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Green ultrasound-assisted extraction of fish oil from rainbow trout intestines and purification with adsorbents

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Abstract

This study explored the application of green ultrasound-assisted technology for the extraction of oil from the intestines of rainbow trout. Purification methodologies were incorporated using adsorbents in order to enhance the quality of the extracted oil, which was evaluated based on its color, peroxide value (POV), free fatty acids, organic pollutants, and fatty acid composition. The extraction condition for maximum oil recovery was 60 °C for 30 min, with the addition of 1 g of sodium chloride and a water-to-sample ratio of 0:2. The analysis indicated that silica gel exhibited the highest efficiency as an adsorbent for the elimination of peroxides from extracted oil, with optimal results achieved after adsorption for 60 min. Despite undergoing purification, the POV of fish oil still exceeded the quality standard established by the CODEX Alimentarius Commission. In order to optimize the extraction process, the incorporation of antioxidants, including gallic acid, tannic acid, and *Aronia* (black chokeberry) powder, was implemented before the oil refining process. The integration of antioxidants and purification further lowered the POV and mitigated the production of organic pollutants, concurrently enhancing oil quality compared to without antioxidants. Notably, the incorporation of antioxidants during the initial stages of the extraction process resulted in a significant increase in the average concentrations of essential polyunsaturated fatty acids (PUFAs) in the final products. Overall, this study revealed that *Aronia* has the potential to serve as a natural, less-costly antioxidant alternative to pure antioxidants, such as tannic acid and gallic acid. Furthermore, the potential nutritional value of the final refined oil sample derived from rainbow trout intestines can be improved in terms of ω-3 fatty acid content by the developed method.

Keywords Adsorbent, Antioxidant, Essential fatty acid, Peroxide value, Rainbow trout oil, Ultrasound-assisted extraction

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Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a crucial fish species in global aquaculture because of its high levels of omega-3 (ω-3) long-chain polyunsaturated fatty acids (PUFAs) like eicosapentaenoic acid (20:5 ω-3, EPA) and docosahexaenoic acid (22:6 ω-3, DHA) [1]. The consumption of ω-3 PUFAs has been linked to the suppression of chronic conditions, as well as the improvement of brain development and function [2, 3]. However, during the processing of harvested rainbow trout, which involves degutting, filleting, skinning, and trimming, only 30–50% of the fish is converted into fillets for consumption by consumers [4, 5]. Consequently, several rainbow trout by-products are either discarded, resulting in environmental pollution, or utilized as low-value products, such as animal feed or fertilizer [6, 7]. In this context, it is important to investigate ways to enhance the nutritional value of rainbow trout by-products for human consumption and address environmental concerns occurring in the fish distribution chain. Bechtel found 19.1%, 8.1%, and 2.0% oil in the viscera of Alaska pollock, Pacific cod, and pink salmon, respectively, highlighting the hidden value of fish-derived by-products [8]. Likewise, Fiori et al. suggested that rainbow trout by-products are valuable sources of oil that is rich in ω-3 fatty acids (FAs) [9]. Therefore, producing oil rich in ω-3 FAs from rainbow trout by-products could be a valuable opportunity to enhance their usefulness and compete with other fish oils in the market.

Various conventional methods have been employed for extracting oil from fish species and fish by-products, including pressing, hydrodistillation, steam distillation, and solvent extraction [10–13]. However, these methods are time-consuming, energy-intensive, and result in a low extraction recovery. Additionally, the high temperatures involved can lead to thermal degradation or hydrolysis of unsaturated compounds [14, 15], and the use of organic solvents in extraction can result in harmful residues in the final product, posing risks to human health and the environment [16]. In order to mitigate this environmental concern and improve the safety and quality of oil, it is crucial to research and develop extraction methods that do not involve the use of organic solvents.

With advances in modern technology and the arrival of the green era, innovative technologies have been developed and used to extract oil from fish species and their by-products. Ultrasound-assisted extraction (UAE) is one of the innovative and environmentally friendly methods [17]. This method can decrease the extraction time and solvent consumption, and increase the penetration of cellular materials, thereby enhancing the oil extraction efficiency [17]. However, the use of UAE at high temperatures for oil extraction can result in the oxidation and degradation of unsaturated FAs, particularly ω-3 PUFAs. The oxidation process not only produces unpleasant odors and flavors but also reduces the nutritional quality and safety of food due to the formation of secondary products [18]. One way to inhibit or retard lipid oxidation is to use antioxidants, which scavenge free radicals, converting them into more stable products [19, 20]. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ) are synthetic antioxidants commonly used to prevent oil oxidation due to their chemical stability and affordability [21, 22]. However, these compounds have been found to have negative effects on human health [23]. Natural antioxidants, such as tocopherols, phenolic compounds, and carotenoids extracted from plants and fruits, are being used as replacements for synthetic antioxidants due to safety concerns. Previous studies have shown that plant-based antioxidants, such as rosemary, green tea leaves, apple, and blueberry fruit extract, have a positive impact on oil stability when exposed to high temperatures [24–26]. *Aronia melanocarpa* cultivation in South Korea is steadily growing. *Aronia* fruits are high in phenolic compounds, specifically those belonging to the anthocyanin subclass, such as cyanidin-3-galactoside, cyanidin-3-arabinoside, cyanidin-3-glucoside, and cyanidin-3-xyloside [27]. Given the antioxidant properties of these compounds [28], it would be beneficial to assess whether *Aronia* extract could be useful for oil preservation. It is important to note that crude oils must also undergo a purification process to meet the quality standards set by the CODEX Alimentarius Commission [29].

The objective of this study was to develop a UAE method and a purification method using adsorbents to enhance the recovery and quality of rainbow trout oil from rainbow trout intestine tissues. Oil quality was assessed by measuring its peroxide value (POV), which indicates its oxidation stability. The FA profile of the final products was analyzed to determine the nutritional potential of the extracted oil.

Materials and methods

Materials

The rainbow trout by-products used in this study were obtained from a seafood restaurant in Gangwon, South Korea. The intestines were stored in Ziploc plastic bags and transported in an insulated icebox at −5 °C to the laboratory. The samples were promptly separated with anatomical scissors and then ground using a grinder. The ground tissues were stored in amber glass bottles at −20 °C until used. *Aronia* fruits were cultivated in

Chuncheon, South Korea, in August 2022. The fresh fruits were stored in a freezer at −24 °C until required. The fresh fruits were lyophilized using a freeze-dryer (MG-VFD20, MG Ind., Gunpo, South Korea) and then pulverized with a blender (HMF-3500TG, Hanil, Seoul, South Korea). The powder was stored at −20 °C until it was used.

