CHINA
\(^2\) School of Grain, Jilin Business and Technology College, Changchun, 130507, CHINA
\(^3\) School of Computer and Information Engineering, Harbin University of Commerce, Harbin, 150028, CHINA

Abstract: Enzyme-assisted aqueous extraction of rice germ oil (RGO) was performed in this study. The physicochemical properties, fatty acid composition, bioactive substances and antioxidant activity of RGO were analyzed. An enzyme composed of alcalase and cellulase (1:1, w/w) was found to be the most effective in the extraction yield of oil. The optimal oil yield of 22.27% was achieved under the conditions of an enzyme concentration of 2% (w/w), incubation time of 5 h, incubation temperature of 50 °C, water to seed ratio of 5:1, and pH 6.0. The predominant fatty acids of RGO were oleic acid (39.60%), linoleic acid (34.20%) and palmitic acid (20.10%). The total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) composition of RGO were 22.50%, 39.60% and 36.00%, respectively. RGO yielded a high content of γ-oryzanol (530 mg/100 g oil), tocotrienol (62.96 mg/100 g oil), tocopherol (23.24 mg/100 g oil) and a significant amount of phytostanol (372.14 mg/100 g oil). It exhibited notable antioxidant activities with IC\(_{50}\) values of 32.37 and 41.13 mg/mL, according to the DPPH radical scavenging assay and β-carotene/linoleic acid bleaching test, respectively.

Key words: enzyme-assisted aqueous extraction, rice germ oil, physicochemical properties, bioactive substances, antioxidant activity

1 Introduction

Rice is one of the most important cereal crops in the world. It is the staple food for more than half of the world’s population\(^1\). Rice bran is an important byproduct of rice processing and is obtained when brown rice is milled to produce polished rice. Commercial rice bran is generally a mixture of rice bran and rice germ. Rice bran contains approximately 12-18% fat, which contains many health-friendly nutrients such as tocopherol, tocotrienol, phytosterol, squalene, oryzanol and phospholipids\(^2\). \(^3\).

Rice bran oil (RBO) is considered to be one of the most nutritious oils because of its balanced fatty acid composition and unique natural bioactive ingredients\(^4\). RBO contains approximately 25.5% saturated fatty acids (SFAs), 48.0% monounsaturated fatty acids (MUFAs), 26.5% polyunsaturated fatty acids (PUFAs), and is rich in γ-oryzanol, γ-tocotrienols and phytosterols. It has a positive effect on human health, such as anti-hyperlipidemia and cholesterol lowering, blood glucose lowering and anti-diabetes and antioxidant properties\(^5\)\(^6\). In recent years, the physiological activities of RBO have been studied by a number of scholars. Pushpan et al. found that Njavara RBO exhibited anti-atherogenic properties by effectively modulating inflammatory mechanisms\(^7\). Devarajan et al. demonstrated that using a blend of sesame oil and RBO as cooking oil showed a significant antihypertensive and lipid-lowering action and had a noteworthy additive effect with antihypertensive medication\(^8\). Noreen Samad’s research indicated a protective antioxidant role of RBO in the prevention of haloperidol-induced extrapyramidal symptoms\(^9\).

Press extraction and solvent extraction methods are widely used in the oil industry. They have a high extraction yield of oil but also have certain limitations. In recent years, supercritical fluid extraction (SFE) and enzyme-assisted aqueous extraction (EAAE) have become effective and more environmentally friendly methods for the extrac-
tion of edible oils and high added value compounds from many different sources. In supercritical fluid extraction, both the temperature and pressure can be controlled to increase the extraction yield of oil while minimizing the thermal degradation of proteins, antioxidants and other biologically active components. However, in comparison with traditional techniques, supercritical fluid extraction requires high pressure and expensive extraction equipment, and it is very difficult to achieve large-scale industrial production. Enzyme-assisted aqueous extraction is an effective method for the simultaneous extraction of oils and proteins from oilseeds. Compared with solvent extraction, the oil extracted by the aqueous enzymatic method does not require further refining, which reduces the processing cost to some extent. Moreover, the process conditions are relatively simple and much safer, environmentally friendly and economical.

In general, cereal germ is high in nutrients, such as wheat germ and corn germ, which are rich in oil, protein, tocopherol, phytosterol and other nutraceuticals. Moreover, the oil content in the germ is significantly higher than that in the bran. Studies have shown that the oil content in wheat germ was more than twice that of wheat bran. In recent years, scholars have successfully extracted wheat germ oil and corn germ oil from wheat germ and corn germ by enzyme-assisted aqueous extraction.

The literature contains only a few systematic studies on rice germ oil (RGO). Therefore, the objective of this study was to investigate the extraction of oil from rice germ using the enzyme-assisted aqueous extraction method. The effects of enzyme concentration, initial pH of the mixture, incubation time, incubation temperature, and water to seed ratio on the extraction yield of RGO were evaluated. In addition, the physicochemical properties, fatty acid compositions, bioactive substances including γ-oryzanol, tocopherol, tocotrienol, phytosterols and the antioxidant activities of RGO were analyzed.

