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Effects of natural and mechanical drying processes on unsaturated fatty acids, vitamin E, estradiol, and physicochemical properties of *Rana chensinensis* ovum

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Abstract

Although *Rana chensinensis* Ovum (RCO) is enriched with high nutritional value, its quality varies due to the different drying methods used by farmers. This study aims to explore the impact of natural and mechanical drying methods on the nutritional compositions and physicochemical properties of RCO. Five different drying methods were studied, including blast drying (BD), mild breeze light drying (MBLD), natural air drying (NAD), vacuum heat drying (VHD), and vacuum freeze drying (VFD). The quality assessment was based on unsaturated fatty acid (UFA) content, iodine value, estradiol, vitamin E, saponification value, thiobarbituric acid value (TBA), acid value, and antioxidant properties. The results showed that VFD had higher UFA content and lower acid value ($139.950 \pm 0.397\ mg \cdot g^{-1}$) and TBA value ($0.097 \pm 0.003$) than others. VFD and NAD had higher iodine values (147.166 ± 1.475 and 146.803 ± 1.209, respectively) than others. There were no significant differences in estradiol, vitamin E, peroxide value and the clearance rates of $ABTS^+$ free radicals and DPPH• free radicals with different drying methods. The findings will provide valuable insights into the industrialization of RCO.

Keywords *Rana chensinensis* ovum, Drying process, Unsaturated fatty acids, Vitamin E, Estradiol, Physicochemical properties

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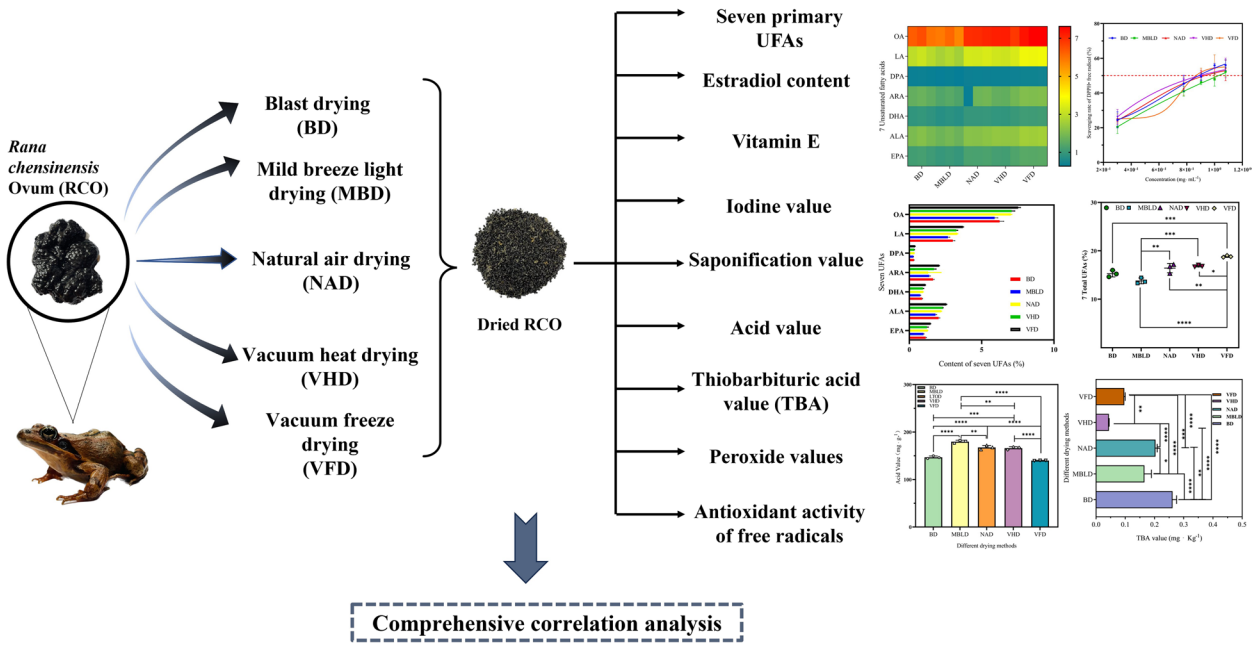
Graphical Abstract



Introduction

Rana chensinensis, a type of frog similar to the Bull Frog (*Rana catesbiana* Shaw), is known for its dietary benefits. *Rana chensinensis* ovum (RCO), obtained from *Rana chensinensis*, is highly nutritious (Liu et al. 2018; Lu et al. 2012; Wang et al. 2022; Zhang, Li et al. 2022; Zhang, Zhang et al. 2022). Previous studies have shown that RCO is rich in unsaturated fatty acids (UFAs) and essential bioactive compounds such as estradiol and progesterone. Zhang (Zhang et al. 2022) studied the principal components of 20 batches of RCO from different origins and identified seven unsaturated fatty acid chromatographic peaks as EPA, ALA, DHA, ARA, DPA, LA and OA. Additionally, RCO has been reported to have the potential to regulate lipid metabolism, prevent and treat cardiovascular diseases, promote growth and development, and provide therapeutic benefits for conditions such as tumors (Dan-Yu et al. 2010) and inflammation (Jing et al. 2008; Li et al. 2022; Wang et al. 2015; Xu et al. 2022; Zhang, Li et al. 2022; Zhao et al. 2019). In vivo and in vitro studies of RCO have demonstrated the antioxidant capacity of RCO oil, unsaturated fatty acids, proteins, melanin, polysaccharides, adhesive membrane polysaccharides and estrogens (Jun-Mei et al. 2018; Rui et al. 2018; Zhang et al. 2022; Zhao et al. 2019). In vitro antioxidant studies of RCO showed that RCO and its components had DPPH• and ABTS+ free radical scavenging activity (Rui et al. 2018). RCO oil has good development and utilization value in natural antioxidants and health food (Yunhou et al. 2018). However, the drying method may have an effect on the antioxidant capacity of the food. Irondi et al. (Ma et al. 2013) found that oil extracted from freeze-dried papaya seeds had better antioxidant capacity relative to air drying (room temperature), sun drying, oven drying, and freeze-drying. Therefore, it is important to test its free radical scavenging ability in drying processes for its further development. A study showed that oven-dried and freeze-dried seed oils had higher DPPH scavenging ability (Fawole et al. 2022). RCO has a similar taste and comparable nutrition to fish roe, but it is primarily sold as a dried agricultural product. Therefore, it is necessary and desirable for RCO processors and consumers of oil on the value chain to explore the effect of RCO drying methods on their free radical scavenging capacity.