Sodium thiosulfate anhydrous (≥95%), potassium iodide (KI, 99.5%), sodium chloride (NaCl, ≥99.5%), sodium hydroxide (NaOH, >98%), and isopropanol (≥99.9%) were purchased from Daejung Chemical & Metals Co., Ltd (Gyeonggi-do, Korea). Starch (soluble), sodium bicarbonate (>99.7%), gallic acid (97.5–102.5%), and tannic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetic acid (≥99.7%), methanol (≥99.9%), and methyl *tert*-butyl ether (MTBE, ≥99.8%) were purchased from Tedia Company, Inc. (Fairfield, OH, USA). Chloroform (99.0%) was obtained from Junsei Chemical (Tokyo, Japan). Aluminum oxide (Al_2O_3, activated neutral, 60-mesh powder, surface area: 205 m^2/g) was obtained from Thermo Fisher Scientific (Ward Hill, MA, USA). Silica gel (SG; 70–230 mesh, surface area: 480–540 m^2/g) was purchased from EMD Millipore Corp. (Merck KGaA, Darmstadt, Germany). Chloroform (99.8%) was obtained from Acros Organics (Thermos Fisher Scientific, NJ, USA). *m*-Cresol purple (indicator grade) was purchased from Sigma-Aldrich Chemicals (Bangalore, India).

Oil extraction from rainbow trout intestines using UAE

Five grams of the wet ground sample was weighed into 40-mL glass vials. Specific amounts of water and NaCl were added to each sample, which were vortex-mixed for 1 min and then placed in an ultrasonic bath (SD-351H, Mujigae, Seoul, South Korea). The ultrasound-treated samples were transferred to 15-mL conical tubes and centrifuged at 9000 rpm for 5 min, resulting in three layers: an oil, residue, and aqueous layer. The upper (oil) layer was collected in a 5-mL glass vial using Pasteur glass pipettes. The quantity of oil extracted was recorded. The experiment was conducted in triplicate for each sample.

The total oil yield was expressed as a percentage based on the weight of the wet ground sample, as defined below:

$$\text{Extraction yield (\%)} = \frac{\text{weight of extracted oil}}{\text{weight of ground sample}} \times 100. \quad (1)$$

The experimental conditions that yielded the highest oil recovery were selected in order to investigate the influence of UAE on oil extraction. Three replicates were performed to determine the standard deviation (SD). Various process parameters can influence the extraction yield of oil, including the temperature, extraction time, sample-to-extraction solvent ratio, and the amount of added salt [30–34]. In this study, the UAE time (5, 15, 30, 45, and 60 min), UAE temperature (50 to 80 °C), water-to-sample ratio (0:2, 1:2, 2:2, and 3:2, v/w), and amount of added salt (0, 1, 2, and 3 g) were investigated, in sequence, to enhance the oil extraction efficiency.

Physicochemical characteristics of oil samples

POV analysis

The POVs of the extracted oils were determined by titration, following the AOAC standard method 965.33 [35]. A 10-mL solution of chloroform and glacial acetic acid (2:3, v/v) was added to 1 g of the extracted oil. The mixture was diluted with 1 mL of saturated KI solution. After adding 75 mL of distilled water and 1 mL of starch solution, the mixture was kept in the dark for 1 min and then titrated with 0.01 N sodium thiosulfate. The POV was calculated by Eq. (2):

$$\text{POV (meq}O_2\text{/kg)} = \frac{\text{Volume of sodium thiosulfate used (mL)} \times \text{Normality of sodium thiosulfate} \times 1000}{\text{Weight of sample (g)}}. \quad (2)$$

Free fatty acids (FFAs), acid value (AV), and moisture content (MC)

Determination of the free fatty acid (FFA) content and AV of the trout oil samples was carried out using the procedure outlined by Seaborn et al. [36], which employs *m*-cresol purple as the indicator. In this experimental procedure, a 100-mL Erlenmeyer flask was used to weigh 1 g of the oil sample. Afterward, a total volume of 75 mL of a ternary mixture consisting of chloroform, methanol, and isopropanol (2:1:2, v/v/v) was added to the flask. Additionally, 4 drops of a 0.5% *m*-cresol purple solution were introduced into the mixture, and the contents were thoroughly mixed. The mixture was titrated using a 0.05 N NaOH solution until the color transitioned from

yellow to purple, indicating the attainment of the titration endpoint.

The FFA percentage and AV (mg KOH/g) were calculated by Eqs. (3) and (4), respectively:

$$\text{FFA (\% as oleic acid)} = \frac{(\text{Titration volume of sample} - \text{titration volume of blank}) \times 1.41}{\text{Weight of sample (g)}} \quad (3)$$

$$\text{AV (mg KOH/g)} = \text{percentage FFAs (as oleic acid)} \times 1.99. \quad (4)$$

Additionally, the moisture content (MC) of the oil samples was determined at 105 °C using a DSH-50-10 electronic moisture analyzer (WANT, Changzhou, China) [37].

Purification of the extracted oil

The purification of the extracted oil was studied using adsorption materials such as SG, Al_2O_3, and a mixture of SG and Al_2O_3. In this study, 3 g of the extracted oil were placed into a 5-mL glass vial, and then the material was added to each vial. The vials were placed in a horizontal shaker (Maxi-Mix III, type 65800, Thermolyne, Dubuque, IA, USA) and shaken at 800 rpm for 60 min. The samples were removed from the shaker, allowed to settle, and then filtered using 0.45 μm syringe filters and transferred to 5-mL glass vials. Optimization of the adsorption material and the adsorption time (10, 30, 60, and 120 min) was conducted in triplicate for each adsorption material. The samples were checked visually for color, and their POVs were analyzed.

Determination of FAs

The prepared extracts obtained from the above procedures were further processed for quantitative analysis of FAs. The FAs in the lipids were chemically transformed into their fatty acid methyl esters (FAMEs) using acid-catalyzed transesterification, following the method described by Nechev et al. [38] with slight modifications. Two hundred microliters of the extract were transferred into a 15-mL glass centrifuge tube. The methylation reaction was performed using 1.5 mL of 10% sulfuric acid in methanol at 60 °C for 2 h. Afterward, 3 mL of 20% aqueous NaCl solution, 20 mg of sodium bicarbonate, and 2 mL of MTBE were added to the tube. The mixture was vortex-shaken for 3 min and then centrifuged at 3500 rpm for 5 min. The upper organic phase was collected into a 2-mL vial with a glass Pasteur pipette and capped for instrumental analysis.