2 Materials and Methods

2.1 Materials and reagents

Rice germ powder: the rice bran produced by Heilongjiang Rice Processing Company was sieved through a 25 mesh sieve (710 μm). The oversize fraction was mainly broken rice hull. The undersize fraction was then sieved through a 30 mesh sieve (600 μm), where the oversize fraction was mainly rice germ, and the undersize fraction was pure rice bran. The oversize fractions of the 30 mesh sieve were picked by hand to obtain pure rice germ. Pure rice germ and pure rice bran were ground and passed through a 60 mesh sieve (250 μm) and stored in a sealed plastic container at −18°C.

The AOAC method was used to determine the moisture, protein, ash, oil, carbohydrate and total fiber content of rice germ samples. The rice germ used in this study initially contained 8.10 ± 0.03% moisture, 25.73 ± 0.02% oil, 15.54 ± 0.02% protein, 4.30 ± 0.07% total ash, and other ingredients were 47.33%.

Pectinase: enzyme activity is 50,000 U/g, the optimum temperature is 40-50°C, the optimum pH is 3.5-4.5, provided by Dongguan Xinyao Biotechnology Co., Ltd. Neutrase 0.8 L: enzyme activity is 0.8 AU/g, provided by Novozymes, the optimum temperature is 45-55°C, and the optimum pH is 5.5-7.5. Cellulase 1.5 L: enzyme activity is 700 EGU/g, provided by Novozymes, the optimum temperature is 50-60°C, the optimum pH is 4.5-6.0. Alcalase 2.4 L: enzyme activity is 2.4 AU/g, provided by Novozymes, the optimum temperature is 55-70°C, and the optimum pH is 6.5-8.5. All reagents used in this study were analytical grade except the methyl-esterification reagent, which was chromatographically grade.

2.2 Enzyme-assisted aqueous extraction process

Distilled water was added to 10 g of rice germ powder, according to a certain water-to-seed ratio (v/w), and the mixture was adjusted to pH 4.0-8.0 with 0.1 mol/L NaOH solution and 0.1 mol/L HCl solution. At a temperature of 35-55°C, a certain amount of biological enzyme (dry weight of rice germ basis) was added, and enzymatic hydrolysis proceeded for 2-6 h. The pH of the mixture was kept constant during the enzymatic hydrolysis, and the mixture was continuously stirred at a speed of 200 r/min to ensure full enzymatic hydrolysis. After the enzymatic hydrolysis was completed, the enzymatic hydrolysate was centrifuged at 10,000 × g for 20 min in a centrifuge tube to separate the free oil and the emulsion. Free oil I was collected and weighed. The emulsion was heated to 95°C for 3 h, then frozen at −20°C overnight, thawed at room temperature, and then the thawed emulsion was centrifuged at 4,000 × g for 20 min in a centrifuge tube. Free oil II was collected, dried and weighed.

In this study, the oil extraction yield was calculated using the formula below and expressed as a percentage of the initial oil content:

\[ \text{The extraction yield of RGO (\%) = } \frac{m_1 + m_2}{m_0} \]

where \( m_1 \) is the mass of free oil I (g), \( m_2 \) is the mass of free oil II (g), and \( m_0 \) is the mass of rice germ (g).

2.3 Physicochemical properties of RGO

The acid value (AV), peroxide value (PV), saponification values (SV), unsaponifiable matter (UM), iodine values (IV) and the refractive index (RI) of RGO at 25°C were determined by AOCS and AOAC standard methods.

2.4 Fatty acid compositions

Fatty acid methyl esters (FAMEs) were slightly modified...
and assayed according to the method proposed by Nehdi et al.\textsuperscript{20}.

FAMEs were prepared by adding 1 mL of \textit{n}-hexane to 40 mg of oil followed by 200 μL of 2 mol/L sodium methoxide. The mixture was heated in the bath at 50°C for a few seconds, and then 200 μL of 2 mol/L HCl was added. Next, 1 μL of the top layer was injected onto a GC (Agilent 6890 N, CA, USA) equipped with a flame ionization detector (FID) and a polar capillary column (HP-innowax polyethylene glycol, 0.25 mm internal diameter, 30 m length and 0.25 mm film in thickness) to obtain individual peaks of fatty acid methyl esters. The detector temperature was 275°C, and the column temperature was 150°C and held for 1 min and increased at the rate of 15°C/min to 200°C and the rate of 2°C/min to 250°C and held for 4 min. The run time was 45 min. The relative percentage of fatty acids was calculated on the basis of the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample.

2.5 Determination of bioactive substances

2.5.1 \(\gamma\)-Oryzanol analysis

The \(\gamma\)-oryzanol analysis was performed according to the protocol described by Sakunpak \textit{et al.}\textsuperscript{24}.

HPLC analysis was performed using an Agilent Technologies 1100 with diode array detector (DAD). The column was an Agilent Zorbax Eclipse XDB-C18 (4.6 × 150 mm, 5 μm). The sample was separated at 40°C using a mobile phase consisting of acetonitrile, methanol and isopropanol (25:70:5 v/v/v) with a flow rate of 1 mL/min (18 min). The injection volume was 20 μL, and the \(\gamma\)-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 following parameters: capillary voltage, 4000 V; nebulizer pressure, 50 psi; gas temperature, 350°C; drying gas and recorded in the mass range of m/z 200-800. The \(\gamma\)-oryzanol was identified by Agilent Mass Hunter software and based on the retention time indicated in the standards.