Despite its high value as a food source, the industrialization of RCO remains challenging, particularly due to quality changes during the drying process. Food drying is a pretreatment method that reduces moisture content and water activity through natural or mechanical means. Studies have shown that food drying can increase the storage time of food and facilitate transportation (Zhang et al. 2018). For example, in the case of roe drying of aquatic products, Topuz et al. found that freeze-drying

successfully inhibited BA formation (Topuz et al. 2021). The drying process protects food from microbial corrosion and oxidation to a certain extent, including aquatic products, seafood, fresh fruits and vegetables (Mahanti et al. 2021). However, the drying process can alter the nutritional and chemical composition of the food, as well as its biological activity. Various studies have shown that different drying methods affect the preservation of specific components in certain foods, such as ginger volatiles, gingerols, total phenolic content, and total flavonoid content (An et al. 2016). Natural sun drying, air drying, indoor drying and hot air drying are the most widely used methods due to their lower cost (Díaz-Maroto et al. 2003). However, the traditional drying method based on solar energy can easily deteriorate due to the change of environmental conditions, which leads to a significant loss of product quality. While hot air drying is a commonly used method because it can produce dried materials in a shorter time compared to sun drying and indoor drying (Zhang et al. 2018). However, antioxidants and active compounds in RCO, especially unsaturated fatty acids, are sensitive to heat, oxygen, and light during drying. However, there is no relevant study on the effects of natural and mechanical drying processes on the main unsaturated fatty acids, estradiol content, vitamin E content, iodine value, saponification value, acid value, TBA value and free radical scavenging ability of RCO.

Therefore, the objective of this research is to firstly explore the impacts of natural drying and mechanical drying methods on the bioactive nutrient composition and physicochemical properties of RCO. Five different methods were employed, including vacuum freeze-drying (VFD), vacuum heat drying (VHD), natural air-drying (NAD), blast drying (BD), and mild breeze light drying (MBLD). The contents of unsaturated fatty acids of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), oleic acid (OA), α-linolenic acid (ALA), Arachidonic acid (ARA), Linoleic acid (LA), iodine value, estradiol, vitamin E, saponification value, 2-thiobarbituric acid value, acid value, peroxide value, scavenging capacity of $ABTS^{+}$ free radicals and DPPH• free radicals were investigated. The results of this research will serve as a foundation for the quality control of RCO products and facilitate the commercialization and industrialization of this valuable food.

Materials and methods

Materials

The RCO used in this study was collected from the Changbai Mountain *Rana chensinensis* Breeding Base (Jilin, China, 2022.12). Fresh RCO of 2.650 g, 2.651 g and 2.649 g were placed in a moisture meter (PC-16A, Shanghai PuchunMeasuring Instrument Co., LTD., Shanghai, China) and dried until the weight remained unchanged within 60 seconds, then drying was stopped. The water loss rate of the sample was obtained and the average water loss rate was calculated, which was the average water content of RCO. The RCO moisture used in this experiment was 67.63%. Samples were stored in a freezer at −80 °C before experiments. All other reagents were purchased commercially and used without further purification. The water used in the experiment was produced by an ultra-pure water machine WP-UP-III-10 (Water Purifier, Sichuan, China). All other reagents and chemicals were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China).

Different drying methods of RCO

BD: RCO (100 g) was placed in a blast drying oven (GZX-9140MBE, Medical Equipment Factory of Shanghai Boxun Industrial Co., LTD., Shanghai, China) and dried at 39 °C until reaching a constant weight. MBLD: RCO (100 g) was placed in mild breezes and light equipment (WD-A, Tianjin Pharmacopeia Standard Instrument Factory, Tianjin, China) at a set temperature of 28 °C humidity of 22%, and default wind and light. NAD: RCO (100 g) was placed outdoors to avoid light and dried to a constant weight (ambient temperature with humidity 57%, force 4 northeast wind). This condition was based on the air-drying condition in Northeast China in winter at the time RCO was harvested. VHD: RCO (100 g) was placed in a vacuum drying oven (DZF-6050, Medical Equipment Factory of Shanghai Bosun Industrial Co., LTD., Shanghai, China) and dried at 39 °C to a constant weight. VFD: RCO (100 g) was dried in a vacuum freeze-dryer at −80 °C (FreeZone 4.5 Plus, LABCONCO, Kansas, USA) to a constant weight.

Sample preparation

Dried RCO (10.0 g) obtained by five different drying methods was weighed separately and ground to powder by a high-speed Chinese medicine grinder (HX-200, Xian Hardware & Medicine Factory, Yongkang, Zhejiang, China) and put into a 250 mL conical bottle. Petroleum ether (160 mL) was added to the bottle to complete the assembly of the extraction device. The solution was extracted by ultrasound for 75 minutes without heating. Extractions of five dried RCOs were then collected by vacuum evaporation of solvent petroleum ether from the extract at 40 °C using a rotary evaporator. The extract was a yellowish oil.

Determination of UFAs

The unsaturated fatty acids (UFA) s content in RCO was determined via high-performance liquid chromatography

(HPLC) with some adjust (Zhang, Li, et al. 2022). Briefly, 0.0325 g of RCO extract was mixed with 3 mL of 2 mol·L^{-1} NaOH solution and stirred for 3 hours, and then the pH was adjusted to 3 using 2 mol·L^{-1} HCl. After extraction with petroleum ether in three steps (each with 2 mL), the upper layer was collected and subjected to rotary evaporation. Following sample collection, each sample was filled with 5 mL of ethanol and underwent filtration through a 0.22 μm membrane (Nylon 66) before injection. An Agilent 1260 series with a TC-C18 column (4.6 × 250 mm, 5 μm) was used to quantify the seven primary UFAs. Mobile phase A was chromatography grade acetonitrile. Mobile phase B was ultrapure water containing 1% phosphoric acid (v / v). A gradient elution was used. The mobile phase change conditions were 0–18 min, linear gradient 87% mobile phase A. 18–20 min, maintaining 87–90% mobile phase A and 20–22 min, 90–96% mobile phase A. 22–26 min, 96% A. 26–30 min, 96–100% A. The flow rate change conditions were 0–14 min, linear change 1.0–0.5 The detection wavelength was 203 nm, and the temperature was 30 °C. The injection volume was 10 μL. The unsaturated fatty acid standards were dissolved in ethanol and filtered through a 0.22 μm microporous membrane (Nylon 66) before injection (Zhang, Li, et al. 2022). The data were recorded and processed using Agilent Chemstation software (Agilent Technologies, Inc., Santa Clara, USA). The content of unsaturated fatty acids is expressed as a percentage in the extract.

Determination of estradiol

Calibration curve of the estradiol standard

Estradiol was analyzed by HPLC, and the conditions were optimized (Cao et al. 2010). Agilent 1260 series liquid chromatograph equipped with a UV detector (Palo Alto, CA, USA) was used for quantitative analysis of estradiol content in RCO extraction. The chromatographic column used was an Agilent TC-C18 (4.6 × 250 mm, 5 μm). Estradiol standard solutions of 0.001 mg · mL^{-1}, 0.002 mg · mL^{-1}, 0.004 mg · mL^{-1}, 0.006 mg · mL^{-1}, 0.008 mg · mL^{-1} were prepared, and the data were recorded and processed using Agilent Chemstation software. Standard curve of estradiol in this experiment: y = 25,592x - 2.2067, R^2 = 0.9839.

Determination of estradiol

RCO samples (about 50 mg) were stored in 1 mL of methanol under the conditions described above. Before injection, samples were filtered through a 0.22 μm membrane (Nylon 66). The estradiol content was calculated using the standard curve, and the data were processed via Agilent Chemstation software.