Organic compounds in the extracted oil were identified by diluting 0.2 g of the oil in MTBE. FAs and other organic compounds were analyzed by gas chromatography–mass spectrometry (GC–MS) using an Agilent 7890 A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent J&W HP-88 column (100 m × 0.25 mm × 0.2 mm) and coupled to an Agilent 5975C inert mass spectrometer. Helium (99.999%) was used as a carrier gas with a flow rate of 1 mL/min. Both the inlet and detector were kept at 250 °C. The oven temperature was set at an initial 120 °C (isothermal for 1 min), increased at 10 °C/min to 175 °C (isothermal for 10 min), then increased at 5 °C/min to 210 °C (isothermal for 5 min), followed by a final increase at 5 °C/min to 245 °C (isothermal for 15 min). The ion source and quadrupole were set at 230 and 150 °C, respectively. Mass spectral data were recorded over the range of 34–650 *m/z* in the full-scan acquisition mode. Quantification of FAMEs was done using extracted ion chromatograms. Peak assignments were made using a FAME mix from Supelco (Bellefonte, PA, USA) and by comparison with the National Institute of Standards and Technology (NIST) mass spectral library.

Statistical analysis

All data are presented as the mean ± SD. One-way analysis of variance (ANOVA) was conducted using the IBM® SPSS Statistics software (version 26; SPSS, Inc., Chicago, IL, USA) to determine significant differences among the means ($p < 0.05$).

Results and discussion

Effects of process parameters on oil recovery using UAE

The impact of extraction time on oil recoveries was examined, and it was observed that recoveries increased up to 30 min and then gradually declined, as shown in Fig. 1A. The oil extraction efficiency was 43.5% at 5 min of ultrasonication time, and the highest extraction yield of 46.5% was obtained at 30 min. The oil recoveries decreased to 44.4% and 42.1% when the duration of the ultrasonic extraction was increased to 45 and 60 min, respectively. A similar trend has been observed in previous research [39–42]. During the initial stage of ultrasonication, cell wall disruption by ultrasonic waves increase the contact area between the solvent and materials, which leads to increased mass transfer rates [30, 43, 44]. After reaching equilibrium, the oil recovery decreases due to a decrease in the mass transfer rate between the oil and the medium. This is owing to the solvent penetration into the cells of the sample, leading to the dissolution and degradation of soluble constituents,

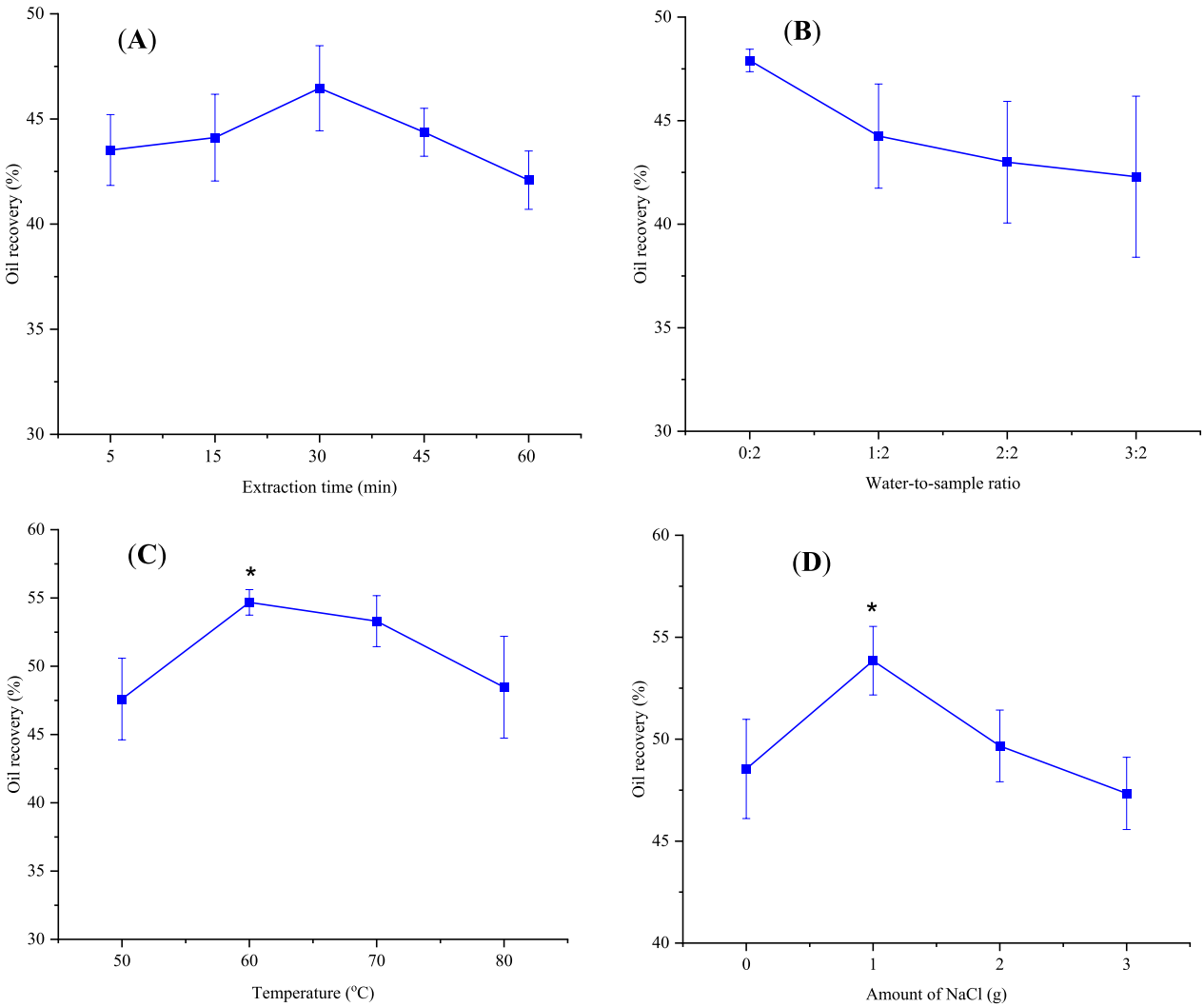

Fig. 1 Effect of **A** extraction time, **B** water-to-sample ratio, **C** temperature, and **D** the amount of salt on oil recovery. The asterisks denote statistically significant differences when compared to the initial values, with a significance level of 0.05

which removes most of the oil [31]. Therefore, a 30-min ultrasonication time was selected for further experiments.

