



## High-oleic rapeseed oil quality indicators and endogenous antioxidant substances under different processing methods

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### ABSTRACT

This study exposed high-oleic rapeseed oil (HORO) to different pretreatment (microwave or roasting) and processing methods to investigate (cold pressing, hexane extraction, subcritical butane extraction, and aqueous enzymatic extraction) the effects of processing technologies on HORO parameters associated with its physico-chemical properties, endogenous antioxidant substances, and antioxidant capacity. The oil yield of various processing technologies was between 35.4% and 59.7%, and the fatty acid composition did not significantly differ. Hierarchical clustering and principal component analyses were used for evaluation. The results revealed that the microwave pretreatment–hexane extraction (M-HE) method resulted in significantly higher levels of tocopherols (688.4 mg/kg), polyphenols (1007.76 mg/kg), and phytosterols (1810.6 mg/kg) in HORO, implying strong free radical scavenging capacity (DPPH-oil: 79.63, DPPH-nonpolar: 71.42, DPPH-polar: 6.65, FRAP: 55.4, ABTS: 3043.7  $\mu\text{mol TE/kg}$ ). Hence, M-HE is a promising method for producing HORO with a higher stability and nutritional value.

### Introduction

Rapeseed oil is an important, edible oil that is cultivated worldwide. Its total yield is approximately 12 million tons in China, accounting for 20% of the world's supply (Chew, 2020). The oleic acid (C18:1) content in ordinary rapeseed oil ranges from 8% to 60%, whereas high-oleic rapeseed oil (HORO) has a C18:1 content of over 72%, with a high nutritional and health value (Zhang, Liu, & Che, 2018). HORO, a high-quality edible vegetable oil with potentially good economic and broad market prospects, does not readily undergo oxidation and rancidification and has a higher commercial value than ordinary rapeseed oil.

Rapeseed pretreatment, oil extraction, and refining are the three fundamental steps in the typical processing technology for the

preparation of rapeseed oil (Ye & Liu 2023). Pretreatment has the potential to improve its composition without violating current regulations (McDowell, Elliott, & Koidis, 2017). The most typical thermal pre-processing techniques used in rapeseed oil production include roasting (Rekas, Wroniak, Siger, & Ścibisz, 2017) and microwaving (Cong, Cheong, Huang, Zheng, Wan, & Zheng, 2019). The utilization of pre-processing techniques confers several advantages, such as a rise in oil yield, a higher concentration of minor compounds with beneficial properties, and improved oxidative stability (Siger, Kaczmarek, & Rudzińska, 2015). Additionally, research has revealed that certain pre-processing methods result in the creation of furan compounds, including 2-furanmethanol, dihydro-2(3H)-furanone, and 5-butyldihydro-2(3H)-furanone, thus changing the flavor of the oil (Ren et al., 2018).

**Abbreviations:** HORO, high-oleic rapeseed oil; HOR, high-oleic rapeseed; CP, cold pressing; HE, hexane extraction; AEE, aqueous enzymatic extraction; SBE, subcritical butane extraction; M-CP, microwave pretreatment-cold pressing; R-CP, roasting-pretreated-cold pressing; M-HE, microwave pretreatment–hexane extraction; R-HE, roasting-pretreated–hexane extraction; M-AEE, microwave pretreatment–aqueous enzymatic extraction; R-AEE, roasting-pretreated–aqueous enzymatic extraction; M-SBE, microwave pretreatment–subcritical butane extraction; R-SBE, roasting-pretreated–subcritical butane extraction; AV, acid values; PV, peroxide values; IV, iodine value; OSI, oxidation stability index; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; PCA, principal component analysis; HCA, hierarchical cluster analysis; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, Ferric reducing ability of plasma; ABTS, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonate).

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At present, the most frequently utilized methods for the preparation of rapeseed oil are either mechanical pressing or solvent extraction (Ye & Liu 2023), such as hexane extraction (HE), which has the advantages of a large processing capacity and low production cost (Guo, Wan & Huang 2019). However, this has created a series of environmental problems. Rapeseed oil obtained by cold pressing (CP) has a higher content of nutritional bioactive components and retains its native functional properties (Chew, 2020); thus, it is widely accepted by consumers. In the past few decades, new rapeseed oil extraction/preparation methods, which typically include subcritical butane extraction (SBE) and aqueous enzymatic extraction (AEE) (Gaber, Tujillo, Mansour, & Juliano, 2018), have been developed. SBE is a new separation technology, which guarantees high quality and productivity, and enables industrialization (Guo et al., 2019). AEE has significant advantages in energy consumption and environmental sanitation. It uses an enzyme to destroy the vegetable cell wall via mechanical disruption, to release the oil (Hu et al., 2022). Alcalase can release oil droplets more efficiently by destroying the cell wall and hydrolyzing lipoproteins to obtain a high oil yield. The composite cell walls polysaccharide enzyme is a kind of non-starch complex glycoenzyme, which can degrade, for example, cellulose, hemicellulose,  $\beta$ -glucan, xylan, and araban and is added to reduce the complexation of polysaccharides and polyphenols and protect the polyphenol content of the oil (Li, Zhang, Xu, Li, Cao, & Liu, 2019).

To sum up, different processing technologies have different effects on oil quality. However, most previous studies focused on common rapeseed oil. Research and development of HORO is at the initial stage, as the industrial production of oil is hindered by a paucity of research regarding the influence of processing methods on the oil's nutritional value and functional properties. This study aimed to investigate the effects of 12 different processing technologies (microwave, roasting combined with CP, HE, AEE and SBE) on the physicochemical properties, endogenous antioxidant substances, and antioxidant capacity of HORO. The goal was to provide guidance for the development of high-quality HORO.

## Methods

### Materials and chemicals

The high-oleic rapeseed (HOR) was peeled using a 6FT-PB3 separating machine (Jinggu Quinoa Machinery, Shandong, China) after harvest, immediately transported to the laboratory and stored in sealed bags at  $-4^{\circ}\text{C}$  until use. Standard mixtures of 40-fatty acid methyl esters, phytosterol (purity > 95%), tocopherols (purity > 95%), bis(trimethylsilyl)trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMCS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing ability plasma (FRAP), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) were purchased from Sigma-Aldrich Chemical Co. Ltd. (Shanghai, China). The composite cell walls polysaccharide enzyme and alcalase were obtained from Ruiyang Biotechnology Co., Ltd. (Jiangsu, China). The other reagents were purchased from Baisende Biology Co., Ltd (Zhengzhou, China).

### Lipid Extraction

#### Pretreatment method

**Microwave pretreatment.** HOR (1500 g) was accurately weighed and exposed to microwave radiation for 7 min using an M1-L213C microwave (frequency 2450 MHz; power 800 W; Midea, Guangdong, China). The seeds were cooled at room temperature (60 min) before processing.

**Roasting pretreatment.** HOR (1500 g) was roasted in an oven (DZF-6021, Shanghai, China) at  $140^{\circ}\text{C}$  for 1 h, followed by cooling at room

temperature (60 min) before processing.

#### Processing method

**Cold pressing.** CP was performed following McDowell et al., (2017) with slight modification. HOR (1500 g) was pressed with an electric screw press (J58-630 T, Qingdao, China). The internal temperature of the screw rod in the press (Yijiayi Mechanical Equipment Co., Ltd, Hubei, China) process was  $60 \pm 10^{\circ}\text{C}$ , and the temperature of the produced oil was  $40 \pm 2^{\circ}\text{C}$ . The oil was subjected to centrifugation for 15 min at 6000 r/min (TG-165, Changsha, China) in a 15 mL centrifuge tube for fraction separation. The cold-pressed oil was stored at  $4^{\circ}\text{C}$  until use.

**Hexane extraction.** HE was carried out according to Zhang et al., (2023) with slight modification. HOR was ground (5 min) using an 800A multi-function grinder electric food processor (Yongkang Sufeng Industry and Trade Co., Ltd., Zhejiang, China). The ground HOR (1500 g) was weighed and added to *n*-hexane (1:7, w/v). The solution was extracted using an ultrasonic unit (SB25-12DTD, Chengteng Biotechnology Co., Ltd., Shandong, China) for 2 h at  $45^{\circ}\text{C}$  with 70% power. The solvent evaporation of the liquid extract was carried out in a rotary evaporator flask (SHZ-III, Waters, Shanghai, China) under vacuum by immersing it in a  $60^{\circ}\text{C}$  water bath and rotating it at 50 r/min (TG-165, Changsha, China). The oil was then filtered using a qualitative filter paper and weighed.