Determination of vitamin E

Calibration curve of vitamin E

The content of vitamin E was determined by HPLC (Prakash et al. 2021). Vitamin E solutions of 0.01, 0.02, 0.04, 0.06, 0.08, and 0.10 mg · mL^{-1} with methanol as the solvent were filtered through a 0.22 μm syringe (Nylon 66) and injected with 10 μL. The Waters e 2695 HPLC system, Waters 2475 FLR Detector, and Amerhyst C18-P column (250 × 4.6 mm, 5 μm) were used for analysis. The excitation and emission wavelengths were 290 nm and 330 nm, respectively. Methanol: water (95: 5, v · v) was used as the mobile phase, and the solvent flow rate was maintained at 1.0 mL · min^{-1}. Drawing of the standard curve of the resulting data. Vitamin E standard curve in this experiment: y = 6675.2x - 2699.8 R^2 = 0.999.

Determination of vitamin E

Samples (0.01 g) were dissolved in 1 mL of methanol, filtered through a 0.22 μm syringe (Nylon 66), and quantified through method described above with the retention time of the standard. The vitamin E content in RCO was determined and expressed as μg·g-1 and calculated using the standard curve.

Determination of iodide value

Iodine value (IV) determination in RCO samples was carried out using spectrophotometry (Kruatian & Jitmanee 2013). For the standard curve, 0.10 mol/L Hanus solution was appropriately diluted with glacial acetic acid to prepare a series of standardized Hanus solutions. 1.00 mL of different concentrations of Hanus solution was added together with 1.00 mL of isooctane and left in the dark for 1 min before 1.00 mL of 15% w/v KI solution was added. One mL of the resulting solution was mixed with 1.00 mL of 15% w/v KI solution and then diluted to 100 mL with deionized water before absorbance was measured in a volumetric flask. All procedures were performed at 25 °C. An absorbance-concentration curve was used to determine the concentration of the unreacted Hanus solution at 350 nm. For the sample solution, after precise weighing, the dried sample extract was dissolved with isooctane into an RCO solution at a concentration of 0.01 g/mL. 1 mL of RCO sample solution was treated in the dark with 1.00 mL of 0.10 mol/L Hanus solution for 1 min, and then the rest of the procedure was performed as described above for standard curve drawing. The sample solution was replaced by isooctane for reagent blank treatment. The concentration of the unreacted Hanus solution was determined by calibration plots. The IV was calculated using Eq. (1), guided by the standard curve: y = 0.2049x - 0.0039 R^2 = 0.9919

$$\text{Iodine value} = \frac{(M_B - M_S) \times 100 \times 126.9 \times 2}{(1000 \times W_S)} \quad (1)$$

where M_B and M_S are the molar concentrations of IBr obtained from blank and sample determinations, respectively, and W_S is the weight in grams of sample.

Determination of saponification value

The saponification value refers to the analytical method used to determine the amount of free fatty acids and glycerides present in oils and fatty acids. In this paper, the saponification value was determined by titration according to the Chinese National Standard (GB/T 5534–2008). The procedure involves boiling the sample with potassium hydroxide-ethanol solution and titrating the excess potassium hydroxide with a calibrated hydrochloric acid solution. Samples (2.0 g) were packed in 250 mL Erlenmeyer flasks with an accuracy of 0.005 g. Subsequently, a solution of 25.0 mL of 0.5 mol·L^{-1} potassium hydroxide-ethanol was added, along with boiling aids. The reflux condenser was attached to the Erlenmeyer flask, which was heated slowly for 2 hours while shaking the mixture periodically. After heating, phenolphthalein indicator was added to the solution and titrated with 0.5 mol·L^{-1} hydrochloric acid standard solution until the pink color disappeared, indicating the endpoint of the reaction. Equation (2) was then used to calculate the saponification value of RCO. A blank test was performed alongside this procedure using 25.0 mL of potassium hydroxide-ethanol solution without a sample.

$$I_S = \frac{(V_{0-}V_1) \times C \times 56.1}{m} \quad (2)$$

where I_S is the saponification value (mg/g), V_o is the volume (mL) of hydrochloric acid standard solution consumed in the blank test, V_i is the volume (mL) of hydrochloric acid standard solution consumed in the sample, c is the concentration (mol/L) of hydrochloric acid standard solution, and m is the mass (g) of the sample.

Determination of acid value

The acid value (AV) was evaluated according to China national standards GB5009.229–2016 (Li et al. 2023). To measure the acid value of a 0.1 g sample of RCO extraction obtained from different drying methods, 60 mL of ethyl ether-isopropanol solution mixed with phenolphthalein indicator was added to a 250 mL Erlenmeyer flask containing the sample. The solution was shaken until the complete dissolution of the sample. A standard titration solution was then titrated onto the sample solution using a graduated burette until the reddish appearance of the solution persisted for 15 seconds, indicating the endpoint of the titration. The volume of standard titration solution consumed was recorded. To conduct a blank test, a mixture of organic solvent and indicator identical to the sample determination was titrated with a standardized titration solution, and the volume of solution consumed, V_0, was recorded. The acid value was calculated using Eq. (3). This method provides a reliable way to determine the acid value of RCO extraction samples.

$$X_{AV} = \frac{(V - V_0) \times C \times 56.1}{m} \quad (3)$$

Determination of TBA

TBA was determined using the method of Lee et al. with some adjustments (Lee & Yoon 2013). A precisely measured amount of RCO extract (100 mg) with a precision of 0.1 mg was directly placed into a 25 mL volumetric flask, followed by the addition of 5 mL – 10 mL of 1-butanol to facilitate sample dissolution and dilution to the mark. Subsequently, a 5 mL aliquot of the prepared sample solution was dispensed into a stoppered test tube, followed by the addition of 5 mL of TBA reagent to the tube. After covering the stopper, the test tube was vigorously shaken to ensure efficient mixing. The stoppered test tube, containing the prepared solution, was subjected to a 2-hour heating process at 95 °C, followed by cooling to room temperature via tap water immersion for approximately 10 minutes. Once cooled, the solution in the stoppered test tube was transferred to a clean and dry 10 mm cuvette for spectrophotometric analysis. The spectrophotometer zero point was calibrated using distilled water at 530 nm before measuring the absorbance of the solution as A.

To prepare the blank, a stoppered test tube filled with 5 mL of 1-butanol, instead of the sample, was supplemented with 5 mL of TBA reagent, capped, and shaken well. Subsequently, the test tube was subjected to a 2-hour heating process at 95 °C, capped and shaken well, and then cooled to room temperature using tap water immersion for approximately 10 minutes. The absorbance of the blank reagent was immediately measured as B. The TBA value of RCO was calculated using formula (4).

$$\text{TBA} = \frac{(A - B) \times 50}{m} \quad (4)$$

Determination of peroxide value

The peroxide value was measured using the method of Mureșan et al. with adjustments (Mureșan et al. 2010). Approximately 10 mg of RCO extract was dissolved in 9.8 ml of a chloroform-methanol mixture, 7:3 (v·v), and 50 µL of ammonium thiocyanate solution was added, followed by 50 µL of Fe (II) solution. After 10 min, a

spectrophotometer (UV-754) was used to measure the absorbance at 520 nm compared to the blank. The blank contains all reagents except RCO extraction. The peroxide value is expressed in m Eq $O_2 \cdot kg^{-1}$ fat and calculated using Eq. (5).

$$\text{Peroxide Value (PV)} = \frac{\text{Abs}}{55.84 \times \text{w}} \times \frac{1}{\text{b}} \left[\text{m EqO}_2 \cdot \text{Kg}^{-1} \text{ fat}\right] \quad (5)$$

Standard curve: 0.1, 0.2, 0.4, 0.6, and 0.8 mg · mL-1 Fe^{3+} standard working solutions were added to five 10 ml dry stoppered graduated test tubes, and the volume was adjusted to 1 ml with 75% ethanol to prepare 0.1, 0.2, 0.4, 0.6, and 0.8 mg · mL^{-1} Fe^{3+} standard series. The stopper was quickly closed and vortexed for 10 seconds. They were placed in the dark at 15–25 °C for 20 minutes, with 75% ethanol as a blank control, and the absorbance was measured at a wavelength of 520 nm.