The impact of the water-to-sample ratio is illustrated in Fig. 1B. Oil recovery from the rainbow trout intestines decreased with the addition of water during UAE. When no water was added, the sample was hydrolyzed and achieved a 47.9% oil recovery. The yield decreased to 44.3% when the water-to-sample ratio (v/w) was 1:1 (v/w) and 42.3% at the ratio of 3:2. Similar studies have shown that the highest lipid yields from whole fish and fish by-products using the hydrolysis method can be achieved without adding water [45]. Reducing the addition of water, or even eliminating this step during the extraction process can minimize the formation of an emulsion layer that absorbs some of the lipids [45, 46].

The temperature also affected the UAE process, as shown in Fig. 1C. Initially, the oil yield increased from 47.6% to 50 °C to 54.7% at 60 °C, but the extraction efficiency decreased when the temperature was increased to 70 and 80 °C, resulting in oil recoveries of 53.3% and 48.5%, respectively. Previous studies have shown that high temperatures can reduce the viscosity and surface tension of extraction solvents, resulting in increased diffusion and penetration in the cell matrix. This leads to improved permeability and solubility of the solvents in the matrix [30, 43, 47]. Elevated temperatures also increase vapor pressures and produce more vapor-filled

bubbles, which cushions the implosion of these bubbles and leads to a decrease in oil yield [30, 48, 49]. Consequently, relatively lower temperatures are preferred, and for this study, a temperature of 60 °C was selected for further experiments.

The impact of NaCl levels on oil recovery is illustrated in Fig. 1D. Without the addition of NaCl to the extraction process, the oil extraction yield was observed to be 48.5%. Adding 1 g of NaCl to the samples during extraction increased the oil recovery to 53.8%. Werman and Neeman [50] found that adding salt to the extraction process can help separate oil from emulsion and increase the difference in specific gravity between the oil and the aqueous phase. As shown in Fig. 1D, the oil recovery decreased when the amount of added NaCl exceeded 1 g, resulting in yields of 49.7% and 47.3% when 2 and 3 g of NaCl were added, respectively. Previous studies have found that a high amount of salt can contribute to increased emulsification [51] and lead to an increased osmotic pressure difference between the external environment and the oil-containing cells [52]. This could hinder the separation of oil from the cells. Therefore, 1 g of NaCl was selected for further experiments.

Quality of the extracted oil

The extracted oil had a reddish-brown color (Fig. 2A) and a MC of 2% (Table 1). As shown in Table 1, the AV and level of FFAs in the crude oil met the criteria for fish oils established by the CODEX Alimentarius Commission, but the POV (29.0 ± 7.2 meqO_2/kg) exceeded the CODEX limit (< 5 meqO_2/kg oil) [29]. The high levels of peroxides in the oil may be attributed to the oxidation process that takes place at elevated temperatures. Consequently, it is necessary to purify crude oil samples in order to meet the color and quality standards and increase the potential value of the oil.

Table 1 Physicochemical characteristics of the crude oil

Quality parameters	This study	Standard[a]
Color	Reddish brown	–
Moisture (%)	2.00	–
FFAs (% as oleic acid)	1.41 ± 0.28	–
AV (mg KOH/g)	2.81 ± 0.56	≤ 3
POV (meqO_2/kg)	29.0 ± 7.2	≤ 5

FFA free fatty acid, *AV* acid value, *POV* peroxide value

[a] Reference [29]

The crude oils underwent a purification process using difference adsorption materials. After purification with SG, a mixture of SG and Al_2O_3 (1:1, w/w), and Al_2O_3 alone, the oil appeared light yellow, golden yellow, and light brown, respectively. The standard color for high-quality fish oil is light or bright yellow [15]. Therefore, SG is the most efficient method for improving the color of extracted crude oil using adsorption technology. The increased adsorbent capacity may be attributed to the large surface area. SG has a higher surface area (480–540 m^2/g) than Al_2O_3 (205 m^2/g). Chu et al. found that the surface area and adsorbate affinity toward the adsorbent are factors that affect the adsorbent capacity [53].

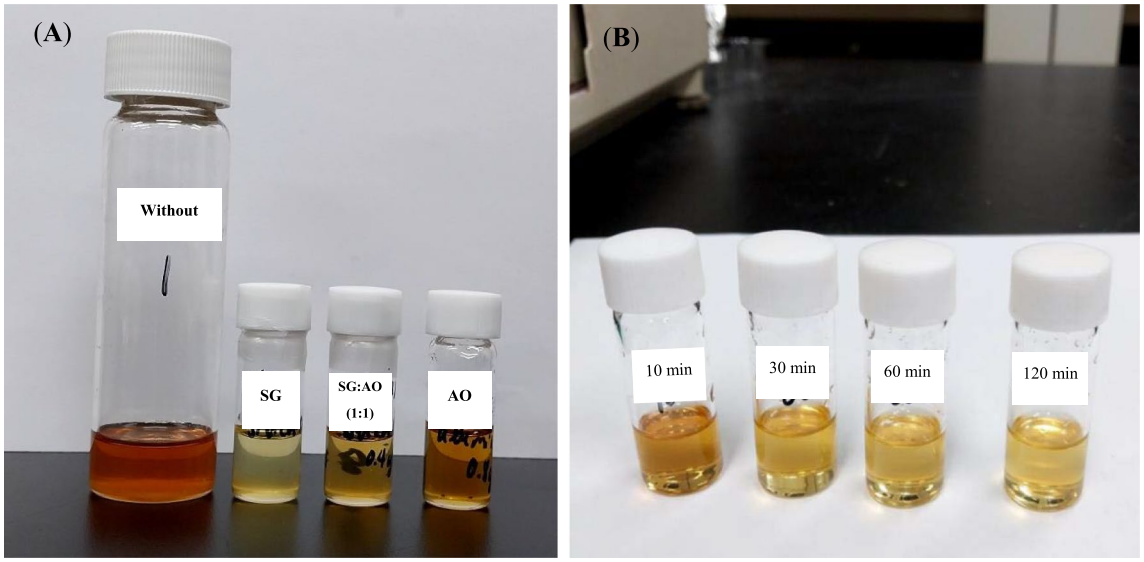

Fig. 2 Appearance of the extracted oil **A** before and after purification using adsorbent materials, and **B** with varying purification times. *SG* silica gel, *AO* aluminum oxide