**Aqueous enzymatic extraction.** The preparation of AEE HORO was according to Zhang et al, (2023) with some modifications. The enzyme in the HOR was deactivated and the pulp was moistened by combining the HOR with boiling water at 1:7 (w/v) ratio. The composite cell walls polysaccharide enzyme (3%, w/w) was added for enzymatic hydrolysis, and the mixture was incubated at  $48^{\circ}\text{C}$  for 5 h. The pH of the slurry was adjusted to 10, with 2 mol/L NaOH, and extracted at  $60^{\circ}\text{C}$  for 1 h. The pH value was then readjusted to 9 and 1.5% (v/v) alcalase was added. Next, the mixture was incubated at  $60^{\circ}\text{C}$  and magnetically stirred at 300 r/min (TG-165, Changsha, China) for 2 h in a water bath. The temperature was kept at  $85^{\circ}\text{C}$  to induce enzymolysis and terminate the reaction. The mixture was poured into separating funnels and allowed to settle for 24 h to collect the upper oil phase.

**Subcritical butane extraction.** Preparation of SBE HORO was according to the Guo et al., (2019) for rapeseed oil extraction. A subcritical fluid extraction system (PLE-5L, Henan Subcritical Extraction Biological Technology Co., Ltd) was used to extract HORO. HOR was put through a 40-mesh sieve into an extraction container and sealed with a lid. The ratio of material to butane was 1:8 (w/v), and the pressure of the vessels was  $-0.01$  MPa. Three extraction cycles of 40 min at  $60^{\circ}\text{C}$  were performed.

### Oil yield and physicochemical properties

#### Determination of oil yield

The weight of HOR and HORO were recorded in the lipid extraction process, and oil yield (R) was calculated according to the following formula

$$R(\%) = \frac{M_1}{M_2} \times 100\%$$

where  $M_1$  is the mass of the HORO (g) and  $M_2$  is the weight (g) of HOR.

#### Determination of physicochemical properties and fatty acid composition

The determination of peroxide value (PV), acid value (AV), iodine value (IV), and moisture content was performed using the AOCS official methods Cd 3d-63, Cd 8-53, Cd 1d-92 and Ca 2a-45, respectively. The

fatty acid composition was determined using the ISO 12966–2:2017 method for edible oil fatty acid detection. Degree of fatty acids unsaturation (DUS) was analyzed according to the calculation formula:

$$DUS(\Delta mol^{-1}) = [1 \times (\%monoene) + 2 \times (\%diene) + 3 \times (\%triene)]/100$$

#### Determination of endogenous antioxidant substance

##### Tocopherol and phytosterols contents

Tocopherol content was determined according to the ISO 9936:2016 Animal and vegetable fats and oils tocopherol detection method. Phytosterols content was determined according to the ISO 12228–1:2014, with the results reported in mg/kg.

##### Polyphenols content

The polyphenols of HORO were determined following Zhang et al., (2023) involving the Folin–Ciocalteu reagent. Polyphenol compounds were briefly extracted three times from HORO (1.0 g) with 5 mL methanol aqueous solutions (60%, v/v). The procedure involved the mixing of extract (0.2 mL) and 0.25 mL Folin–Ciocalteu reagent for 10 min in the dark, followed by the addition of 0.75 mL of 20% sodium carbonate and incubation in the dark for 1 h. Next, the solution was determined using a UV-250 Spectrophotometer at 750 nm. A similar approach was used to measure the standard curve of the gallic acid solution, and the results are reported in mg/kg.

##### Antioxidant capacity assays

Oxidation stability index of the oils was determined using an 892 Rancimat equipment (Metrohm, Switzerland) according to the AOCs method Cd 12b-92. The test was carried out at a constant temperature at 120 °C with air flow of 20 L/h, using 3.0 g oil samples and 60 mL distilled water in a conductometric vessel. The results were expressed in hours.

About 0.15 g of HORO was added in 10 mL of methanol to extract the polar part of oil, and ethyl acetate was used to extract nonpolar part. The FRAP, ABTS, and DPPH radical cation scavenging capacity tests were performed according to the method described in our previous study (Zhang et al., 2023). Trolox standards were used for quantification, and the results are expressed as  $\mu\text{mol}$  of Trolox equivalents per kg of sample ( $\mu\text{mol TE/kg}$ ).

##### Statistical analysis

Data are reported as mean  $\pm$  standard error of three determinations calculated using Microsoft Excel (Microsoft, USA). SPSS 26.0 (IBM, USA) software was used for statistical analysis, and the differences between groups were tested using one-way analysis of variance (ANOVA) and Duncan's tests. Means were compared and were considered significant when  $p < 0.05$ . The tree diagram was constructed by analyzing the physicochemical properties and endogenous antioxidant substances of HORO through various processing technologies and systematic cluster analysis. To determine relationships among the gathered quantitative data, chemometric analysis and principal component analysis (PCA) of the data on the endogenous antioxidants beneficial to HORO and the antioxidant capacity were conducted. Data from the following analyses of oils were deemed suitable for PCA classification according to the generated factor loading matrix: FRAP (X1), ABTS (X2),  $\alpha$ -tocopherol (X3),  $\beta$ -tocopherol (X4), DPPH-nonpolar (X5), polyphenol content (X6),  $\gamma$ -tocopherol (X7), DPPH-the oil (X8), DPPH-polar (X9),  $\delta$ -tocopherol (X10), and phytosterol content (X11).

## Results and discussion

### Oil yield

Table 1 displays the oil yield and physicochemical properties of HORO that were extracted using the various processing techniques. From the processing technology perspective, a decrease in yield was observed in the following order: AEE (56.4–59.7%) > HE (56.5–59.5%) > SBE (45.8–47.6%) > CP (35.4–39.9%). The high AEE yield revealed that the enzyme degraded the HOR cell wall more effectively, leading to a higher release of oil and other materials trapped within the cells, into the aqueous medium, resulting in an improved yield (Hu et al., 2022). The lowest oil yield was obtained from roasting-pretreated-CP (R-CP) oil, because roasting may decrease the water content of HOR. The water could lead to the repulsion of hydrophobic (from oil) and hydrophilic (from proteins and sugars) forces, and the formation of hydrogen bonds, contributing to oil separation (Lv & Wu, 2019). The reduced moisture content of HORO using R-CP made oil separation more difficult, resulting in a lower oil yield. Meanwhile, during mechanical pressing, there was residual oil of the cake and oil loss in the press chamber (Matskevich, Nevzorov, Kolomeitsev, & Kapsargina, 2021). The pretreatment method has a small impact on the yield of HORO in comparison to the processing technology.

### Physicochemical indices

As shown in Table 1, the AV and PV of oils extracted using different methods were significantly different ( $p < 0.05$ ). The AV (0.98–3.13 mg/g) and PV (0.79–4.34 mmol/kg) of oils were below the Codex Standard 210–2019 limit (AV  $\leq 4$  mg/g, PV  $\leq 7.5$  mmol/kg). The AV of HORO obtained from preprocessing was higher than that without pretreatment. This suggests that preprocessing techniques have negative effects on HORO. The high temperatures of microwaving or roasting pretreatment accelerated the hydrolysis of triglycerides and increased the amount of free fatty acids. The PV of CP was the highest, owing to the partial destruction of the seed structure after pressing, the activation of natural enzymes in the seed, and the oil outflow after cell destruction, which contributed to the rapid deterioration of the oil quality (Zhang, Li, Xu, Wang & Wang 2022).

The IV is frequently viewed as a rough standard for the stability of oil during heat treatment, as oils with a greater concentration of unsaturated fatty acids (UFA) are more prone to thermal oxidation (Ren et al., 2018). The lowest IV of HORO was found in the microwave-pretreated-HE (M-HE) oil (117.3 g/100 g) and the highest in the roasting-pretreated-HE (R-HE) oil (129.3 g/100 g), indicating that pretreatment influences the quality of HORO processed by HE. In terms of processing technology without pretreatment, the IV of oil produced using AEE (118.0–118.5 g/100 g) was the lowest, indicating that this process improved resistance to rancidity and an extended storage period.