The determination of each content was repeated 3 times, and the standard curve was drawn with the mean value of absorbance against the corresponding content of Fe^{3+} (mg · mL^{-1}). The experimentally measured Fe^{3+} standard curve is $y = 0.0735x + 0.0083$, $R^2 = 0.9931$.

Antioxidant activity

DPPH• free radicals scavenging

The DPPH• free radical scavenging test was performed using the reported method (Christodouleas et al. 2015). The RCO extraction of different drying methods was prepared into solutions with mass concentration gradients of 12.0, 10.0, 8.0, 6.0, and 2.0 mg · mL^{-1}. A 95% ethanol solution of DPPH• free radicals with a concentration of 0.1 mmol · L^{-1} was prepared. Then, 100 µL of different concentration gradient solutions of RCO extract was added to 100 µL of DPPH• free radicals solution, shaken well, and reacted in the dark at room temperature for 30 min, and finally, the absorbance value of the above solution A_S was measured at 517 nm, with the solvent 95% ethanol replacing the sample as the blank, absorbance A_0. The DPPH• free radicals scavenging clearance rates of different concentrations of RCO extraction by different drying methods were calculated according to Eq. (6).

$$\text{DPPH} \bullet \text{ free radicals scavenging activity (\%)} = \frac{A_0 - A_s}{A_0} \times 100 \quad (6)$$

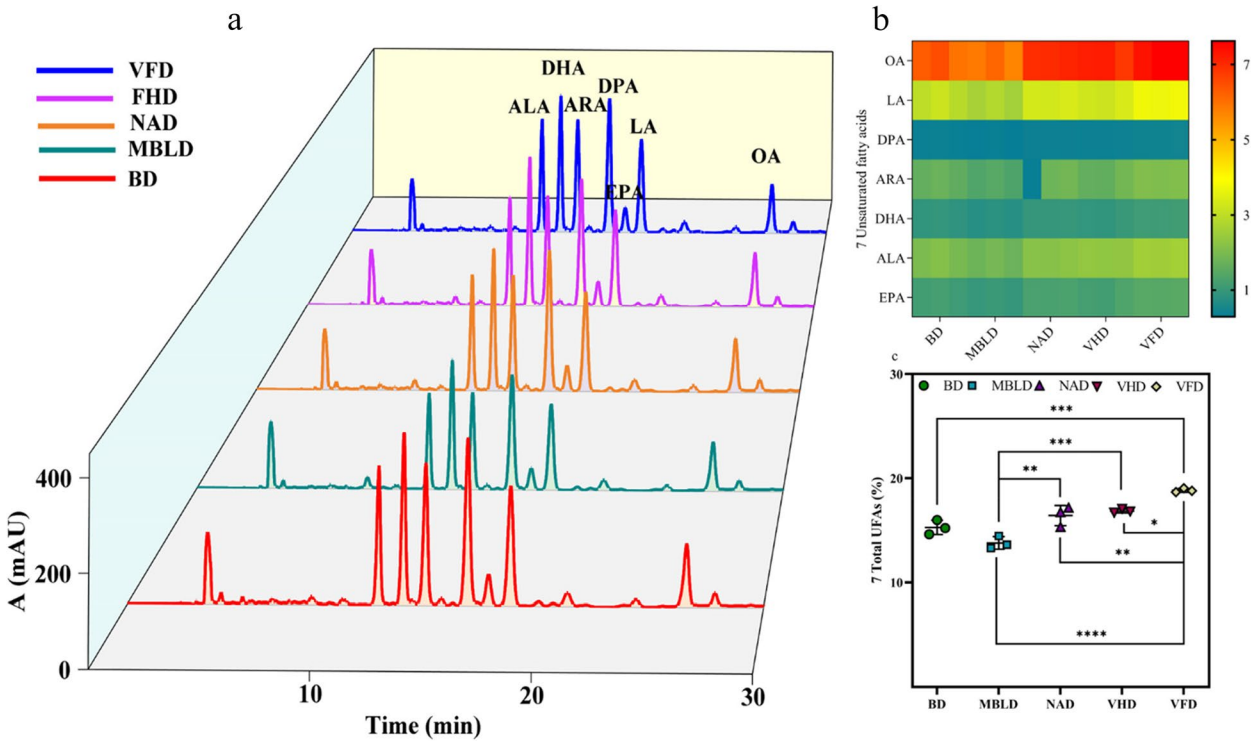


Fig. 1 **a** High-performance liquid chromatography spectra of seven main unsaturated fatty acids of RCO extract by five drying methods. **b** Heatmap of seven unsaturated fatty acid contents (%) of RCO extract by five drying methods. **c** The total content (%) of RCO extract by five drying methods. The number of * represents the difference: * represents $0.01 < P < 0.05$, ** represents $0.001 < P < 0.01$, *** represents $0.0001 < P < 0.001$, **** represents $P < 0.0001$ (Tukey's multiple comparisons test). BD = blast drying, MBLD = mild breeze light drying, NAD = natural air drying, VHD = vacuum heating drying, VFD = vacuum freeze drying, UFAs = unsaturated fatty acids, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), oleic acid (OA), α-linolenic acid (ALA), Arachidonic acid (ARA), Linoleic acid (LA)

Table 1 The content of seven main unsaturated fatty acids (UFAs) in RCO extract

	BD (%)	MBLD (%)	NAD (%)	VHD (%)	VFD (%)
EPA	1.14 ± 0.05^{a}	0.98 ± 0.05^{b}	1.26 ± 0.02^{a}	1.22 ± 0.10^{a}	1.47 ± 0.02^{c}
ALA	2.03 ± 0.09^{a}	1.81 ± 0.09^{b}	2.20 ± 0.04^{c}	2.32 ± 0.02^{cd}	2.56 ± 0.04^{e}
DHA	0.89 ± 0.04^{a}	0.76 ± 0.04^{b}	0.96 ± 0.02^{a}	0.93 ± 0.07^{ad}	1.11 ± 0.02^{c}
ARA	$1.66 \pm .076^{a}$	1.38 ± 0.08^{a}	1.31 ± 0.89^{a}	1.70 ± 0.15^{a}	2.06 ± 0.02^{a}
DPA	0.32 ± 0.02^{a}	0.28 ± 0.02^{b}	0.35 ± 0.01^{a}	0.35 ± 0.01^{a}	0.40 ± 0.01^{c}
LA	3.01 ± 0.14^{a}	2.67 ± 0.13^{b}	3.31 ± 0.05^{bd}	3.25 ± 0.10^{a}	3.71 ± 0.04^{c}
OA	6.24 ± 0.29^{a}	5.90 ± 0.22^{a}	7.02 ± 0.07^{b}	7.07 ± 0.22^{b}	7.52 ± 0.16^{b}
Total of 7 UFAs	15.27 ± 0.69^{a}	13.78 ± 0.61^{a}	16.41 ± 0.97^{ac}	16.84 ± 0.19^{ac}	18.83 ± 0.19^{b}