2.5.2 Total tocopherols analysis

The tocopherol and tocotrienol analyses were performed according to the methods reported by Chen and Bergman\textsuperscript{25}.

The HPLC system consisted of an Agilent Technologies 1100 with a diode array detector (DAD). A total of 20 μL of the samples was injected and separated at 40°C on a Luna CN 100A column (4.6 × 250 mm, 5 μm). The initial mobile phase conditions of the extraction were 94% MeOH and 6% deionized water, at a flow rate of 1 mL/min, for 12 min. The mobile phase was then changed linearly to 25% acetonitrile, 70% MeOH, 5% IsOH, and 0.1% of acetic acid and held there for 8 min. The tocopherol and tocotrienol (\(\alpha\), \(\beta\), \(\gamma\) and \(\delta\) forms) were determined by UV/UV-Vis detection at wave-lengths of 298 and 328 nm. Tocopherols and tocotrienols were identified based on the retention time indicated by the standards.

2.5.3 Phytosterol analysis

The phytosterol analysis was performed according to the protocol described by Thanh \textit{et al.}\textsuperscript{20}.

The fused silica capillary GC column was a DB-5ms (30 m × 0.25 mm i.d. × 0.25 μm film thickness). One microliter of the samples was injected using an Agilent Technologies 7893 Auto Sampler, and the split ratio was 1:50. Programmed oven temperatures were originally set at 100°C (1 min) and then raised to 300°C (14 min) at a rate of 10°C/min, using helium 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. Phytosterols were identified and quantified by SIM (single ion monitoring) mode according to their retention times and MS spectra. Cholestanol was used for the internal standard.

2.6 Determination of antioxidant activity

2.6.1 DPPH radical scavenging assay

The DPPH radical scavenging activity was determined according to the method reported by Zhang \textit{et al.}\textsuperscript{27} The antioxidant activities of RGO and RBO are reported as the IC\textsubscript{50} value.

A total of 100 μL of seed oil was mixed with 1.4 mL of ethanol and then added to 1 mL of 0.004% DPPH in ethanol. The mixture was shaken vigorously and immediately placed in a UNICO UV-2100 spectrophotometer (UNICO, Shanghai, China) to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min until the reaction reached a plateau. Vitamin C (VC), a stable antioxidant, was used as a synthetic reference. The radical scavenging activities of the samples, expressed as percentage inhibition of DPPH, were calculated according to the formula:

\[
\text{Inhibition percentage (Ip)} = 100 \left( \frac{A_1 - A_o}{A_1} \right)
\]

where \(A_1\) and \(A_o\) are the absorbance values of the blank and of the tested samples, respectively, checked after 70 min.

2.6.2 \(\beta\)-Carotene/linoleic acid bleaching test

The \(\beta\)-carotene/linoleic acid bleaching test was done according to the method reported by Zhang \textit{et al.}\textsuperscript{27} The antioxidant activities of RGO and RBO are reported as IC\textsubscript{50} value.

Approximately 10 mg of \(\beta\)-carotene was dissolved in 10 mL of chloroform. Next, 20 mg of linoleic acid and 200 mg of Tween 40 were added to 0.2 mL of carotene–chloroform solution. After mixing, chloroform was removed under vacuum using a rotary evaporator at 40°C. Then, 50 mL of distilled water was added to the residue with vigorous shaking to form an emulsion. Five milliliters of the emulsion was added to a tube containing 0.2 mL of oil, and the absorbance was immediately measured at 470 nm against a
blank consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50°C, and the oxidation of the emulsion was determined by measuring the absorbance at 470 nm over a 60 min period. The control samples contained 200 μL of water instead of oil. Butylated hydroxytoluene (BHT), a stable antioxidant, was used as a reference. The antioxidant activity was expressed as the inhibition percentage with reference to the control after a 60 min incubation using the following equation:

\[
\text{Antioxidant activity (AA)} = \frac{100(DR_c - DR_s)}{DR_c}
\]

where \( DR_c \) is the degradation rate of the control, \( DR_s = \frac{\ln (A/B)}{60} \); \( DR_s \) is the degradation rate in the presence of the sample, \( DR_s = \frac{\ln (A/B)}{60} \), where \( A \) is the absorbance at time zero; and \( B \) is the absorbance at 60 min.

2.7 Statistical analysis

All tests were performed in triplicate, and the results are expressed as the mean ± standard deviation. The data were statistically analyzed using SPSS statistical software 17.0. The difference was considered statistically significant at \( p < 0.05 \). The test values for each compound were compared to the mean of all tests by calculating the confidence intervals.

3 Results and discussion

3.1 Enzyme-assisted aqueous extraction of RGO

3.1.1 Effect of enzymes on the extraction yield of RGO

To study the effects of different enzymes on the extraction yield of RGO, enzymes including pectinase, proteinase, alcalase, cellulase and an enzyme complex consisting of alcalase and cellulase (1:1, w/w) were selected. Extractions were conducted using individual enzymes and the enzyme complex (each at 1%, w/w) using an incubation time of 5 h, an incubation temperature of 50°C, pH 6.0, and a water to seed ratio of 5:1. The results are shown in Fig. 1.