The order described below shows a decrease in moisture content: AEE (7.18–7.92%) > HE (1.64–5.91%) > CP (0.09–5.65%) > SBE (1.09–2.00%). The low moisture content of HORO after roasting or microwaving may be due to the heat-caused removal of some of the moisture from the seeds during the pretreatment process. Furthermore, the moisture content of HORO made using AEE and HE was significantly higher than that of CP, which is consistent with the results for oil yield. The high moisture content of oils extracted using AEE was associated with technology-related conditions. The standard procedure typically involves extracting the crushed materials using an aqueous solution and enzymes. Afterwards, the mixture is separated through centrifugation into the oil and emulsion, as well as the aqueous and solid components. Hence, the aqueous environment led to the high moisture content (Jiamphun & Chaiyana 2022). During AEE, the presence of moisture can enhance the diffusion of enzymes, making it easier to break down the cotyledon cell wall (Mwaurah et al., 2020).

**Table 1**  
The physicochemical properties of high-oleic rapeseed oil with different processing technology.

	Oil yield/%	AV/mg/g	PV/mmol/kg	IV/g/100 g	Moisture content/%
CP	39.9 ± 0.4 <sup>e</sup>	1.03 ± 0.07 <sup>g</sup>	4.34 ± 0.40 <sup>a</sup>	121.9 ± 1.6 <sup>de</sup>	5.65 ± 0.08 <sup>c</sup>
M-CP	37.7 ± 0.7 <sup>f</sup>	1.49 ± 0.02 <sup>d</sup>	0.79 ± 0.40 <sup>f</sup>	126.1 ± 0.8 <sup>b</sup>	0.09 ± 0.01 <sup>j</sup>
R-CP	35.4 ± 0.3 <sup>g</sup>	1.27 ± 0.01 <sup>f</sup>	1.18 ± 0.00 <sup>ef</sup>	124.2 ± 1.4 <sup>bc</sup>	0.73 ± 0.02 <sup>i</sup>
HE	59.5 ± 0.7 <sup>a</sup>	1.35 ± 0.02 <sup>ef</sup>	3.16 ± 0.00 <sup>b</sup>	120.1 ± 0.6 <sup>ef</sup>	4.04 ± 0.06 <sup>d</sup>
M-HE	57.3 ± 1.6 <sup>b</sup>	1.39 ± 0.02 <sup>e</sup>	1.18 ± 0.00 <sup>ef</sup>	117.3 ± 1.4 <sup>g</sup>	5.91 ± 0.02 <sup>c</sup>
R-HE	56.5 ± 1.1 <sup>b</sup>	1.49 ± 0.01 <sup>d</sup>	3.16 ± 0.00 <sup>b</sup>	129.3 ± 0.7 <sup>a</sup>	1.64 ± 0.08 <sup>g</sup>
SBE	46.1 ± 0.2 <sup>cd</sup>	0.98 ± 0.03 <sup>g</sup>	1.58 ± 0.00 <sup>cd</sup>	122.5 ± 0.7 <sup>cd</sup>	1.94 ± 0.02 <sup>ef</sup>
M-SBE	45.8 ± 0.8 <sup>d</sup>	1.27 ± 0.05 <sup>f</sup>	1.58 ± 0.00 <sup>cd</sup>	122.6 ± 0.5 <sup>cd</sup>	1.09 ± 0.02 <sup>h</sup>
R-SBE	47.6 ± 0.6 <sup>c</sup>	1.37 ± 0.07 <sup>e</sup>	1.58 ± 0.00 <sup>c</sup>	119.4 ± 0.7 <sup>fg</sup>	2.00 ± 0.01 <sup>e</sup>
AEE	59.7 ± 0.6 <sup>a</sup>	2.03 ± 0.06 <sup>c</sup>	1.18 ± 0.00 <sup>de</sup>	118.5 ± 1.5 <sup>fg</sup>	7.92 ± 0.23 <sup>a</sup>
M-AEE	56.5 ± 0.4 <sup>b</sup>	2.51 ± 0.09 <sup>b</sup>	0.79 ± 0.00 <sup>f</sup>	118.0 ± 2.6 <sup>fg</sup>	7.64 ± 0.55 <sup>a</sup>
R-AEE	56.4 ± 1.1 <sup>b</sup>	3.13 ± 0.06 <sup>a</sup>	1.18 ± 0.00 <sup>ef</sup>	118.3 ± 0.7 <sup>fg</sup>	7.18 ± 0.16 <sup>b</sup>

The superscript letters indicate the statistical difference in rows in significant level at 5%.

### Fatty acid composition

Table 2 illustrates the fatty acid composition of the HORO, which was acquired using various processing methods. As per statistical analysis, no significant differences ( $p > 0.05$ ) were found in the fatty acid composition of the HORO. Furthermore, the application of pretreatment in the extraction process was inconsequential to the oil's fatty acid composition. The major fatty acids of the HORO were oleic acid (C18:1, 74.12%-79.03%) and linoleic acid (C18:2, 8.39-12.18%). Erucic acid (C22:1, 1.83%-2.51%) is a common fatty acid in rapeseed oil with negative effects on human health (Schwarzinger, Feichtinger, Blank-Landeshammer, Weghuber, & Schwarzinger, 2022). HORO is characterized by a high content of C18:1 and low content of C22:1. Moreover, saturated fatty acids (SFA) contributed <7% to the total fatty acids. Nutrition studies have shown that saturated fatty acids can increase the level of low-density lipoprotein cholesterol in human blood, leading to

the occurrence of cardiovascular diseases (Gu, Liu, Liu, Pang, & Qin, 2017). Therefore, HORO is a healthy edible oil.

### Endogenous antioxidant substances

#### Tocopherol content

Tocopherols serve as endogenous antioxidants in oil. The tocopherol content of the oils extracted using the different extraction methods are presented in Table 3. A total of four tocopherol homologues ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -) were identified in HORO. Clearly, the  $\gamma$ -tocopherol content was 240.3-472.4 mg/g, accounting for 52.72%-73.29% of the total tocopherol content. This indicated that  $\gamma$ -tocopherol was the primary tocopherol in HORO. Moreover, the tocopherol content obtained using SBE only comprised  $\alpha$ -tocopherol (150.4-197.9 mg/kg) and  $\gamma$ -tocopherol (412.7-472.4 mg/kg). It is possible that the  $\beta$ -tocopherol and  $\delta$ -tocopherol levels were below the detection limit because the mild