7 unsaturated fatty acids (7 UFAs): eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), oleic acid (OA), α-linolenic acid (ALA), Arachidonic acid (ARA), Linoleic acid (LA), *BD* blast drying, *MBLD* mild breeze light drying, *NAD* natural air drying, *VHD* vacuum heating drying, *VFD* vacuum freeze drying. Data were evaluated by ANOVA followed by Tukey's test. Different letters horizontally indicate significant differences ($p < 0.05$) according to Tukey's test

$ABTS^+$ free radicals scavenging

The $ABTS^+$ free radical scavenging ability was tested according to a previous method (Wojtunik-Kulesza et al. 2018). $ABTS^+$ free radical solution (7.4 mM) and $K_2S_2O_8$ stock solution (2.6 mM) were mixed at a volume ratio of 1:1 and placed in a dark environment at room temperature for 12–16 hours. Ethanol (95%) was used to dilute the solution to make the absorbance value at 734 nm 0.7 ± 0.05, and this was used as the $ABTS^+$ working solution. The sample solutions were 20, 15, 12, 10, 8, 6, and 4 mg·mL^{-1}. $ABTS^+$ free radical (100 µL) working solution and 100 µL sample solution were reacted in the dark for 6 minutes in a microwell plate, and the absorbance A_S at 734 nm was measured. Ethanol (95%) was used as a blank instead of the sample, and the absorbance A_0 at 734 nm was measured. According to Eq. (7), the $ABTS^+$ radical clearance rate of different concentrations of RCO extraction in each batch was calculated.

$$\text{ABTS}^+ \text{ free radical scavenging activity (\%)} = \frac{A_0 - A_s}{A_0} \times 100 \quad (7)$$

Statistical analysis

For each experiment, three parallel replicates were set up. All data from each experiment are presented as the mean ± standard deviation (SD). All statistical analyses were performed using the SPSS software package (SPSS version 19.0 for Windows). The statistical significance level was set at $p < 0.05$.

Results and discussion

Seven primary unsaturated fatty acids (UFAs)

The biological activities of RCO primarily stem from its UFAs. In particular, RCO is rich in EPA, DHA, ALA, ARA, DPA, LA, and OA (Gan et al. 2020). Of those, EPA and DHA play crucial roles in vision and nervous system development, respectively (Hamilton et al. 2020; Kris-Etherton et al. 2009). DHA and DPA can improve metabolic disorders, inhibit lipogenesis (Wei et al. 2020), regulate human blood flow velocity and lipid levels, and reduce the probability of cardiovascular risk events (Asztalos et al. 2016). ALA, on the other hand, maintains normal physiological activities of the body, reduces blood lipids and blood pressure, improves cardiovascular diseases, improves memory, protects nervous tissue, prevents allergic diseases, and delays aging (Naghshi et al. 2021). DPA has been demonstrated to improve immunity, protect vision, have anti-thrombosis and antitumor effects, reduce cardiovascular and cerebrovascular diseases, and prevent neurodegenerative diseases (Lorente-Cebrian et al. 2015; Perez-Cornago et al. 2020; Woodman et al. 2003). LA maintains skin elasticity and health, treats and prevents dermatitis caused by radiation, and improves other wounds (Hartop & Prottey 2010). Furthermore, it interacts with essential fatty acids, correcting skin impermeability caused by water deficiency. OA has a preventive and controlling effect on metabolic syndrome, obesity, cardiovascular disease, diabetes, inflammation, and other diseases (Sales-Campos et al. 2013).

As shown in Fig. 1a, the peak time of unsaturated fatty acids in RCO remained consistent across five distinct drying methods. The comparative analysis of seven main unsaturated fatty acids across five distinct drying methods showed significant differences ($P < 0.05$). The addition of seven unsaturated fatty acids also resulted in a significant difference ($P < 0.05$). As shown in Table 1, the UFA content of VFD was the highest, while MBLD and BD had small UFA contents due to the sensitivity of UFA molecules to heat and light and susceptibility to oxidative degradation by oxygen. This result suggested that high temperatures and oxygen-rich environments may lead to a reduction in UFAs and hinder their retention in RCO. The oxidation products of unsaturated fatty acids

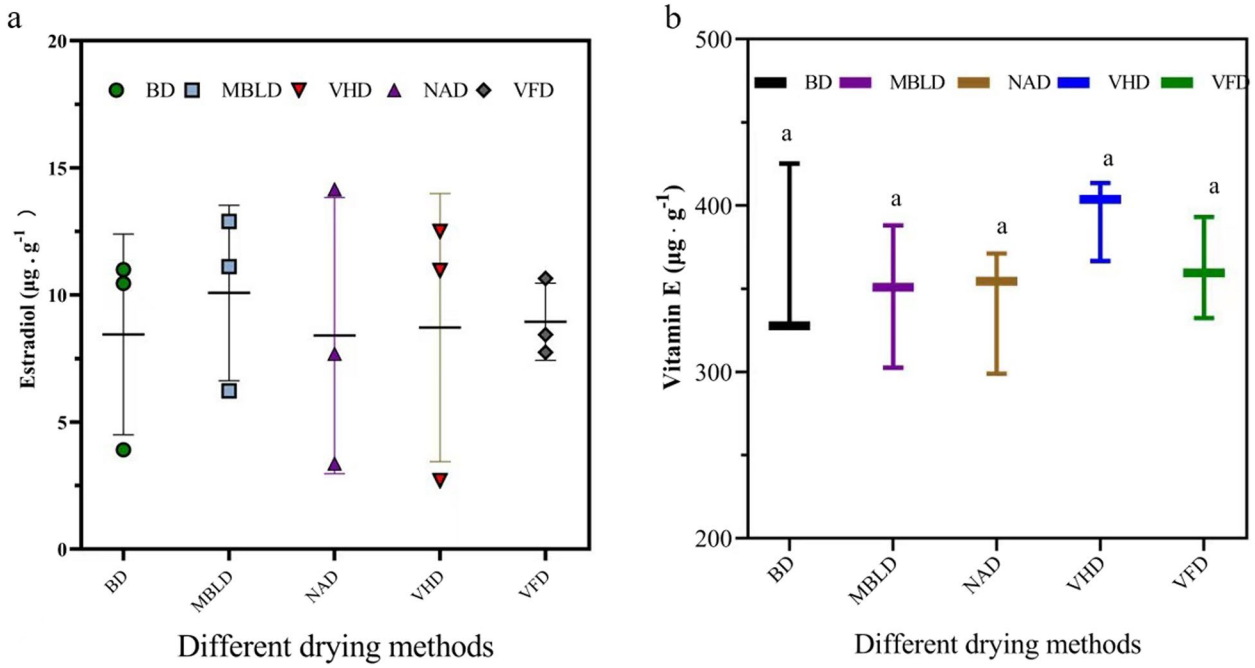


Fig. 2 **a** Estradiol in RCO extract of five drying methods (μg·g^{-1}), **b** Vitamin E in RCO extract of five drying methods (μg·g^{-1}). Both $p > 0.05$, Tukey's multiple comparisons test. BD = blast drying, MBLD = mild breeze light drying, NAD = natural air drying, VHD = vacuum heating drying, VFD = vacuum freeze drying

affect the quality of RCO, reducing its nutritional value and shortening its shelf life. As shown in Fig. 1b, OA, LA, DHA, ALA, ARA, EPA, DHA, and DPA were found in all samples in descending order. Among them, the ARA of NAD is much lower. Therefore, it is essential to choose a suitable method to dry RCO to retain its unsaturated fatty acids, while VFD, VHD, and NAD are beneficial to protect UFAs.