As presented in Fig. 1, the oil yield is relatively low (9.78%) when no enzyme is added to the system. More oil was extracted using alcalase (13.89%) than cellulase (12.63%), proteinase (13.13%) and pectinase (11.38%). This finding is consistent with studies by Hamnoungjai et al., who indicated that protease recovered more oil than cellulase in the extraction from rice bran. This is related to the hydrolysis of proteins around the oil by protease. Protease can also increase the extraction rate of oil by reducing the emulsifying ability of soluble proteins.

The enzyme complex was found to be the best enzyme offering the optimal oil yield (15.37%) and was used for subsequent work. The combination of cellulase and alcalase can degrade cellulose and proteins while destroying the structural integrity of the rice germ cell wall. In addition, the enzymatic treatment may cause a breakdown in the protein networks of cotyledon cells and in the oleosin-based membranes that surround lipid bodies, thereby releasing more oil.

3.1.2 Effect of the enzyme complex concentration on the extraction yield of RGO

To study the effect of enzyme concentration on the extraction yield of RGO, extractions were conducted using an enzyme complex (alcalase/cellulase = 1:1, w/w), using an incubation time of 5 h, an incubation temperature of 50°C, a water to seed ratio of 5:1, and pH 6.0. The results are shown in Fig. 2.

As shown in Fig. 2, when raising the enzyme complex concentration from 1.0% to 2.0%, the extraction yield of the RGO increased significantly. However, when the enzyme complex concentration increased from 2.0% to 3.0%, the extraction yield increased slightly. This may be because more enzyme complexes were added to the system, and the more active sites on the enzyme complex could have interactions with the cell wall polysaccharides, lipoproteins and lipopolysaccharides, so that the oil in the cotyledon cells can be released. However, when the substrate and the enzyme are fully effective, the excess enzyme will no longer increase the oil extraction yield. In addition, the enzyme complex concentration should be a compromise
between the extraction yield and the cost. Therefore, the optimal enzyme complex concentration was 2% (w/w), and this concentration was used for subsequent work.

Other scholars have found that an enzyme cocktail (cellulase/proteinase/pectinase 1/1/1, w/w/w) was the most effective enzyme in aqueous enzymatic extraction of oil from Isatis indigotica seeds and that the optimal enzyme concentration was 1.82% (w/w)\(^3\). Gai et al. reported that the optimal oil yield in enzyme-assisted aqueous extraction of oil from Forsythia suspense seed was achieved by using a 1.5% (w/w) enzyme cocktail (cellulase/pectinase/proteinase 1/1/1, w/w/w).\(^3\)

3.1.3 Effect of the incubation time on the extraction yield of RGO

To study the effect of incubation time on the extraction yield of RGO, extractions were conducted using a 2% enzyme complex concentration (alcalase/cellulase = 1:1, w/w), an incubation temperature of 50°C, pH 6.0, and a water to seed ratio of 5:1. The results are shown in Fig. 3.

As presented in Fig. 3, the extraction yield of RGO increased with the prolongation of enzymatic hydrolysis time. When enzymatic hydrolysis was done for 5 h, the extraction yield of RGO reached 22.27%. With longer hydrolysis time, there were more enzyme complex interactions with rice germ, resulting in a greater extraction yield of oil. However, increasing the extraction time to 6 h did not significantly increase the oil extraction yield, which might be caused by depletion of the substrates and/or product inhibition of enzymes. In this study, a time of 5 h was adopted and used for subsequent work. The trend is consistent with the research about enzyme-assisted aqueous extraction of wheat germ oil\(^1\). The optimal incubation time of wheat germ oil extraction was 6 h.

3.1.4 Effect of the incubation temperature on the extraction yield of RGO

To study the effect of the incubation temperature on the extraction yield of RGO, extractions were conducted using a 2% enzyme complex concentration (alcalase/cellulase = 1:1, w/w), an incubation time of 5 h, pH 6.0, and a water to seed ratio of 5:1. The results are shown in Fig. 4.

As shown in Fig. 4, elevating the incubation temperature from 35°C to 50°C dramatically increased the extraction yield of RGO. This is because the activity of the enzyme is strongly dependent on the optimal temperature range. Elevating the temperature can increase the activity of the enzyme complex, promote the flow of oil and lead to an enhancement in the extraction yield. However, when the enzymatic temperature was higher than 50°C, the extraction yield began to decrease. This may be due to the enzyme activity being inhibited and the reaction rate of enzymatic hydrolysis being lowered. Furthermore, an excessive temperature will inactivate the enzyme, which results in a reduced extraction yield. In the present study, an incubation temperature of 50°C was found to be adequate for the extraction process. According to the literature, the optimal incubation temperature of this study is close to the observed results for Iranian Wild Almond.\(^3\)
3.1.5 Effect of the initial pH of the mixture on the extraction yield of RGO

To study the effect of the initial pH of the mixture on the extraction yield of RGO, extractions were conducted using a 2% enzyme complex concentration (alcalase/cellulase = 1:1, w/w), an incubation time of 5h, an incubation temperature of 50°C, and a water to seed ratio of 5:1. The results are shown in Fig. 5.