**Table 2**  
The fatty acid composition (%) of high-oleic rapeseed oil with different processing technology.

	CP	M-CP	R-CP	HE	M-HE	R-HE	SBE	M-SBE	R-SBE	AEE	M-AEE	R-AEE
C16:0	3.24 ± 0.12 <sup>c</sup>	3.34 ± 0.03 <sup>c</sup>	3.36 ± 0.07 <sup>c</sup>	3.52 ± 0.03 <sup>c</sup>	3.53 ± 0.11 <sup>c</sup>	3.58 ± 0.07 <sup>c</sup>	3.36 ± 0.13 <sup>c</sup>	2.25 ± 0.06 <sup>c</sup>	3.29 ± 0.17 <sup>c</sup>	3.36 ± 0.11 <sup>c</sup>	2.17 ± 0.06 <sup>cd</sup>	2.29 ± 0.17 <sup>cd</sup>
C16:1	0.13 ± 0.01 <sup>e</sup>	0.14 ± 0.01 <sup>e</sup>	0.15 ± 0.03 <sup>f</sup>	0.17 ± 0.01 <sup>e</sup>	0.20 ± 0.01 <sup>e</sup>	0.21 ± 0.02 <sup>c</sup>	0.83 ± 0.01 <sup>e</sup>	0.29 ± 0.01 <sup>f</sup>	0.21 ± 0.02 <sup>f</sup>	0.21 ± 0.02 <sup>f</sup>	0.02 <sup>f</sup>	0.19 ± 0.01 <sup>e</sup>
C18:0	2.09 ± 0.09 <sup>d</sup>	2.13 ± 0.01 <sup>d</sup>	2.14 ± 0.01 <sup>d</sup>	2.15 ± 0.04 <sup>d</sup>	2.14 ± 0.03 <sup>d</sup>	2.11 ± 0.11 <sup>c</sup>	2.19 ± 0.16 <sup>d</sup>	2.58 ± 0.29 <sup>c</sup>	2.03 ± 0.06 <sup>d</sup>	2.03 ± 0.06 <sup>f</sup>	2.03 ± 0.21 <sup>cd</sup>	2.06 ± 2.12 <sup>cd</sup>
C18:1	75.87 ± 0.62 <sup>a</sup>	75.03 ± 2.05 <sup>a</sup>	74.50 ± 0.16 <sup>a</sup>	74.94 ± 0.79 <sup>a</sup>	74.55 ± 1.13 <sup>a</sup>	74.25 ± 0.84 <sup>a</sup>	76.03 ± 1.06 <sup>a</sup>	79.03 ± 0.11 <sup>a</sup>	74.12 ± 0.71 <sup>a</sup>	74.90 ± 0.14 <sup>a</sup>	76.16 ± 0.55 <sup>a</sup>	76.35 ± 0.4 <sup>a</sup>
C18:2	11.18 ± 0.19 <sup>b</sup>	11.35 ± 0.39 <sup>b</sup>	11.73 ± 0.23 <sup>b</sup>	11.58 ± 0.51 <sup>b</sup>	11.88 ± 0.59 <sup>b</sup>	12.18 ± 0.24 <sup>b</sup>	8.39 ± 0.22 <sup>b</sup>	9.70 ± 0.43 <sup>b</sup>	12.07 ± 0.13 <sup>b</sup>	11.17 ± 0.14 <sup>b</sup>	11.20 ± 0.19 <sup>b</sup>	10.82 ± 0.54 <sup>b</sup>
C18:3	2.08 ± 0.07 <sup>d</sup>	2.19 ± 0.17 <sup>d</sup>	2.24 ± 0.11 <sup>d</sup>	2.28 ± 0.08 <sup>d</sup>	2.22 ± 0.21 <sup>d</sup>	2.26 ± 0.24 <sup>c</sup>	1.94 ± 0.5 <sup>d</sup>	1.26 ± 0.07 <sup>e</sup>	2.18 ± 0.18 <sup>d</sup>	2.34 ± 0.15 <sup>d</sup>	2.38 ± 0.11 <sup>c</sup>	2.14 ± 0.18 <sup>cd</sup>
C20:0	0.63 ± 0.02 <sup>c</sup>	0.61 ± 0.03 <sup>c</sup>	0.63 ± 0.02 <sup>c</sup>	0.62 ± 0.03 <sup>e</sup>	0.62 ± 0.04 <sup>e</sup>	0.61 ± 0.04 <sup>c</sup>	2.58 ± 0.43 <sup>d</sup>	1.20 ± 0.10 <sup>c</sup>	1.09 ± 0.03 <sup>e</sup>	1.09 ± 0.03 <sup>e</sup>	1.03 ± 0.03 <sup>e</sup>	1.15 ± 0.04 <sup>e</sup>
C20:1	2.20 ± 0.08 <sup>d</sup>	2.16 ± 0.04 <sup>d</sup>	2.21 ± 0.03 <sup>d</sup>	2.15 ± 0.04 <sup>d</sup>	2.09 ± 0.16 <sup>d</sup>	2.13 ± 0.03 <sup>c</sup>	2.02 ± 0.11 <sup>d</sup>	1.23 ± 0.07 <sup>c</sup>	1.96 ± 0.12 <sup>d</sup>	1.99 ± 0.11 <sup>f</sup>	1.86 ± 0.07 <sup>d</sup>	1.86 ± 0.05 <sup>d</sup>
C22:0	0.29 ± 0.01 <sup>e</sup>	0.30 ± 0.02 <sup>e</sup>	0.30 ± 0.00 <sup>f</sup>	0.30 ± 0.02 <sup>e</sup>	0.31 ± 0.02 <sup>e</sup>	0.30 ± 0.01 <sup>c</sup>	0.28 ± 0.06 <sup>e</sup>	0.29 ± 0.01 <sup>f</sup>	0.25 ± 0.03 <sup>f</sup>	0.25 ± 0.03 <sup>f</sup>	0.22 ± 0.01 <sup>f</sup>	0.23 ± 0.02 <sup>e</sup>
C22:1	1.92 ± 0.04 <sup>d</sup>	2.02 ± 0.07 <sup>d</sup>	2.21 ± 0.02 <sup>d</sup>	1.91 ± 0.02 <sup>d</sup>	2.00 ± 0.08 <sup>d</sup>	1.98 ± 0.07 <sup>c</sup>	1.95 ± 0.78 <sup>d</sup>	1.83 ± 0.15 <sup>d</sup>	2.45 ± 0.20 <sup>d</sup>	2.31 ± 0.09 <sup>d</sup>	2.37 ± 0.13 <sup>c</sup>	2.51 ± 0.12 <sup>cd</sup>
C24:0	0.18 ± 0.01 <sup>e</sup>	0.17 ± 0.02 <sup>e</sup>	0.19 ± 0.01 <sup>f</sup>	0.18 ± 0.01 <sup>e</sup>	0.17 ± 0.01 <sup>e</sup>	0.19 ± 0.01 <sup>c</sup>	0.17 ± 0.02 <sup>c</sup>	0.14 ± 0.01 <sup>f</sup>	0.15 ± 0.01 <sup>f</sup>	0.15 ± 0.01 <sup>f</sup>	0.15 ± 0.01 <sup>f</sup>	0.15 ± 0.01 <sup>e</sup>
C24:1	0.19 ± 0.01 <sup>e</sup>	0.23 ± 0.01 <sup>e</sup>	0.32 ± 0.17 <sup>f</sup>	0.19 ± 0.02 <sup>e</sup>	0.21 ± 0.01 <sup>e</sup>	0.21 ± 0.02 <sup>c</sup>	0.18 ± 0.02 <sup>c</sup>	0.19 ± 0.02 <sup>f</sup>	0.19 ± 0.01 <sup>f</sup>	0.19 ± 0.01 <sup>f</sup>	0.21 ± 0.02 <sup>f</sup>	0.20 ± 0.01 <sup>e</sup>
SFA	6.43 ± 0.06 <sup>c</sup>	6.55 ± 0.02 <sup>d</sup>	6.62 ± 0.02 <sup>cd</sup>	6.77 ± 0.00 <sup>c</sup>	6.77 ± 0.01 <sup>c</sup>	6.79 ± 0.00 <sup>c</sup>	8.58 ± 0.02 <sup>a</sup>	6.46 ± 0.01 <sup>c</sup>	6.81 ± 0.02 <sup>b</sup>	6.88 ± 0.02 <sup>b</sup>	5.60 ± 0.01 <sup>f</sup>	5.88 ± 0.01 <sup>f</sup>
MUFA	80.31 ± 0.06 <sup>a</sup>	79.58 ± 0.01 <sup>b</sup>	79.39 ± 0.01 <sup>b</sup>	79.36 ± 0.02 <sup>b</sup>	79.05 ± 0.02 <sup>b</sup>	78.78 ± 0.02 <sup>c</sup>	81.01 ± 0.02 <sup>a</sup>	82.57 ± 0.01 <sup>a</sup>	78.83 ± 0.01 <sup>c</sup>	79.60 ± 0.01 <sup>b</sup>	80.80 ± 0.02 <sup>a</sup>	81.11 ± 0.02 <sup>a</sup>
PUFA	13.26 ± 0.01 <sup>d</sup>	13.54 ± 0.00 <sup>c</sup>	13.97 ± 0.06 <sup>b</sup>	13.86 ± 0.02 <sup>b</sup>	14.10 ± 0.01 <sup>a</sup>	14.44 ± 0.01 <sup>a</sup>	10.33 ± 0.01 <sup>d</sup>	10.96 ± 0.06 <sup>e</sup>	14.25 ± 0.08 <sup>a</sup>	13.51 ± 0.00 <sup>c</sup>	13.58 ± 0.00 <sup>c</sup>	12.96 ± 0.03 <sup>c</sup>
DUS	1.09 ± 0.01 <sup>ab</sup>	1.09 ± 0.03 <sup>ab</sup>	1.10 ± 0.01 <sup>ab</sup>	1.09 ± 0.02 <sup>ab</sup>	1.10 ± 0.02 <sup>ab</sup>	1.10 ± 0.02 <sup>ab</sup>	1.04 ± 0.03 <sup>c</sup>	1.06 ± 0.01 <sup>bc</sup>	1.10 ± 0.02 <sup>ab</sup>	1.10 ± 0.01 <sup>ab</sup>	1.10 ± 0.01 <sup>ab</sup>	1.09 ± 0.02 <sup>a</sup>

The superscript letters indicate the statistical difference in rows in significant level at 5%.