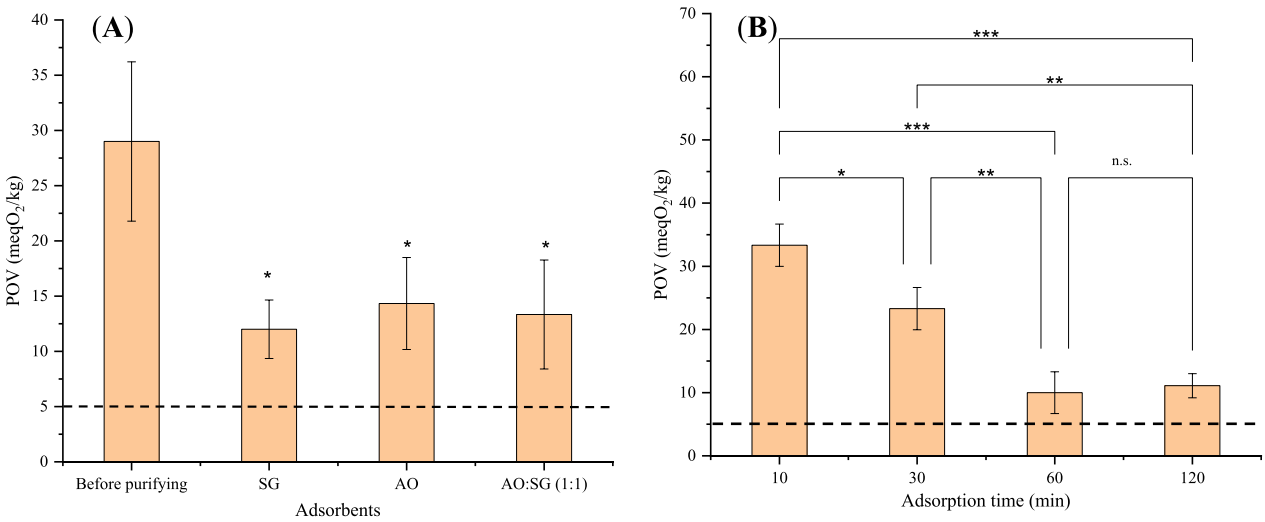

Fig. 3 Effect of adsorbent materials (**A**) and adsorption time (**B**) on the POV of the extracted oil. The asterisks denote significant differences in the values. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ns: no significant difference. *POV* peroxide value, *SG* silica gel, *AO* aluminum oxide

Besides the color of the extracted oil, oil oxidation is also a critical factor in determining oil quality. This study assessed how the extraction process affects oil oxidation and oil refinement efficiency, specifically in terms of POV. The POV indicates oxidation during the initial stages of lipid deterioration [54]. The impact of various adsorbents on the POV of the extracted oil is shown in Fig. 3A. After the refining process using SG, Al_2O_3, and a mixture of SG and Al_2O_3 (1:1, w/w), the average POVs were 12.0, 14.3, and 13.3 meqO_2/kg, respectively, which were significantly lower compared to pre-purification ($p < 0.05$). These findings showed that using adsorbents in the purification process significantly reduced the POV of the extracted oil. SG was found to be the most effective adsorbent among the material tested, lowering the POV by up to 58.6%, followed by a mixture of SG and Al_2O_3, with a 54.0% decrease in the POV, and Al_2O_3 was the least effective, lowering the POV by 50.6% compared to the pre-purified oil; albeit, no significant differences in the POVs were observed among the adsorbents (Fig. 3A). SG is the optimal choice for refining based on the observed color and measured POV of the oil. However, even after the adsorbent purification process, the POV of the extracted oil still did not comply with the CODEX standard [29]. Therefore, additional experiments were conducted to optimize the adsorption time using SG as the material for a further refining process.

After undergoing purification for 10 and 30 min, the color of the extracted oil before purification (reddish brown) changed to bright brown or pale orange. Increasing the adsorption time to 60 and 120 min resulted in the oils acquiring a bright yellow color (Fig. 2B). As shown in Fig. 3B, the POV of the extracted oil after 10 min of adsorption was measured to be 33.3 meqO_2/kg. After extending the adsorption time to 30 min, the POV significantly decreased to 23.3 meqO_2/kg, with a removal rate of 29.2% compared to the oil after 10 min of adsorption ($p < 0.05$). Extending the adsorption time to 60 min led to a measured POV of 10 meqO_2/kg, with a removal efficiency of 69.1%. These findings suggest that increasing the adsorption time can improve the removal rate of peroxides in extracted oil when using SG as an adsorbent. However, as shown in Fig. 3B, the POVs of oils after 60 and 120 min of adsorption were not significantly different ($p > 0.05$). Therefore, a 60-min adsorption time was selected for further experiments. Despite a significant decrease in POV after increasing the adsorption time, it still exceeded the CODEX standard [29]. Antioxidants were added during the lipid extraction process to address the issue.

After adding antioxidants during the extraction process and purifying with SG, the extracted oil appeared light or bright yellow (Fig. 4A). The addition of antioxidants during the extraction process did not impact the color quality of the oil. The impact of adding antioxidants during the extraction process to inhibit peroxide formation is shown in Fig. 4B. Compared to the extracted oil without added antioxidants (21.3 ± 3.21 meqO_2/kg), the inclusion of antioxidants tannic acid, gallic acid, and *Aronia* during the extraction process, decreased the POV significantly to 5.67 ± 1.53, 9.00 ± 0.00, and 8.00 ± 2.00 meqO_2/kg, lowering the POV by 73.4%, 62.5%, and 57.8%, respectively, before purification through adsorption technology. Marine oils, such as rainbow trout oil, contain a high