As presented in Fig. 5, the extraction yield of RGO increased within the pH range of 4.0-6.0, but the yield decreased with an increase beyond pH 6.0. The optimum pH values of alcalase and cellulase were 6.5-8.5 and 4.5-6.5, respectively. When the pH value was approximately 6.0, the activity of the enzyme complex was high. There were more effective enzyme complex interactions with rice germ, resulting in a greater extraction yield of oil. When the pH increased beyond 6.0, it inhibited the activity of the enzyme complex and reduced the extraction yield. In this study, a pH of 6.0 was adopted and used for subsequent work. According to the literature, pH 5.85 was the optimum for enzyme-assisted aqueous extraction of maize germ oil15.

3.1.6 Effect of the water to seed ratio on the extraction yield of RGO

To study the effect of the water to seed ratio on the extraction yield of RGO, extractions were conducted using a 2% enzyme complex concentration (alcalase/cellulase = 1:1, w/w), an incubation time of 5h, an incubation temperature of 50°C, and pH 6.0. The results are shown in Fig. 6.

As shown in Fig. 6, when the water to seed ratio was either relatively high or low, it was not conducive to the extraction of RGO. When the water to seed ratio was 5:1, the extraction yield of RGO was higher than at other levels.
This is probably because when the amount of solvent was too low, the viscosity of the enzymatic hydrolysate was high, which is not conducive to the migration of oil molecules. In addition, the opportunity for interaction between the enzyme and the substrate molecules was at a low level. Therefore, increasing the water to seed ratio was beneficial to the dissolution of the protein and the enzyme. When the water to seed ratio was at a high level, the concentration of the mixed solution was diluted, and it was difficult to maintain the mixture homogeneity. This also reduces the collision probability of the enzyme and the substrate molecules, which caused a decrease in extraction efficiency. In the present study, a water to seed ratio of 5:1 was found to be adequate for the extraction process. The trend is consistent with the research regarding enzyme-assisted aqueous extraction of wheat germ oil.

### 3.2 Physicochemical properties

Based on the above experiments, the optimal yield of RGO was 22.27\% by enzyme-assisted aqueous extraction. The optimal experimental conditions were as follows: enzyme complex concentration 2\% (alcalase/cellulase = 1:1, w/w), incubation time 5 h, incubation temperature 50°C, pH 6.0, and water to seed ratio 5:1. RBO was extracted under the same conditions. The physicochemical properties of RGO and RBO are illustrated in Table 1.

As seen in Table 1, the acid value of RGO is 2.76 mg KOH/g oil lower than that of RBO, which is only approximately 50\% of RBO. The peroxide value was 1.68 mmol/kg lower than that of RBO. This indicated that the RGO had a relatively stronger antioxidative activity. The saponification value of RGO, the content of unsaponifiable matter, the iodine value and the refractive index were similar to that of RBO. The physicochemical properties of RBO were similar to those determined by Shukla et al.\(^{34}\). The slight difference in these results may be due to different varieties and processing methods.

### 3.3 Fatty acid profile of RGO

The fatty acid composition of RGO extracted by the enzyme-assisted aqueous extraction under the optimal conditions was compared with that of RBO. The results are summarized in Table 2.

As seen in Table 2, the main unsaturated fatty acids in RGO were oleic acid (39.60 ± 0.96\%) and linoleic acid (34.20 ± 1.15\%). The saturated fatty acids in RGO were mainly palmitic acid and stearic acid, whose contents were 20.10 ± 0.37\% and 1.68 ± 0.14\%, respectively. The fatty acid content and composition of RGO and RBO were similar. Both oils contained large amounts of unsaturated fatty acids, which were 75.60 ± 2.34\% and 75.87 ± 2.35\%, respectively. Compared with the study by Bhatnagar et al.\(^{2}\), the fatty acid composition was slightly different, which may be caused by differences in the different varieties, climatic conditions and processing methods. The contents of saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid in RGO and RBO were 22.50 ± 0.63\%, 39.60 ± 0.96\%, 36.00 ± 1.38\% and 22.00 ± 0.85\%, 40.96 ± 1.13\%, 34.91 ± 1.22\% respectively, which was closer to the 1:1.5:1 recommended by the FAO/WHO\(^{35}\). The fatty acid composition of RBO was similar to that of Amarasinge et al.\(^{40}\).

### 3.4 γ-Oryzanol, total tocopherol and phytosterol contents of RGO

The bioactive substances including γ-oryzanol, tocopherol...
Table 3  The γ-oryzanol, tocotrienol, tocopherol and phytosterols contents of RGO and RBO.