SFA, C16:0 + C18:0 + C20:0 + C22:0 + C24:0; MUFA, C16:1 + C18:1 + C20:1 + C22:1 + C24:1; PUFA, C18:2 + C18:3.



Table 3

The tocopherol content (mg/kg), polyphenol content and phytosterol content (mg/kg) of high-oleic rapeseed oil with different processing technology.

	CP	M-CP	R-CP	HE	M-HE	R-HE	SBE	M-SBE	R-SBE	AEE	M-AEE	R-AEE
$\alpha$ -Tocopherol	177.4 ± 3.6 <sup>c</sup>	195.5 ± 1.5 <sup>c</sup>	194.3 ± 0.6 <sup>c</sup>	166.2 ± 0.5 <sup>c</sup>	208.4 ± 1.1 <sup>c</sup>	189.3 ± 1.0 <sup>c</sup>	150.4 ± 1.2 <sup>c</sup>	197.9 ± 2.5 <sup>c</sup>	192.3 ± 2.3 <sup>d</sup>	168.2 ± 1.7 <sup>a</sup>	217.8 ± 1.8 <sup>b</sup>	198.2 ± 2.0 <sup>b</sup>
$\beta$ -Tocopherol	8.7 ± 0.0 <sup>c</sup>	0.0 ± 0.0 <sup>d</sup>	4.1 ± 0.0 <sup>a</sup>	0.0 ± 0.0 <sup>d</sup>	15.1 ± 0.0 <sup>b</sup>	13.1 ± 0.1 <sup>b</sup>	0.0 ± 0.0 <sup>d</sup>	0.0 ± 0.0 <sup>d</sup>	0.0 ± 0.0 <sup>d</sup>	11.7 ± 0.0 <sup>a</sup>	15.6 ± 0.0 <sup>b</sup>	13.5 ± 0.0 <sup>c</sup>
$\gamma$ -Tocopherol	436.1 ± 1.0 <sup>bc</sup>	459.3 ± 0.5 <sup>de</sup>	452.8 ± 1.0 <sup>def</sup>	447.1 ± 1.0 <sup>ef</sup>	459.1 ± 3.1 <sup>f</sup>	442.3 ± 1.0 <sup>def</sup>	412.7 ± 1.0 <sup>b</sup>	455.0 ± 0.7 <sup>g</sup>	472.4 ± 1.1 <sup>def</sup>	240.3 ± 1.2 <sup>a</sup>	264.1 ± 3.7 <sup>cd</sup>	270.1 ± 1.0 <sup>a</sup>
$\delta$ -Tocopherol	3.7 ± 0.2 <sup>ab</sup>	0.0 ± 0.0 <sup>d</sup>	8.7 ± 0.1 <sup>ab</sup>	6.0 ± 0.1 <sup>ab</sup>	5.7 ± 0.1 <sup>c</sup>	7.0 ± 0.1 <sup>ab</sup>	0.0 ± 0.0 <sup>d</sup>	0.0 ± 0.0 <sup>d</sup>	0.0 ± 0.0 <sup>d</sup>	2.1 ± 0.0 <sup>c</sup>	3.5 ± 0.1 <sup>a</sup>	2.3 ± 0.1 <sup>ab</sup>
Total tocopherol	625.8 ± 0.0 <sup>c</sup>	654.8 ± 0.0 <sup>b</sup>	659.9 ± 0.1 <sup>b</sup>	619.4 ± 0.0 <sup>c</sup>	688.4 ± 0.0 <sup>a</sup>	651.6 ± 0.0 <sup>b</sup>	563.0 ± 0.0 <sup>d</sup>	653.9 ± 0.0 <sup>b</sup>	664.6 ± 0.0 <sup>b</sup>	413.4 ± 0.0 <sup>g</sup>	500.9 ± 0.0 <sup>e</sup>	484.1 ± 0.1 <sup>f</sup>
Polyphenol	1047.27 ± 0.61 <sup>a</sup>	909.35 ± 0.61 <sup>d</sup>	688.54 ± 0.61 <sup>i</sup>	862.08 ± 0.61 <sup>e</sup>	1007.76 ± 0.61 <sup>b</sup>	751.68 ± 0.61 <sup>g</sup>	429.63 ± 1.83 <sup>l</sup>	518.52 ± 1.06 <sup>j</sup>	467.02 ± 0.61 <sup>k</sup>	800.35 ± 1.22 <sup>f</sup>	741.09 ± 0.61 <sup>h</sup>	953.79 ± 1.22 <sup>c</sup>
Brassicasterol	186.3 ± 0.9 <sup>f</sup>	177.0 ± 1.1 <sup>g</sup>	178.6 ± 0.6 <sup>g</sup>	210.3 ± 1.2 <sup>c</sup>	205.9 ± 1.3 <sup>c</sup>	185.5 ± 1.1 <sup>f</sup>	853.5 ± 3.5 <sup>a</sup>	181.9 ± 1.7 <sup>f</sup>	244.3 ± 1.2 <sup>a</sup>	200.2 ± 1.4 <sup>e</sup>	202.1 ± 0.3 <sup>d</sup>	181.1 ± 1.7 <sup>f</sup>
Campesterol	592.6 ± 2.1 <sup>e</sup>	562.5 ± 0.7 <sup>f</sup>	550.0 ± 1.6 <sup>g</sup>	706.5 ± 2.1 <sup>b</sup>	646.9 ± 1.8 <sup>d</sup>	558.6 ± 2.4 <sup>f</sup>	60.3 ± 0.5 <sup>h</sup>	589.2 ± 1.4 <sup>e</sup>	717.5 ± 2.4 <sup>a</sup>	695.5 ± 0.7 <sup>c</sup>	647.5 ± 1.2 <sup>d</sup>	553.3 ± 2.8 <sup>g</sup>
Stigmasterol	12.3 ± 0.5 <sup>b</sup>	8.0 ± 0.3 <sup>d</sup>	9.5 ± 0.1 <sup>c</sup>	3.5 ± 0.2 <sup>fg</sup>	12.0 ± 0.4 <sup>b</sup>	4.1 ± 0.1 <sup>ef</sup>	215.5 ± 0.8 <sup>a</sup>	10.0 ± 0.1 <sup>c</sup>	4.7 ± 0.1 <sup>e</sup>	3.0 ± 0.1 <sup>g</sup>	4.0 ± 0.3 <sup>ef</sup>	10.2 ± 0.7 <sup>c</sup>
Clerosterol	1.7 ± 0.3 <sup>g</sup>	4.3 ± 0.4 <sup>e</sup>	5.8 ± 0.7 <sup>ab</sup>	6.3 ± 0.4 <sup>a</sup>	5.0 ± 0.5 <sup>cd</sup>	5.2 ± 0.6 <sup>bc</sup>	2.4 ± 0.2 <sup>g</sup>	3.5 ± 0.2 <sup>f</sup>	6.1 ± 0.1 <sup>ab</sup>	5.8 ± 0.2 <sup>ab</sup>	4.8 ± 0.1 <sup>de</sup>	4.8 ± 0.2 <sup>de</sup>
Sitosterol	1.7 ± 0.3 <sup>g</sup>	4.3 ± 0.4 <sup>g</sup>	5.8 ± 0.7 <sup>e</sup>	6.3 ± 0.4 <sup>b</sup>	5.0 ± 0.5 <sup>d</sup>	5.2 ± 0.6 <sup>b</sup>	2.4 ± 0.2 <sup>a</sup>	3.5 ± 0.2 <sup>f</sup>	6.1 ± 0.1 <sup>d</sup>	5.8 ± 0.2 <sup>c</sup>	4.8 ± 0.1 <sup>d</sup>	4.8 ± 0.2 <sup>f</sup>
$\Delta$ 5-Avenasterol	19.3 ± 0.5 <sup>g</sup>	19.6 ± 0.5 <sup>g</sup>	23.3 ± 0.9 <sup>e</sup>	30.9 ± 0.7 <sup>b</sup>	25.1 ± 0.2 <sup>d</sup>	16.8 ± 0.5 <sup>b</sup>	290.7 ± 1.1 <sup>a</sup>	21.7 ± 0.7 <sup>e</sup>	26.1 ± 0.4 <sup>d</sup>	29.0 ± 0.9 <sup>c</sup>	26.5 ± 0.2 <sup>d</sup>	15.3 ± 0.5 <sup>i</sup>
Stigmastadienol	9.6 ± 0.7 <sup>d</sup>	5.5 ± 0.2 <sup>ik</sup>	5.2 ± 0.1 <sup>k</sup>	15.1 ± 0.7 <sup>b</sup>	7.8 ± 0.1 <sup>f</sup>	5.0 ± 0.3 <sup>h</sup>	14.3 ± 0.5 <sup>c</sup>	6.2 ± 0.1 <sup>gh</sup>	6.7 ± 0.2 <sup>g</sup>	16.9 ± 0.4 <sup>a</sup>	8.7 ± 0.1 <sup>c</sup>	5.0 ± 0.4 <sup>k</sup>
Stigmastatrienol	31.2 ± 0.6 <sup>cf</sup>	25.1 ± 0.2 <sup>h</sup>	29.5 ± 0.1 <sup>fg</sup>	44.2 ± 0.7 <sup>a</sup>	33.8 ± 0.7 <sup>cd</sup>	32.1 ± 1.4 <sup>de</sup>	3.1 ± 0.2 <sup>i</sup>	28.8 ± 0.7 <sup>g</sup>	42.2 ± 0.1 <sup>ab</sup>	40.2 ± 0.7 <sup>b</sup>	31.1 ± 1.4 <sup>ef</sup>	34.8 ± 2.1 <sup>c</sup>
$\Delta$ 7-Avenasterol	1.6 ± 0.1 <sup>cde</sup>	1.7 ± 0.1 <sup>cde</sup>	1.8 ± 0.1 <sup>cd</sup>	1.8 ± 0.1 <sup>cd</sup>	1.1 ± 0.1 <sup>e</sup>	1.3 ± 0.1 <sup>de</sup>	11.6 ± 0.7 <sup>a</sup>	2.5 ± 0.5 <sup>b</sup>	2.1 ± 0.1 <sup>bc</sup>	1.6 ± 0.1 <sup>cde</sup>	1.1 ± 0.2 <sup>e</sup>	1.2 ± 0.1 <sup>de</sup>
Other sterols	6.7 ± 0.1 <sup>e</sup>	5.7 ± 0.1 <sup>fg</sup>	5.5 ± 0.7 <sup>g</sup>	11.4 ± 0.8 <sup>d</sup>	6.5 ± 0.1 <sup>ef</sup>	13.6 ± 0.7 <sup>b</sup>	3.7 ± 0.6 <sup>h</sup>	6.6 ± 0.4 <sup>ef</sup>	4.4 ± 0.4 <sup>b</sup>	20.0 ± 0.2 <sup>a</sup>	6.0 ± 0.1 <sup>ef</sup>	12.6 ± 0.1 <sup>c</sup>
Total phytosterol	1633.3 ± 0.0 <sup>e</sup>	1549.3 ± 0.0 <sup>f</sup>	1550.6 ± 0.0 <sup>f</sup>	1999.4 ± 0.0 <sup>a</sup>	1810.6 ± 0.1 <sup>c</sup>	1560.3 ± 0.0 <sup>f</sup>	1456.0 ± 0.0 <sup>h</sup>	1665.1 ± 0.1 <sup>c</sup>	2013.0 ± 0.1 <sup>a</sup>	1902.4 ± 0.0 <sup>b</sup>	1769.8 ± 0.0 <sup>d</sup>	1585.2 ± 0.0 <sup>g</sup>