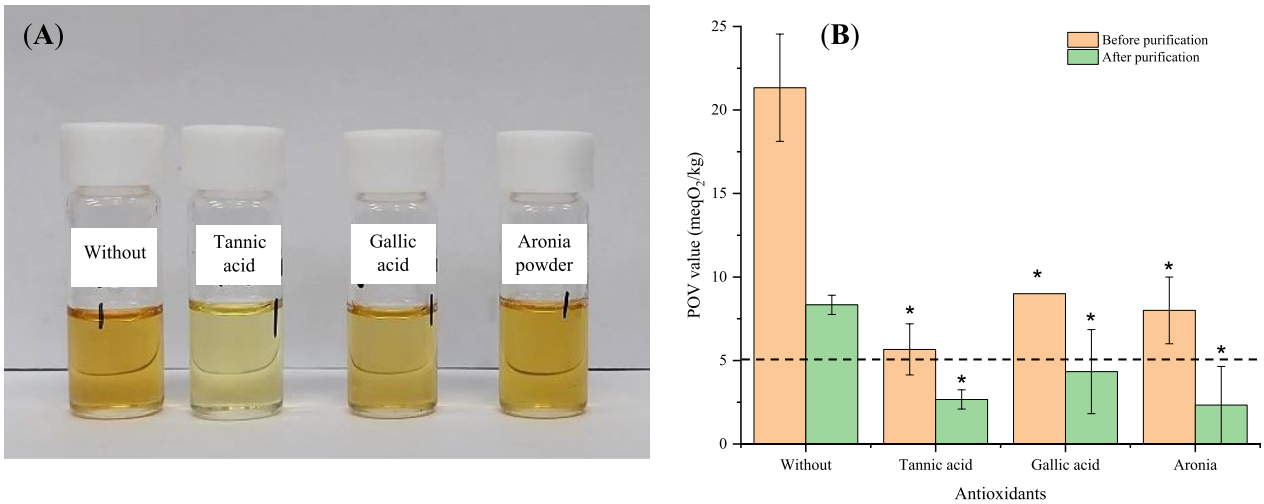

Fig. 4 **A** Appearance of the oils after extraction, with the addition of antioxidants and subsequent purification, and **B** impact of antioxidant addition and purification on the peroxide value (POV) in the oils. The asterisks indicate significant differences in values ($p < 0.05$) when antioxidants are added to the oil

amount of PUFAs, which makes them susceptible to oxidation when exposed to high temperatures. As a result, the highest POV was found in the extracted oil without the addition of antioxidants. The findings that the inclusion of tannic acid, gallic acid, and *Aronia* enhance the oxidation stability of lipids during the extraction process are consistent with previous research. Gülçin et al. found that adding tannic acid to an emulsion with linoleic acid inhibited 97.7% of lipid peroxidation [55]. Asnaashari et al. demonstrated the free radical scavenging capacity and antioxidant activity of gallic acid in both bulk Kilka fish oil and its oil-in-water emulsion [56]. Denev et al. found that *Aronia* fruits have a high antioxidant capacity, with 40% anthocyanins present in the fruits [57].

Unnecessary organic compounds (organic pollutants) produced during the extraction of unsaturated FAs in oil were identified using GC–MS. The analytical results in Fig. 5 show that 2,4-heptadienal, 2,4-dodecadienal, 2-decenal, 2,4-decadienal, 3-decyn-2-ol, and 8-methylene-3-oxatricyclo[5.2.0.0(2,4)]nonane were products of oxidation in the rainbow trout oil without the addition of antioxidants. These polyunsaturated fatty aldehydes are known for their strong fat odor and fish smell and are generally generated by the dehydrogenation of alcohols [58, 59]. They are by-products of lipid peroxidation in cell membranes and are also found in cooking oil fumes. The compounds have previously detected in sardine oil and sardine by-product oil [18]. The analytical results indicated a significant decrease in the fractions of the organic compounds after the addition of antioxidants during extraction. Specifically, the removal rates were 67.1% with tannic acid, 50.3% with gallic acid, and 43.7% with the addition of *Aronia* powder.

The purification process decreased the MC of the oil samples to zero (Table 2). The oil color quality was also assessed, and the AV of the purified oil met the international standard for the quality of fish oils set by the CODEX Alimentarius Commission [29], as shown in Table 2. The POV of the extracted oil without antioxidants during extraction was measured as 8.33 ± 0.58 meqO_2/kg, but this decreased significantly to 2.67 ± 0.58, 4.33 ± 2.52, and 2.33 ± 2.31 meqO_2/kg with the addition of the antioxidants tannic acid, gallic acid, and *Aronia* during the extraction process, respectively, as shown in Fig. 4B. The addition of *Aronia*, tannic acid, and gallic acid removed up to 89.2%, 87.5%, and 79.7% of the peroxides, respectively. The study discovered that by adding antioxidants during the extraction process and purifying the extracted oils, the MC was decreased to zero, the color of the oil samples improved, and the FFA contents, AV, and POV decreased. These levels complied with the CODEX standard [29].

Levels of FAs in the final products

The FA concentrations in the extracted oils added with antioxidants during extraction and subsequent purification are shown in Table 3. The analysis revealed that palmitic acid (C16:0), palmitoleic acid (C16:1 ω-7), *cis*-9-oleic acid (C18:1 ω-9), linoleic acid (C18:2 ω-6), EPA (C20:5 ω-3), and DHA (C22:6 ω-3) were found in high concentrations, accounting for approximately 75% of the FAs in the samples. As shown in Fig. 6, there were no significant differences in the saturated fatty acid (SFA)