| Bioactive substances        | RGO (mg/100 g) | RBO (mg/100 g) |
|----------------------------|----------------|----------------|
| γ-oryzanol                 | 530 ± 3.63     | 1400 ± 13.57   |
| Tocotrienol                | 6.78 ± 0.31    | 7.37 ± 0.53    |
| α-Tocotrienol              | ND             | ND             |
| β-Tocotrienol              | 50.45 ± 1.35   | 42.50 ± 1.00   |
| γ-Tocotrienol              | 5.73 ± 0.16    | 6.19 ± 0.12    |
| δ-Tocotrienol              | 62.96 ± 1.82   | 56.05 ± 1.65   |
| Tocopherol                 | 9.49 ± 0.23    | 6.21 ± 0.25    |
| α-Tocopherol               | 1.41 ± 0.11    | 0.37 ± 0.01    |
| β-Tocopherol               | 11.03 ± 0.37   | 4.28 ± 0.10    |
| γ-Tocopherol               | 1.31 ± 0.22    | 0.63 ± 0.17    |
| δ-Tocopherol               | 23.24 ± 0.93   | 11.49 ± 0.53   |
| Total Tocopherol           | 86.20 ± 2.75   | 67.54 ± 2.18   |
| Total (Tocotrienol+Tocopherol) | 68.22 ± 2.65 | 77.69 ± 2.31   |
| Campesterol                | 95.74 ± 3.89   | 93.23 ± 3.27   |
| Stigmasterol               | 57.41 ± 2.58   | 59.53 ± 2.41   |
| β-Sitosterol               | 199.37 ± 5.13  | 187.95 ± 4.76  |
| Sitostanol                 | 19.62 ± 1.31   | 18.68 ± 1.17   |
| Total phytosterol          | 372.14 ± 12.91 | 359.39 ± 11.61 |

Each value represents the mean ± SD. ND means not detected.

The content of γ-tocopherol in RGO was found to be predominant. It was 2.58 times that of RBO. The total tocopherol content in RGO was 2.02 times that of RBO. The contents of tocotrienols and tocopherols in RBO were slightly different from those found by Nantiyakul et al.27.

As seen in Table 3, the phytosterols in the RGO mainly include campesterol, stigmasterol, β-sitosterol and sitostanol. Among them, the content of β-sitosterol was the highest, accounting for more than 50% of the total phytosterols content. Comparing the two oils, the content of phytosterols in RGO was slightly higher than that in RBO. This result is consistent with the research results of Bhatnagar et al.21.

Comprehensively comparing the contents of γ-oryzanol, tocopherol and tocotrienol, phytosterols in RGO and RBO, it can be concluded that γ-oryzanol, tocopherol and tocotrienol had significantly different distributions in RGO and RBO.

3.5 Antioxidant activity of RGO

The antioxidant activity of RGO extracted by the enzyme-assisted aqueous extraction under the optimal conditions was compared with that of RBO. The antioxidant ac-
Enzyme-assisted Aqueous Extraction of Oil from Rice Germ and its Component Analysis

J. Oleo Sci.

The activity of both oils was evaluated by the DPPH free radical scavenging test and β-carotene/linoleic acid bleaching test. The results are shown in Fig. 7.

As illustrated in Fig. 7A and 7B, DPPH free radical scavenging activity and β-carotene bleaching inhibition increased as the oil concentration increased. The IC_{50} values of RGO in the DPPH free radical scavenging test and the β-carotene/linoleic acid bleaching test were 32.37 ± 0.85 mg/mL and 41.13 ± 0.91 mg/mL, respectively. In addition, the IC_{50} values of VC in the DPPH free radical scavenging experiments and BHT in the β-carotene/linoleic acid experiments were 8.71 ± 0.25 and 4.89 ± 0.17 μg/mL, respectively. These findings indicated that the free radical scavenging efficiency of RGO was weaker than that of VC, and the antioxidant activity was lower than that of the synthetic antioxidant BHT. In this study, RGO showed superior antioxidant activity compared to BHT. Compared with the reported data of some edible oils such as corn oil, olive oil, soybean oil, sunflower oil and rapeseed oil, the RGO extracted in this study exhibited an advantage in antioxidant properties.

Studies have indicated that the antioxidant potential of vegetable oils can be attributed to PUFAs, tocopherols and phenolic in RGO. The antioxidant activity of RGO was superior to that of RBO. On the one hand, the PUFA (36.00 ± 1.38%) of RGO was higher than that of RBO (34.91 ± 1.22%); on the other hand, RGO contained a large amount of tocopherol (23.24 ± 0.93 mg/100 g) and tocotrienol (62.96 ± 1.82 mg/100 g). These are the main reasons why RGO had a high antioxidant activity.

4 Conclusions

An optimal yield of RGO (22.27%) was obtained through the enzyme-assisted aqueous extraction method. Comparing the physiochemical characteristics of enzyme-assisted aqueous extracted oil showed that there were no considerable differences between RGO and RBO. The contents of saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid in RGO were closer to the ratio of 1:1:5:1 recommended by the FAO/WHO. γ-oryzanol in RGO was approximately 37.86% of that of RBO. The content of γ-tocopherol in RGO had an overwhelming superiority of 2.58 times that of RBO. The content of phytosterols in RGO was slightly higher than that in RBO. RGO also showed good effects in DPPH free radical scavenging activity and β-carotene bleaching inhibition, indicating that RGO had an advantage in antioxidant properties. In summary, we believe that RGO is a good source of edible oil and can be used as a valuable source of products in the food, pharmaceutical and other industries.