The superscript letters indicate the statistical difference in rows in significant level at 5%.

temperature, pressure, and butane (used as a solvent in the SBE process) may influence the solvent selectivity for tocopherol (Gu et al., 2019). The processing and pretreatment methods had a significant influence ( $p < 0.05$ ) on the total tocopherol content of HORO. The highest total tocopherol content was obtained using M-HE (688.4 mg/kg), followed by roasting-pretreated-SBE (R-SBE) (664.6 mg/kg) and R-CP (659.9 mg/kg), while the lowest was obtained using AEE (413.4 mg/kg). The inconsistent endogenous antioxidant content obtained using the different processing methods may be affected by thermal tolerance and oil solubility under different processing conditions. Meanwhile, the oil extracted using microwaving and roasting had higher contents of all the homologue tocopherols because the thermal pretreatment evaporated the moisture distributed in the microstructure inside the oilseeds, damaged cell walls, and ruptured cell membranes (caused by the increased water pressure inside HOR), which subsequently increased the release of tocopherols and improved their concentration in the extracted oil (Ren et al., 2018).

#### Polyphenol content

Polyphenols are plant bioactive metabolites with antioxidative functions (Orfali et al., 2021). The polyphenol content of HORO was in the range of 429.63–1047.27 mg/kg (Table 3). The processing technology used had a significant effect ( $p < 0.05$ ) on the polyphenol content. CP led to the highest polyphenol content (mean of 881.72 mg/kg), followed by HE (mean of 873.84 mg/kg) and AEE (mean of 831.74 mg/kg). CP achieved the highest polyphenol content due to the protective effects of the relatively low pressing temperature on the natural bioactive compounds. Pretreatment reduced the polyphenols in CP oil because heat degradation decreases heat-sensitive polyphenols (Ye & Liu 2023). For the CP and HE groups, oil preprocessing led to a lower polyphenol content. The microwaving and roasting preprocessing techniques significantly decreased the polyphenol levels. This may have

been attributable to the roasting temperature of 140°C, which destroyed the phenolic substance's structure, leading to the dissociation of polyphenols. In contrast, the polyphenol content of HORO using roasting-pretreated-AEE (R-AEE) (953.79 mg/kg) was 1.19-fold higher than that in AEE (800.35 mg/kg). In the SBE groups, the polyphenol content using microwave-pretreated-SBE (M-SBE) (518.52 mg/kg) and R-SBE (467.02 mg/kg) was higher than that in SBE (429.63 mg/kg). The increase in phenolic acid abundance in rapeseed oil can be efficiently achieved through thermal preprocessing (McDowell et al., 2017), either by roasting or microwaving. This allows for the decarboxylation of sinapic acid and the formation of the main phenols in the oil (Cong et al., 2019). Moreover, there was cellular experiments proved that the phenolic content and phytosterols of walnut oil increased the activity of the cholesterol reverse efflux gene (ABCG1) thus having cholesterol-lowering effects (Gao, Liu, Jin & Wang, 2022). Therefore, to improve the nutritional value and quality of the final oil products, the processing technologies and operation parameters of HORO must be optimized with the goal of increasing the total polyphenol levels.