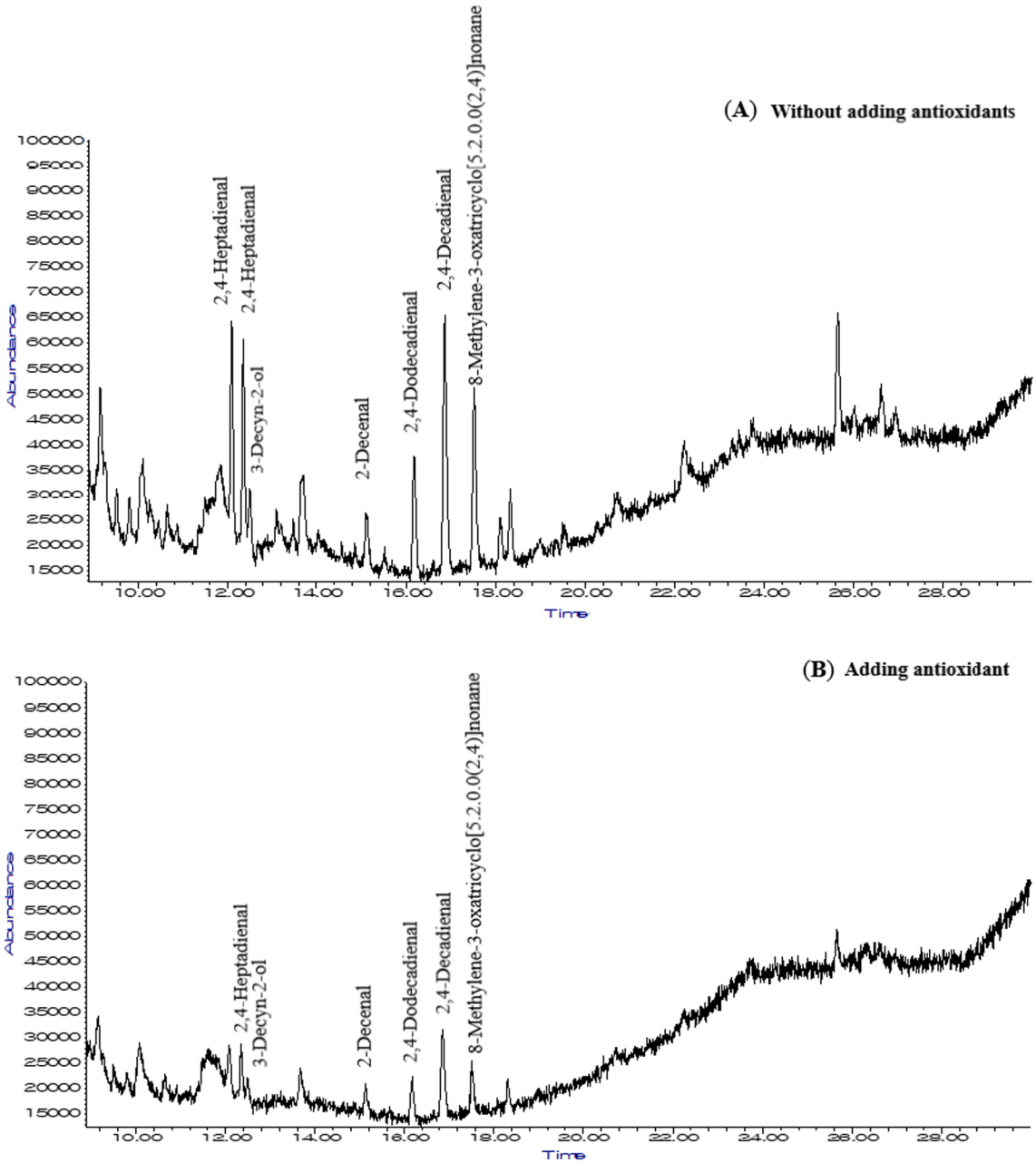

Fig. 5 Chromatograms of organic compounds found in the extracted oil **A** without the addition of an antioxidant and **B** with the addition of an antioxidant

Table 2 Physicochemical characteristics of the purified oils with and without antioxidants added at the beginning of the extraction process

Oil samples	Color	Moisture (%)	FFAs (% as oleic acid)	AV (mg KOH/g)	POV (meqO_2/kg)
Without addition of antioxidant	Bright yellow	0.0	0.71 ± 0.20	1.40 ± 0.40	8.33 ± 0.58
Addition of tannic acid	Light yellow	0.0	0.56 ± 0.00	1.12 ± 0.00	2.67 ± 0.58
Addition of gallic acid	Bright yellow	0.0	0.71 ± 0.20	1.40 ± 0.40	4.33 ± 2.52
Addition of *Aronia* powder	Bright yellow	0.0	0.71 ± 0.20	1.40 ± 0.40	2.33 ± 2.31

FFA free fatty acid, *AV* acid value, *POV* peroxide value

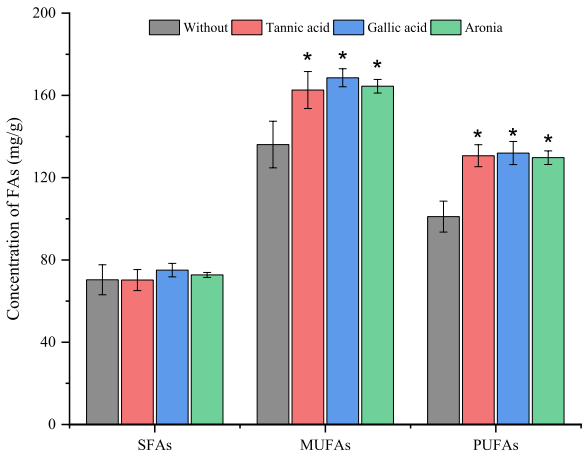

Fig. 6 Concentrations of FAs in the final products. The asterisks denote significant differences compared to the oil without antioxidants at $p < 0.05$. *FA* fatty acid, *SFA* saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid

concentrations among the oils depending on whether antioxidants were added during extraction. The mean concentration of SFAs in the sample without added antioxidants was 70.4 ± 7.3 mg/g. The SFAs content in the extracted samples added with tannic acid, gallic acid, and *Aronia* were 70.2 ± 5.1, 75.0 ± 3.3, and 72.7 ± 1.2 mg/g, respectively. The study found that adding antioxidants during the oil extraction process did not impact the concentrations of SFAs. However, adding antioxidants during the early stages of oil extraction significantly increased the concentrations of monounsaturated FAs and PUFAs (Fig. 6). The concentrations of PUFAs in the extracted oil increased from 101 ± 8 mg/g without added antioxidants to 131 ± 5 mg/g with tannic acid, 132 ± 6 mg/g with gallic acid, and 130 ± 3 mg/g with the addition of *Aronia* during the early stages of extraction.

According to Simopoulo [60], ω-6 FAs tend to promote inflammation, while ω-3 FAs have anti-inflammatory properties. The ratio of ω-6 to ω-3 FAs is commonly used as an indicator of the nutritional value of fish oil [61]. The recommended ratio of ω-6 to ω-3 FAs in human nutrition is less than 5:1 [2, 62]. The ratios of ω-6 to ω-3 FAs in oils added with tannic acid, gallic acid, *Aronia* powder, and without antioxidants were 0.596, 0.599, 0.594, and 0.634, respectively (Table 3). These ratios indicate that the oils have a nutritionally favorable composition.

Conclusions

This study optimized the parameters of UAE to obtain the highest oil yields from trout intestines. The study also examined the purification of extracted oils using adsorbents to remove substances that could accelerate

Table 3 Concentrations (mg/g) of FAs in the extracted oil resulting from the addition of antioxidants and the purification process