Acknowledgments

This work was supported by a grant from Rice bran high-value steady-state processing technology and intelligent equipment development and demonstration (No: 2018YFD0401101). Additionally, this work was supported by a grant from the Provincial Education Department R&D Project of Heilong Jang: Development of rapid prosecution technology for key control points in soybean processing (No: TSTAU-R2018010, TSTAU-C2018011). The authors
would like to thank the anonymous reviewers and the editor for their comments of this paper.

Conflicts of interest
There are no conflicts of interest to declare.

References
1) Nesterenko, A.; Alric, I.; Silvestre, F.; Durrieu, V. Vegetable proteins in micro-encapsulation: A review of recent interventions and their effectiveness. *Ind. Crops Prod.* **42**, 469-479 (2013).

2) Bhatnagar, A.S.; Prabhakar, D.S.; Prasanth Kumar, P.K.; Raja Rajan, R.G.; Gopala Krishna, A.G. Processing of commercial rice bran for the production of fat and nutraceutical rich rice brokens, rice germ and pure bran. *LWT-Food Sci. Technol.* **58**, 306-311 (2014).

3) Moongngarm, A.; Daomukda, N.; Khumpika, S. Chemical compositions, phyto-chemicals, and antioxidant capacity of rice bran, rice bran layer, and rice germ. *APCBBEE Procedia* **2**, 73-79 (2012).

4) Capellini, M.C.; Giacomini, V.; Cuevas, M.S.; Rodrigues, C.E.C. Rice bran oil extraction using alcoholic solvents: Physicochemical characterization of oil and protein fraction functionality. *Ind. Crops Prod.* **104**, 133-143 (2017).

5) Salar, A.; Faghih, S.; Pishdad, G.R. Rice bran oil and canola oil improve blood lipids compared to sunflower oil in women with type 2 diabetes: A randomized, single-blind, controlled trial. *J. Clin. Lipidol.* **10**, 299-305 (2016).

6) Ali, A.; Devarajan, S. Nutritional and Health Benefits of Rice Bran Oil. *Brown Rice*. Springer, Cham Press, pp. 137-138 (2017).

7) Pushpan, C.K.; Shalini, V.; Sindhu, G.; Rathnam, P.; Jayalekshmy, A.; Helen, A. Attenuation of atherosclerotic complications by modulating inflammatory responses in hypercholesterolemic rats with dietary Njavara rice bran oil. *Biomed. Pharmacother.* **83**, 1387-1397 (2016).

8) Devarajan, S.; Singh, R.; Chatterjee, B.; Zhang, B.; Ali, A. A blend of sesame oil and rice bran oil lowers blood pressure and improves the lipid profile in mild-to-moderate hypertensive patients. *J. Clin. Lipidol.* **10**, 339-349 (2016).

9) Samad, N. Rice bran oil prevents neuroleptic-induced extrapyramidal symptoms in rats: Possible antioxidant mechanisms. *J. Food Drug Anal.* **23**, 370-375 (2015).

10) Benito-Román, O.; Rodríguez-Perrino, M.; Sanz, M.T.; Melgosa, R. Supercritical carbon dioxide extraction of quinoa oil: Study of the influence of process parameters on the extraction yield and oil quality. *J. Supercrit. Fluids* **139**, 62-71 (2018).

11) Sparks, D.; Hernandez, R.; Zappi, M.; Blackwell, D.; Fleming, T. Extraction of rice bran oil using supercritical carbon dioxide and propane. *J. Am. Oil Chem. Soc.* **83**, 885-891 (2006).

12) Yusoff, M.M.; Gordon, M.H.; Niranjan, K. Aqueous enzyme assisted oil extraction from oilseeds and emulsion de-emulsifying methods: A review. *Trends Food Sci. Tech.* **41**, 60-82 (2015).

13) Zou, Y.; Gao, Y.; He, H.; Yang, T. Effect of roasting on physico-chemical properties, antioxidant capacity, and oxidative stability of wheat germ oil. *LWT-Food Sci. Technol.* **90**, 246-253 (2018).

14) Kneecz, I.; Varga, Z.; Székely, E. One pot kinetic resolution and product separation with corn germ oil and supercritical carbon dioxide. *J. Supercrit. Fluids* **141**, 218-223 (2018).

15) Shende, D.; Sidhu, G.K. Response surface methodology to optimize enzyme-assisted aqueous extraction of maize germ oil. *J. Food Sci. Technol.* **53**, 3282-3295 (2016).

16) Kumar, G.S.; Krishna, A.G. Studies on the nutraceuticals composition of wheat derived oils wheat bran oil and wheat germ oil. *J. Food Sci. Technol.* **52**, 1145-1151 (2015).

17) Li, H.; Song, C.; Zhou, H.; Wang, N.; Cao, D. Optimization of the aqueous enzymatic extraction of wheat germ oil using response surface methodology. *J. Am. Oil Chem. Soc.* **88**, 809-817 (2011).

18) Moreau, R.A.; Johnston, D.B.; Powell, M.J.; Hicks, K.B. A comparison of commercial enzymes for the aqueous enzymatic extraction of corn oil from corn germ. *J. Am. Oil Chem. Soc.* **81**, 1071-1075 (2004).

19) AOAC. *Official methods of analysis of AOAC international*. Association of Official Agricultural Chemists, AOAC International, VA, USA (1995).