Estradiol content

Estradiol is a crucial estrogen hormone and bioactive component in the human body that is primarily produced by the ovaries in women. It has both positive and negative impacts on the body. On the one hand, estradiol acts as a neuroprotective factor in the brain, shielding it from neurodegenerative diseases, emotional disorders, and cognitive decline (Arevalo et al. 2015). Moreover, it has beneficial effects on heart function, improves cardiovascular health, and protects human vascular endothelial cells (Esmailidehaj et al. 2020). Estradiol also safeguards spinal motor neurons from excitatory injury (Nakamizo et al. 2000). On the other hand, excessive intake of estradiol can lead to obesity and eating disorders, particularly in women, and increase the risk of endocrine system cancer, reproductive system abnormalities, and immune system dysfunction (Eckel 2011; Heger et al. 2013). As shown in Fig. 2a, the estradiol content in RCO was not significantly different among the five drying methods tested. Estradiol of RCO was less interfered by five drying conditions in the experiment. The cost of NAD, BD and MBLD was lower than that of VFD and VHD. Therefore, from the perspective of protecting estradiol, NAD, BD, and MBLD are more appropriate.

Vitamin E

Vitamin E is a pale-yellow oil-like substance that is generally resistant to heat, acids, and bases in the absence of oxygen or oxidants but can be destroyed by UV light. However, during food processing, storage, and packaging, vitamin E and its physiological activity may be lost due to processing and oxidation (Ottaway 2010). Vitamin E is essential for human health and has unique physiological functions. Research indicates that a daily intake of 7–9 mg of vitamin E is required for normal physiological function of human muscles and the central nervous system, while an intake of 100–1000 mg can effectively prevent aging (Erdi et al. 2019). Adequate daily intake of vitamin E can also prevent and treat chronic diseases such as cancer, cardiovascular diseases, cataracts, and eye diseases and can benefit muscle repair, liver function,

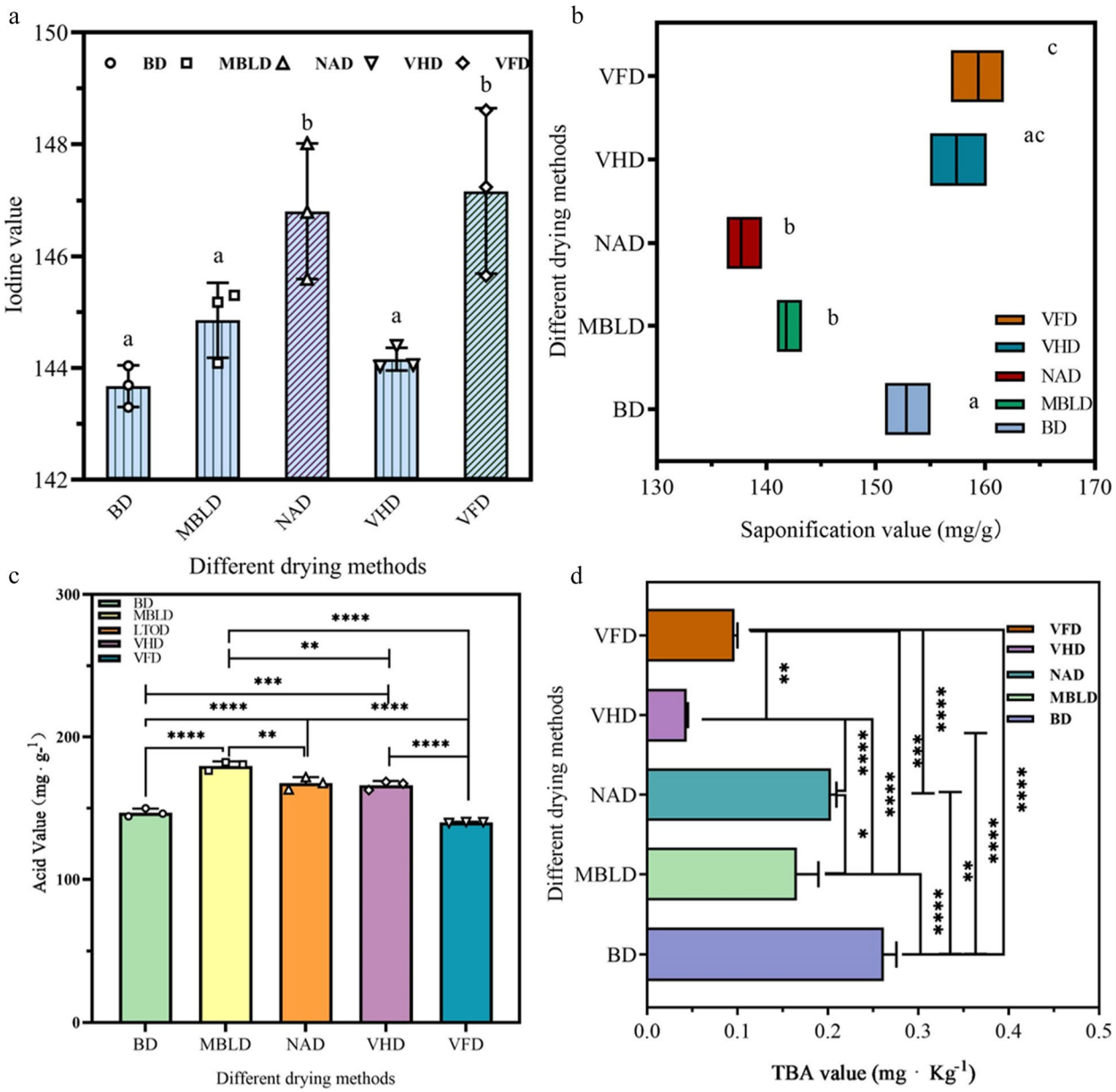


Fig. 3 **a** Iodine values of RCO extract by five drying methods. **b** Saponification value of RCO extracted after five drying methods (mg·g^{-1}). **c** Acid value of RCO of five drying methods (mg · g^{-1}). **d** TBA values of RCO with different drying methods (mg·kg^{-1}). BD = blast drying, MBLD = mild breeze light drying, NAD = natural air drying, VHD = vacuum heating drying, VFD = vacuum freeze drying. The number of * represents the difference: * represents $0.01 < P < 0.05$, ** represents $0.001 < P < 0.01$, *** represents $0.0001 < P < 0.001$, **** represents $P < 0.0001$ (Tukey's multiple comparisons test)

and lung function (Hanson et al. 2016; Sui et al. 2020; Zhan et al. 2020). As shown in Fig. 2b, there were no significant differences in vitamin E content among five drying methods ($P > 0.05$), indicating that vitamin E in RCO is relatively stable. This may be due to the coating effect of RCO, which leads to its vitamin E content being less affected. Hence, selecting a drying method that is both economical and environmentally friendly appears to be a relatively better alternative.