#### Phytosterol content

The phytosterol contents of HORO produced by different methods are shown in Table 3. Ten phytosterols were detected in this study. The range of total phytosterols was from 1456.0 mg/kg (SBE) to 2013.0 mg/kg (R-SBE). The type and quantity of phytosterols in this study are inconsistent with those of Siger et al., (2015) which may be related to the use of different rapeseed varieties, origin, natural environment, or experiment conditions (Ozturk, Ozcan, & Uslu, 2022). Campesterol was the dominant phytosterol (60.3–706.5 mg/kg), and the SBE oil had a notably lower content than other samples. However, the SBE oil had the highest brassicasterol (853.5 mg/kg), stigmasterol (215.5 mg/kg), and  $\Delta$ 5-avenasterol (290.7 mg/kg) contents. Although SBE oil had the highest levels of these sterols, its campesterol content was the lowest,

leading to the lowest content of total sterol. The highest total phytosterol content was found in HE oil (mean of 1790.1 mg/kg), which was approximately 13.5% more than in CP oil (mean of 1577.7 mg/kg). The solubility of phytosterol in *n*-hexane during extraction enables it to leach with the oil, contributing to the high phytosterol content found in HE oil (Kyselka et al., 2014). The sterol content in the SBE groups with different pretreatments exhibited a difference of approximately 557.0 mg/kg, proving that SBE had a significant effect on the phytosterol content in terms of processing technologies and pretreatment.

#### Antioxidant capacity assays

The results of the evaluation of the antioxidant ability of HORO manufactured using various approaches are shown in Table 4. Without pretreatment, the oil extracted using SBE had a higher OSI than that of other processing methods, which was confirmed by the high induction time (10.95 h). This result may have depended on the high SFA (8.58%) content of SBE, as the rate of lipid oxidation is related to the degree of fatty acid saturation; the higher the saturation degree, the less easily lipid is oxidized (Guo, Wan, Huang, & Wei, 2021). Furthermore, the microwave pretreatment of CP, HE, SBE, and AEE led to a noticeable time increase in the OSI to 12.32 h, 7.83 h, 16.11 h, and 9.23 h, respectively. It is possible that microwave pretreatment facilitated the inactivation of oxidative enzymes, resulting in a higher oil oxidative stability (Rekas et al., 2017).

The results of the DPPH-oil test, presented in Table 4, varied from 75.08 to 97.24  $\mu\text{mol TE/kg}$ . The order of decreasing antioxidant capacity was CP > HE > AEE > SBE. In contrast to their oil yield results, the CP groups had the highest antioxidant capacity as measured by the HORO test. Meanwhile, SBE led to the weakest antioxidant capacity based on these five assays in HORO. A possible reason is that as the HORO was not pretreated, some bioactive substances were lost due to its miscibility with the organic solvent during SBE. When comparing the effects of pretreatments, the stability of the oils pretreated using the roasting method was better than that of oils pretreated using the microwaving method in the DPPH-polar assay, while microwave pre-processing was superior in the ABTS and FRAP assays. Previous studies (Tang et al., 2022) proved that tocopherol, polyphenols and phytosterols played a major role in antioxidation and there had synergistic and antagonistic effects among three minor constituents. Furthermore, the same combination of minor constituents showed diverse antioxidant interaction in different lipid environments and the concentration of minor constituents had a great influence on the oxidation stability and the ability of scavenging free radicals (Liu et al., 2020). According to the study by Ozcan, Al-Juhaimi, Ahmed, Osman, and Gassem (2019), a highly positive and significant correlation between total phenolic and antioxidant activities was observed. Despite the differences in heat transfer methods, the formation of polyphenol and tocopherol was the cause of the increased antioxidant activity in HORO, through both microwave and roasting pretreatments (Ye & Liu 2023). Therefore, the

results of the DPPH-nonpolar scavenging activity demonstrated that M-SBE and M-HE had the strongest activity, leading to their high tocopherol content. Thus, the beneficial health properties of HORO could be better realized through preprocessing.

#### Hierarchical cluster analysis

The differences between oils resulting from various processing methods were confirmed by cluster analysis, which was performed by using cosine and centroid clustering, and considering physiochemical properties and endogenous antioxidant substances as variables (Fig. 1). The formation structure was split into three distinct sections, B, C, and D, as shown by the hierarchical tree analysis at a 20-distance threshold. B consisted of M-CP, M-SBE, R-CP, and R-SBE; C was comprised microwave pretreatment-AEE (M-AEE), R-AEE, M-HE, and R-HE; and D was composed of four processing technologies without pretreatment. Part A was formed by C and D combined.

The result of selecting the 16-distance threshold was that M-AEE and R-AEE were grouped with M-HE and R-HE. The microwaving and roasting pretreatments of HORO extracted using AEE and HE had similar effects. This may have been attributed to the similar physiochemical properties of HORO. Additionally, the division of cluster B resulted in the formation of two sub-clusters: M-CP, M-SBE and R-CP, R-SBE. Microwave pretreatment resulted in the lack of  $\beta$ -tocopherol,  $\delta$ -tocopherol, and the contents of similar individual tocopherols collected from the oil samples through CP and SBE. Moreover, the pretreatment methods exerted large effects on the HORO's chemical composition and

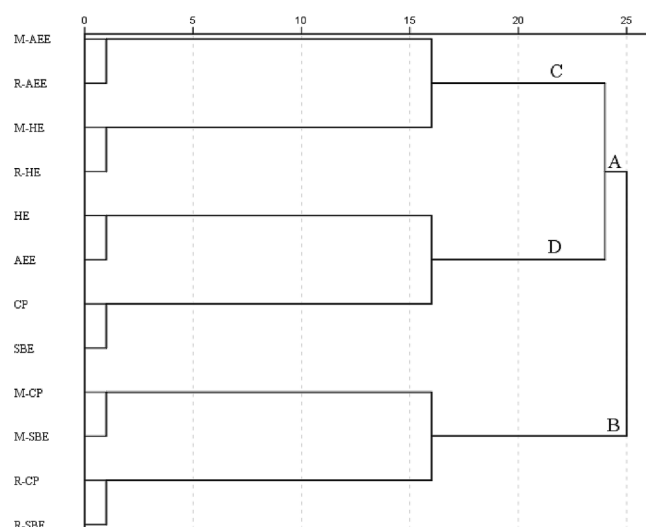


Fig. 1. The hierarchical cluster analysis diagram of high-oleic rapeseed oil.

Table 4

The oxidative stability index (h) and free radical scavenging capacity ( $\mu\text{mol TE/kg}$ ) of high-oleic rapeseed oil with different processing technology.