20) Lamsal, B.P.; Johnson, L.A. Separating oil from aqueous extraction fractions of soybean. *J. Am. Oil Chem. Soc.* **84**, 785-792 (2007).

21) AOCS. *Official methods and recommended practices of the American Oil Chemists’ Society*, 5th ed. AOCS press, Champaign, USA (1997).

22) AOAC. *Official methods of analysis of AOAC international*. Association of Official Agricultural Chemists, AOAC International, VA, USA (2005).

23) Nehdi, I.; Omri, S.; Khalil, M.I.; Al-Resayes, S.I. Characteristics and chemical composition of date palm (Phoenix canariensis) seeds and seed oil. *Ind. Crops Prod.* **32**, 360-365 (2010).

24) Sakunpak, A.; Suksaeree, J.; Pathompak, P.; Sermkaew, N. Antioxidant individual γ-Oryzanol screening in cold pressed rice bran oil of different Thai rice varieties by HPLC-DPPH method. *J. Pharm. Pharm. Sci.*
Enzyme-assisted Aqueous Extraction of Oil from Rice Germ and its Component Analysis

J. Oleo Sci.

25) Chen, M.H.; Bergman, C.J. A rapid procedure for analysing rice bran tocopherol, tocotrienol and γ-oryzanol contents. J. Food Comp. Anal. 18, 139-151 (2005).

26) Thanh, T.T.; Vergnes, M.F.; Kaloustian, J.; El-Moselhy, T.F.; Amiot-Carlin, M.J.; Portugal, H. Effect of storage and heating on phytosterol concentrations in vegetable oils determined by GC/MS. J. Sci. Food Agric. 86, 220-225 (2006).

27) Zhang, S.; Zu, Y.G.; Fu, Y.J.; Luo, M.; Liu, W.; Li, J.; Effertih, T. Supercritical carbon dioxide extraction of seed oil from yellow horn (Xanthoceras sorbifolia Bunge.) and its anti-oxidant activity. Bioresour. Technol. 101, 2537-2544 (2010).

28) Hanmoungjai, P.; Pyle, D.L.; Niranjan, K. Enzyme-assisted water extraction of oil and protein from rice bran. J. Chem. Technol. Biotechnol. 77, 771-776 (2002).

29) Latif, S.; Anwar, F. Aqueous enzymatic sesame oil and protein extraction. Food Chem. 125, 679-684 (2011).

30) Latif, S.; Anwar, F. Effect of aqueous enzymatic processes on sunflower oil quality. J. Am. Oil Chem. Soc. 86, 393-400 (2009).

31) Gai, Q.Y.; Jiao, J.; Mu, P.S.; Wang, W.; Luo, M.; Li, C.Y.; Zu, Y.G.; Wei, F.Y.; Fu, Y.J. Microwave-assisted aqueous enzymatic extraction of oil from Isatis indigotica seeds and its evaluation of physicochemical properties, fatty acid compositions and antioxidant activities. Ind. Crops Prod. 45, 303-311 (2013).

32) Gai, Q.Y.; Jiao, J.; Wei, F.Y.; Luo, M.; Wang, W.; Zu, Y.G.; Fu, Y.J. Enzyme-assisted aqueous extraction of oil from Forsythia suspense seed and its physicochemical property and antioxidant activity. Ind. Crops Prod. 51, 274-278 (2013).

33) Balvardi, M.; Rezaei, K.; Mendiola, J.A.; Ibáñez, E. Optimization of the aqueous enzymatic extraction of oil from Iranian wild almond. J. Am. Oil Chem. Soc. 92, 985-992 (2015).

34) Shukla, H.S.; Prapat, A. Comparative studies between conventional and micro-wave assisted extraction for rice bran oil. J. Oleo Sci. 66, 973-979 (2017).

35) WHO. Interim Summary of Conclusions and Dietary Recommendations on Total Fat and Fatty Acids. The Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition, Geneva, CH, Champaign (2008).

36) Amarasinghe, B.M.W.P.K.; Kumarasiri, M.P.M.; Gandonvilage, N.C. Effect of method of stabilization on aqueous extraction of rice bran oil. Food Bioprod. Process. 87, 108-114 (2009).

37) Nantiyakul, N.; Furse, S.; Fisk, I.; Foster, T.J.; Tucker, G.; Gray, D.A. Phyto-chemical composition of Oryza sativa (Rice) bran oil bodies in crude and purified isolates. J. Am. Oil Chem. Soc. 89, 1867-1872 (2012).

38) Chan, K.W.; Ismail, M. Supercritical carbon dioxide fluid extraction of Hibiscus cannabinus L. seed oil: A potential solvent-free and high antioxidative edible oil. Food Chem. 114, 970-975 (2009).

39) Rezig, L.; Chouaibi, M.; Msaada, K.; Hamdi, S. Chemical composition and profile characterisation of pumpkin (Cucurbita maxima) seed oil. Ind. Crops Prod. 37, 82-87 (2012).

40) Amarowicz, R.; Pegg, R.B.; Rahimi-Moghaddam, P.; Barl, B.; Weil, J.A. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chem. 84, 551-562 (2004).

J. Oleo Sci.