Iodine value

Iodine values are crucial in gauging the level of unsaturation in oils and fats. In this study, iodine values were used to determine the total unsaturated fatty acid content of RCO under different drying methods. A higher

iodine value indicates greater unsaturation, which is beneficial for human health (Pereira & Rocha 2014; Prieto et al. 2014). Oxidation causes a decrease in the iodine values of oils and fats. As shown in Fig. 3a, VHD and NAD retained the highest total unsaturated fatty acid content ($P > 0.05$), with no significant difference between the two methods. BD, on the other hand, resulted in the greatest oxidation of UFAs, leading to the smallest total UFA content. This might be attributed to the high temperature and prolonged oxygen exposure in the BD method. The iodine values of MBLD and VHD were between those of BD and NAD / VFD. VFD and NAD were found to be more effective in retaining total UFAs.

Saponification value

The saponification value indicates the amount of potassium hydroxide required to completely saponify 1 g of oil and indirectly reflects the composition of fatty acids and glycerol content. It is an important indicator of the purity of edible oils, with higher values indicating smaller molecular weights of fatty acids, increased hydrophilicity, and better stability and flavor during cooking (Gopinath et al. 2009). Lower saponification values can result in decreased oil quality and increased impurities. The saponification value also affects the technical parameters of oil derivatives. As shown in Fig. 3b, VFD and VHD had the highest saponification values, likely due to the purity ensured by anaerobic vacuum drying conditions. NAD had the lowest saponification value and worst flavor and purity, possibly due to oxygen exposure and longer exposure time. BD had a higher saponification value than MBLD and NAD but lower than VFD and VHD, possibly due to losses during brief periods of oxygen exposure and high-temperature drying. VFD and VHD were found to have less impact on RCO extract quality and were relatively healthier but more expensive, while BD had a slightly lower saponification value loss than VFD and VHD but a higher loss than NAD and MBLD and was relatively cheaper.

Acid value

The acid value reflects rancidity and the amount of free fatty acids present during the RCO drying process, serving as an indicator of oil quality and oxidation changes during storage (Shen et al. 2020). An increase in free fatty acids in RCO signals triglyceride hydrolysis and greater susceptibility to oxidation (Mihaylova et al. 2020). As shown in Fig. 3c, the results suggest that unsaturated fatty acids in RCO breakdown due to high temperatures and sunlight, leading to the production of free fatty acids. The higher the drying temperature is, the faster the RCO oxidation reaction, and the more free fatty acids are produced (Ren et al. 2021). Additionally,

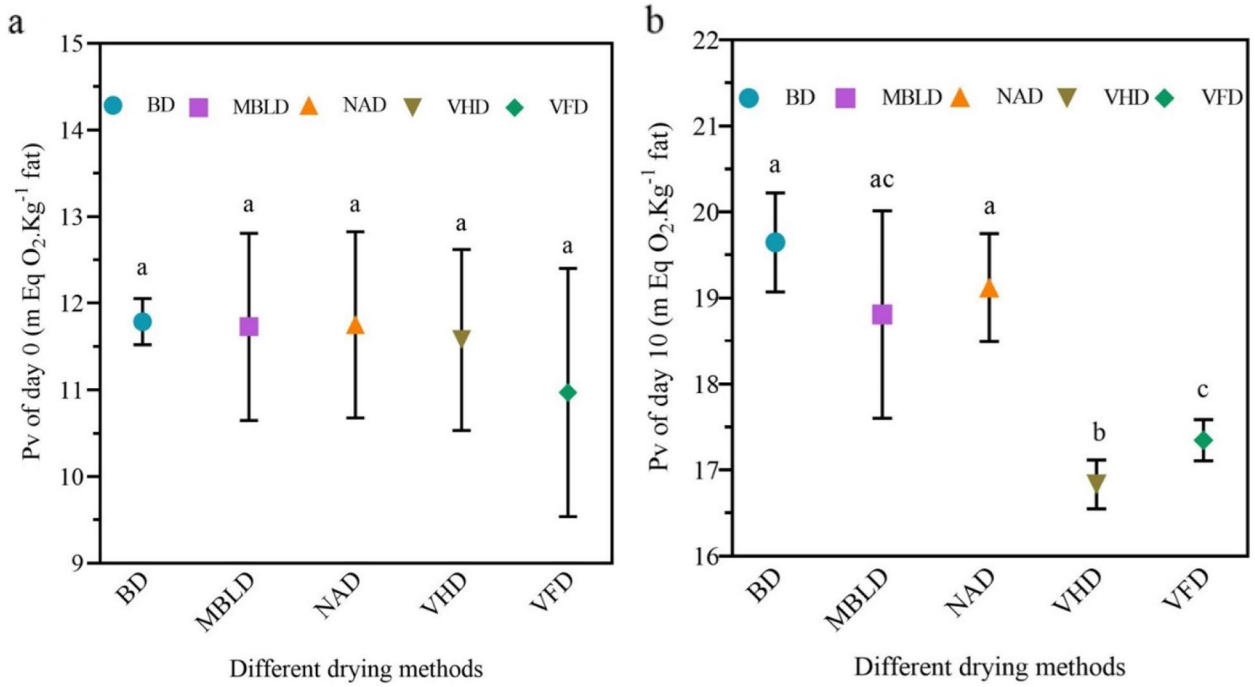


Fig. 4 **a** Peroxide value (Pv) of RCO extract by five drying methods in day 0, $P > 0.05$. **b** Peroxide value (Pv) of RCO extract by five drying methods in day 10, $P < 0.05$ (Tukey's multiple comparisons test). BD = blast drying, MBLD = mild breeze light drying, NAD = natural air drying, VHD = vacuum heating drying, VFD = vacuum freeze drying