	OSI	DPPH-oil	DPPH-nonpolar	DPPH-polar	ABTS	FRAP
CP	8.30 $\pm$ 0.52 <sup>ef</sup>	97.24 $\pm$ 1.67 <sup>a</sup>	66.07 $\pm$ 0.64 <sup>e</sup>	11.03 $\pm$ 0.14 <sup>c</sup>	2898.6 $\pm$ 11.6 <sup>g</sup>	32.0 $\pm$ 0.4 <sup>h</sup>
M-CP	12.32 $\pm$ 0.59 <sup>b</sup>	77.93 $\pm$ 0.55 <sup>efg</sup>	67.25 $\pm$ 0.46 <sup>d</sup>	8.12 $\pm$ 0.00 <sup>d</sup>	3185.7 $\pm$ 3.2 <sup>a</sup>	49.9 $\pm$ 0.2 <sup>c</sup>
R-CP	10.62 $\pm$ 0.40 <sup>c</sup>	78.29 $\pm$ 0.90 <sup>ef</sup>	68.80 $\pm$ 0.92 <sup>c</sup>	11.08 $\pm$ 0.25 <sup>c</sup>	3156.7 $\pm$ 3.2 <sup>b</sup>	32.2 $\pm$ 0.1 <sup>sh</sup>
HE	7.65 $\pm$ 0.30 <sup>def</sup>	86.54 $\pm$ 0.65 <sup>b</sup>	64.04 $\pm$ 0.50 <sup>f</sup>	11.20 $\pm$ 0.14 <sup>c</sup>	3024.7 $\pm$ 9.7 <sup>f</sup>	41.5 $\pm$ 0.2 <sup>f</sup>
M-HE	7.83 $\pm$ 0.57 <sup>f</sup>	79.63 $\pm$ 1.12 <sup>ef</sup>	71.42 $\pm$ 0.56 <sup>b</sup>	6.65 $\pm$ 0.00 <sup>de</sup>	3043.7 $\pm$ 3.5 <sup>e</sup>	55.4 $\pm$ 0.1 <sup>b</sup>
R-HE	7.58 $\pm$ 0.23 <sup>f</sup>	77.68 $\pm$ 0.80 <sup>efg</sup>	64.35 $\pm$ 0.61 <sup>f</sup>	13.01 $\pm$ 0.14 <sup>bc</sup>	3130.5 $\pm$ 3.5 <sup>c</sup>	26.9 $\pm$ 0.7 <sup>i</sup>
SBE	10.95 $\pm$ 0.55 <sup>c</sup>	75.08 $\pm$ 0.89 <sup>g</sup>	63.92 $\pm$ 0.51 <sup>f</sup>	5.16 $\pm$ 2.22 <sup>e</sup>	2392.5 $\pm$ 9.7 <sup>h</sup>	10.9 $\pm$ 0.1 <sup>j</sup>
M-SBE	16.11 $\pm$ 0.48 <sup>a</sup>	82.61 $\pm$ 4.29 <sup>cd</sup>	73.54 $\pm$ 0.43 <sup>a</sup>	13.46 $\pm$ 0.14 <sup>b</sup>	3054.7 $\pm$ 11.7 <sup>e</sup>	49.3 $\pm$ 0.1 <sup>d</sup>
R-SBE	8.41 $\pm$ 0.53 <sup>def</sup>	78.90 $\pm$ 0.85 <sup>ef</sup>	68.41 $\pm$ 0.69 <sup>c</sup>	16.02 $\pm$ 0.03 <sup>a</sup>	3016.9 $\pm$ 5.5 <sup>f</sup>	34.3 $\pm$ 0.3 <sup>g</sup>
AEE	7.63 $\pm$ 0.55 <sup>f</sup>	83.41 $\pm$ 1.08 <sup>c</sup>	71.78 $\pm$ 0.90 <sup>b</sup>	8.06 $\pm$ 1.22 <sup>d</sup>	3150.4 $\pm$ 2.2 <sup>b</sup>	47.6 $\pm$ 0.2 <sup>e</sup>
M-AEE	9.33 $\pm$ 1.53 <sup>d</sup>	75.81 $\pm$ 1.61 <sup>fg</sup>	67.72 $\pm$ 0.39 <sup>cd</sup>	8.12 $\pm$ 2.78 <sup>d</sup>	3072.5 $\pm$ 6.5 <sup>d</sup>	61.8 $\pm$ 0.8 <sup>a</sup>
R-AEE	8.93 $\pm$ 0.12 <sup>de</sup>	80.44 $\pm$ 2.11 <sup>cd</sup>	66.16 $\pm$ 0.81 <sup>e</sup>	13.01 $\pm$ 0.15 <sup>bc</sup>	3071.4 $\pm$ 5.8 <sup>d</sup>	32.8 $\pm$ 0.4 <sup>h</sup>

The superscript letters indicate the statistical difference in rows in significant level at 5%.

endogenous antioxidant substances. Therefore, although the data of the four processing technologies without pretreatment differ greatly, they are still clustered into the same group.

### Principal component analysis

A principal component analysis (PCA) was performed on the collected data to extensively display the impact of various processing and pretreatment methods on the nutritional quality and antioxidant capacity of HORO. The PCA was performed on 11 factors connected to HORO samples prepared using various processing methods. The first four principal components described a substantial 75.084% of the total variability (PC1, PC2, PC3 and PC4 were 30.505%, 17.331%, 15.406%, and 11.842%, respectively). This indicated that they could represent 11 indices to analyze the endogenous antioxidant content and in vitro antioxidant level of HORO prepared by different processing methods. Fig. 2 depicts the initial factor load matrix model of the principal components. In the first two principal components, FRAP, ABTS,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol, DPPH-nonpolar, phytosterol, and polyphenol were the decisive factors in the factor loading matrix that indicated a strong correlation between the antioxidant capacity of HORO and its  $\alpha$ -tocopherol,  $\beta$ -tocopherol, and total phenol contents. Different individual tocopherols in HORO have distinct roles in scavenging disparate free radicals. Of these,  $\alpha$ -tocopherol has the strongest ability, which depends on its molecular structure. A phenol hydroxyl group in  $\alpha$ -tocopherol can provide an H-atom to DPPH to become a stable tocopherol quinone radical (Rubio, Sopelana, Ibargoitia, & Guillén, 2018). The same substance having different effects on scavenging different free radicals may be related to the potential interactions between different types of lipophilic and hydrophilic antioxidants. As such, the synergistic or antagonistic action between antioxidants may lead to the enhancement or attenuation of antioxidant activity (Liu et al., 2022).

Standardized data were used to analyze and rank the four calculated principal components, which allowed for the weight calculation of each principal component and the overall score ranking. The overall scores of the oils produced from the 12 combinations of processing technologies were calculated, among which M-HE had the highest score. Consequently, HORO prepared using M-HE had the best nutritional value and functional properties.

### Conclusions

The research aimed to evaluate the impact of 12 different processing technologies on the physicochemical properties, endogenous antioxidant substance content, and antioxidant capacity of HORO. The findings indicated that the extraction process was more efficient at producing lipids from HORO than the pressing process. HORO produced using AEE had the highest oil yield (59.7%), while that produced using R-CP had the lowest (35.4%). The processing methods had little effect on the fatty acid composition of HORO, with high C18:1 and low C22:1 content. The analysis revealed that M-HE oil had a higher tocopherol content (688.4 mg/kg) than the others, and CP-processed oil was highest in polyphenol content (1047.27 mg/kg). The total phytosterol concentration in SBE oil (1456.0 mg/kg) was less than that in other samples, and the same extraction process with roasting pretreatment (R-SBE) led to the highest phytosterol content (2013.0 mg/kg). Thus, the processing method has more influence on the physicochemical properties of HORO than the pretreatment method. However, the effects of pretreatment methods on nutrient content and antioxidant capacity vary according to different processing methods. Among them, those without preprocessing are classified into one group, AEE and HE are grouped together, while CP and SBE are grouped together; this was further demonstrated in the HCA analysis. Generally, microwave pretreatment performed better than roasting. PCA based on antioxidant capacity and nutrients showed that HORO prepared using M-HE had better nutritional characteristics and stability. The results of this study are a valuable resource for the

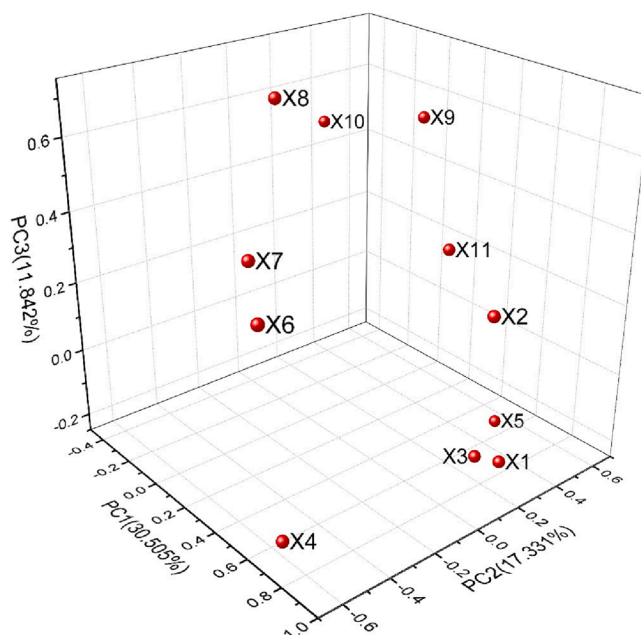


Fig. 2. Principal component analysis loading plot.

optimization of industrial production and processing methods for HORO. In the future, high-quality HORO can be developed by optimizing its processing methods.

### CRediT authorship contribution statement

**Huihui Zhang:** Writing – original draft. **Pan Gao:** Conceptualization, Writing – original draft. **Huiwen Fang:** Formal analysis. **Man Zou:** Formal analysis. **Jiaojiao Yin:** Investigation. **Wu Zhong:** Investigation. **Zhi Luo:** Investigation. **Chuanrong Hu:** Conceptualization. **Dongping He:** Resources. **Xingguo Wang:** Supervision.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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### Consent for publication

The work described has not been published before; it is not under consideration for publication elsewhere; its publication has been approved by all co-authors; its publication has been approved by the responsible authorities at the institution where the work is carried out.

### Availability of data and material

The data and material set supporting the materials and results of this article are included within the article.

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