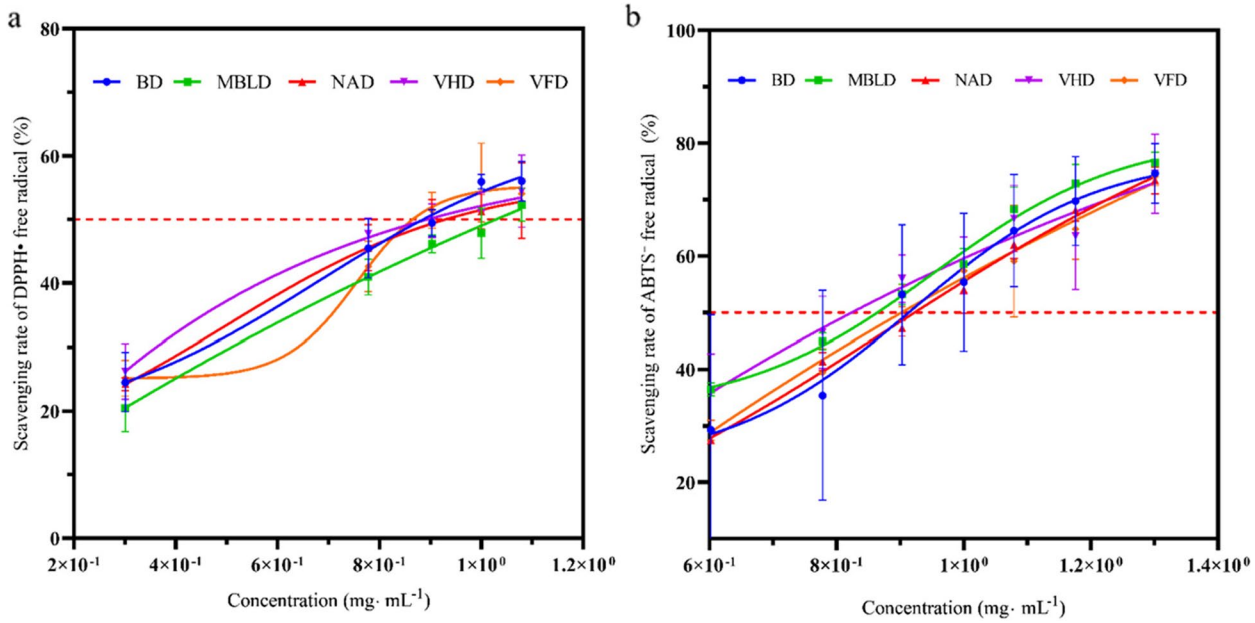


Fig. 5 **a** Scavenging rate of DPPH• free radicals. **b** Scavenging rate of $ABTS^{+}$ free radicals ($P > 0.05$, Tukey's multiple comparisons test). BD = blast drying, MBLD = mild breeze light drying, NAD = natural air drying, VHD = vacuum heating drying, VFD = vacuum freeze drying

the complex oxidation of oils and fats can produce small molecules of aldehydes and acids, which can also contribute to an elevated acid value (Cao et al. 2014). Therefore, RCO should steer clear of prolonged high-temperature drying with oxygen and light conditions.

Thiobarbituric acid value (TBA)

The TBA value is an important indicator of lipid oxidation secondary products. TBA values differ with drying methods and there is a significant difference. As shown in Fig. 3d, of these five methods, three showed the highest TBA, in order from highest to lowest BD, NAD, and MBLD. The increase in TBA values with temperature may be attributed to the fatty acid structure of RCO and its higher sensitivity to lipid oxidation. The formation of lipid oxidation end products and secondary oxidation products such as aldehydes may also undergo polymerization reactions or interact with other polymers (e.g., proteins). To ensure better edible quality, it is recommended to use VFD and VHD for drying RCO to reduce the increase in the TBA value.

Peroxide values

The results of peroxide values showed that there was no statistically significant difference ($P > 0.05$) in the effect of the five drying conditions on the peroxide values of day 0 RCO as shown in Fig. 4a. However, as shown in Fig. 4a, statistically significant differences ($p < 0.05$) were observed when RCO of different drying methods placed in a refrigerator at −20°C for 10 days was tested for peroxide values. The increase in peroxide value is attributed to the formation of hydroperoxides, which is an indicator of the initial stage of oxidation (Umeda & Jorge 2021). The peroxide values of RCO on day 0 under different drying conditions are not significantly different, as shown in Fig. 5a, which indicates to some extent that RCO is relatively stable under the five drying conditions. However, the peroxide value of day 10 is shown in Fig. 4b. VHD and VFD have less effect on the peroxide value of RCO, while BD and NAD make the peroxide value of RCO increase significantly. It can be assumed that the stability of the peroxide values of the RCO and fats deteriorated with increasing drying temperature and oxygen exposure, and there were significant differences (Gómez-Alonso et al. 2004). In summary, there was no significant difference in the peroxide values of RCO extracts extracted by the five drying processes, but the oxidative properties of RCO extracts may be altered during storage after extraction.

Antioxidant activity of free radicals

DPPH• free radicals scavenging ability

The effects of the five drying methods on the DPPH• free radicals scavenging ability of RCO were studied, and no statistically significant difference was found, as shown in Fig. 5a. This suggests that RCO has relatively stable properties under these drying conditions, which preserves its antioxidant ability. The antioxidant activity of RCO is crucial for its industrial utilization. For example,

previous studies on grape seed oil using the DPPH• free radicals method provided a basis for its exploitation and utilization (Bail et al. 2008). This study found that RCO's oxidation resistance was stable, possibly due to the shell protection of RCO's anti-free radical substances during the drying process. Many polar and nonpolar compounds in oils have antioxidant effects similar to those of some synthetic oxidants.

$ABTS^+$ free radicals scavenging ability

During the reaction process, the electrons of $ABTS^+$ free radicals interacted with the antioxidant components in RCO extraction, resulting in the pairing of their respective electrons and the subsequent elimination of $ABTS^+$ free radicals from the reaction solution. The $ABTS^+$ free radical scavenging ability of RCO remained relatively stable under five different drying conditions, with no statistically significant difference observed, as shown in Fig. 5b. Recent experimental evidence suggests that oxidative stress is the primary mechanism underlying the development of chronic diseases such as inflammation, cardiovascular diseases, and cancer (Shi et al. 2018). Epidemiological studies have confirmed that regular consumption of antioxidant-rich foods can effectively reduce the risk of these diseases (Cilla et al. 2009). There was no significant difference in the peroxide value of Day 0, which was consistent with the results of antioxidant free radicals, reflecting the relative stability of RCO to a certain extent under different drying methods. The antioxidant capacity of foods may reflect their ability to prevent disease. As different drying methods have little impact on RCO's free radical scavenging ability, it has significant application value.

Conclusion

This research investigated the impact of natural and mechanical drying processes on RCO. The results showed that drying methods that involve oxygen, light, and high temperatures lead to a loss of unsaturated fatty acids. The stability of estradiol and vitamin E in RCO remained consistent throughout the experiment, with no significant differences observed between the five drying methods. VFD and BD had higher saponification values, while VFD and BD had lower acid values, and VHD and VFD had lower TBA values than the others. The peroxide value showed no significant difference. In the ABTS+ free radicals and DPPH• free radicals clearance rate tests, the RCO extractions of the five drying methods did not show a significant difference. Among the five drying techniques, VHD shows potential for industrial applications due to its balance of production cost and quality. Future research can explore optimizing drying conditions to enhance the value of drying methods, such as controlling the oxygen content, temperature, time, pressure, and light exposure. Additionally, exploring combination drying methods is an idea worth considering. This work will not only provide essential information for RCO industrialization but also for other dietary frogs.

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Authors' contributions
Xinxin Xu: data management, formal analysis, investigation, validation, visualization, writing - original draft, writing - review and editing. Yongsheng Wang: Conceptualization, funding acquisition, methodology, project management, resources, supervision, writing - review and editing. Yue Zhou: Investigation, Validation, Formal Analysis, Writing - Original Draft, Writing - Review and Editing. Yang Xu: investigation, funding acquisition, writing - review and editing. Meiru Zhang: Investigation, Validation, Writing - Review and Editing. Changli Zhang: data curation, investigation, writing - original draft. Shihan Wang: investigation, visualization, writing - review and editing. Bin Yao: visualization, writing - review and editing. Junting Chen: review and editing. Zhihan Wang*: Conceptualization, funding acquisition, methodology, project management, resources, supervision, writing - review and editing.

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Availability of data and materials
Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors hereby declare no conflict of interest.

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