FAs	Synonyms	Without antioxidants	Addition of tannic acid	Addition of gallic acid	Addition of *Aronia*
Myristic acid	C14:0	11.7 ± 1.3	11.3 ± 1.0	12.3 ± 0.5	11.9 ± 0.3
Pentadecanoic acid	C15:0	1.27 ± 0.13	1.24 ± 0.15	1.32 ± 0.05	1.29 ± 0.04
Palmitic acid	C16:0	44.1 ± 4.4	44.4 ± 2.88	47.1 ± 2.26	45.6 ± 0.6
Margaric acid	C17:0	1.37 ± 0.17	1.41 ± 0.11	1.46 ± 0.07	1.43 ± 0.03
Stearic acid	C18:0	11.9 ± 1.2	11.9 ± 1.0	12.9 ± 0.4	12.5 ± 0.24
Palmitoleic acid	C16:1 ω-7	24.6 ± 2.2	28.6 ± 1.42	29.0 ± 1.78	28.5 ± 0.82
cis-9-Oleic acid	C18:1 ω-9	86.5 ± 6.6	103 ± 6	107 ± 2	106 ± 1.8
11-Eicosenoic acid	C20:1 ω-9	18.1 ± 1.7	23.0 ± 0.9	23.9 ± 0.4	22.4 ± 0.6
Erucic acid	C22:1 ω-9	6.86 ± 0.84	8.27 ± 0.46	8.39 ± 0.16	7.87 ± 0.09
Linoleic acid	C18:2 ω-6	31.1 ± 2.5	38.6 ± 1.7	39.2 ± 1.6	38.5 ± 0.43
γ-Linolenic acid	C18:3 ω-6	0.76 ± 0.05	0.92 ± 0.03	0.91 ± 0.00	0.89 ± 0.01
α-Linolenic acid	C18:3 ω-3	7.22 ± 0.62	9.21 ± 0.33	9.45 ± 0.12	9.14 ± 0.06
8,11-Eicosadienoic acid	C20:2 ω-9	2.20 ± 0.23	2.96 ± 0.14	3.12 ± 0.11	2.87 ± 0.21
8,11,14-Eicosatrienoic acid	C20:3 ω-6	1.26 ± 0.08	1.55 ± 0.16	1.61 ± 0.08	1.58 ± 0.03
Arachidonic acid	C20:4 ω-6	5.23 ± 0.43	6.58 ± 0.16	6.53 ± 0.15	6.29 ± 0.21
Eicosapentaenoic acid	C20:5 ω-3	19.6 ± 1.1	24.7 ± 1.1	24.4 ± 1.7	24.1 ± 1.3
Docosahexaenoic acid	C22:6 ω-3	33.7 ± 2.5	46.2 ± 1.8	46.7 ± 1.8	46.4 ± 1.1
Total		308 ± 26	364 ± 20	376 ± 13	367 ± 8
ω-6/ω-3 FA ratio		0.634	0.596	0.599	0.594

The values are presented as mean ± standard deviation

FA fatty acid

the oxidation process. SG was determined to be the most effective adsorbent for removing the peroxides in extracted oil. The highest purification efficiency was achieved when the adsorption step was carried out for 60 min. However, even after purification, the POVs still exceeded the limit for the quality of fish oils set by the CODEX Alimentarius Commission. The study discovered that adding antioxidants (gallic acid, tannic acid, and *Aronia* powder) in the initial stages of oil extraction, followed by refining, can effectively reduce oxidation and meet the POV standard for fish oil established by the CODEX Alimentarius Commission. Additionally, the present study has revealed that the incorporation of antioxidants not only removes unnecessary substances and enhances the oxidation stability of the oil but also increases the concentrations of unsaturated FAs, particularly PUFAs, in comparison to oil devoid of added antioxidants. *Aronia* powder derived from natural sources has the potential to serve as a natural antioxidant supplement for oil extraction from fish by-products.

Abbreviations

AV	Acid value
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
FAME	Fatty acid methyl ester
GC–MS	Gas chromatography–mass spectrometry
MC	Moisture content
MUFA	Monounsaturated fatty acid
POV	Peroxide value
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
SG	Silica gel
UAE	Ultrasound-assisted extraction

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Author contributions
TTHM: conceptualization, methodology, formal analysis, data curation, visualization, writing—original manuscript and editing. YC and HP: sampling, analysis. JLC: funding acquisition. J-SC: resources, visualization. DP: funding acquisition, resources. HK: conceptualization, visualization, validation, supervision, writing—review and editing. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Competing interests
The authors declare that they have no competing interests.

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References
1. Turchini GM, Hermon KM, Francis DS (2018) Fatty acids and beyond: fillet nutritional characterisation of rainbow trout (*Oncorhynchus mykiss*) fed different dietary oil sources. Aquaculture 491:391–397. https://doi.org/10.1016/j.aquaculture.2017.11.056
2. Ruxton CH, Reed SC, Simpson MJ et al (2004) The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. J Hum Nutr Diet 17:449–459. https://doi.org/10.1111/j.1365-277X.2004.00552.x
3. Shahidi F, Ambigaipalan P (2018) Omega-3 polyunsaturated fatty acids and their health benefits. Annu Rev Food Sci Technol 9:345–381. https://doi.org/10.1146/annurev-food-111317-095850
4. Olsen RL, Toppe J, Karunasagar I (2014) Challenges and realistic opportunities in the use of by-products from processing of fish and Shellfish. Trends Food Sci Technol 36:144–151. https://doi.org/10.1016/j.tifs.2014.01.007
5. Rustad T, Storrø I, Slizyte R (2011) Possibilities for the utilisation of marine by-products. Int J Food Sci Technol 46:2001–2014. https://doi.org/10.1111/j.1365-2621.2011.02736.x
6. Stevens JR, Newton RW, Tlusty M et al (2018) The rise of aquaculture by-products: increasing food production, value, and sustainability through strategic utilisation. Mar Policy 90:115–124. https://doi.org/10.1016/j.marpol.2017.12.027
7. Esteban MB, García AJ, Ramos P et al (2007) Evaluation of fruit-vegetable and fish wastes as alternative feedstuffs in pig diets. Waste Manag 27:193–200. https://doi.org/10.1016/j.wasman.2006.01.004
8. Bechtel PJ (2003) Properties of different fish processing by-products from pollock, cod and salmon. J Food Process Preserv 27:101–116. https://doi.org/10.1111/j.1745-4549.2003.tb00505.x
9. Fiori L, Solana M, Tosi P et al (2012) Lipid profiles of oil from trout (*Oncorhynchus mykiss*) heads, spines and viscera: trout by-products as a possible source of omega-3 lipids? Food Chem 134:1088–1095. https://doi.org/10.1016/j.foodchem.2012.03.022
10. Suseno SH, Rizkon AK, Jacoeb AM et al (2021) Fish oil extraction as a by-product of Tilapia (*Oreochromis* sp.) fish processing with dry rendering method. IOP Conf Ser Earth Environ Sci 679:012009. https://doi.org/10.1088/1755-1315/679/1/012009
11. Pigott GM (1967) Chap. 13: Production of fish oil. In: Stansby ME (ed) Fish oils. AVI Publishing Co, Westport, pp 183–192
12. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917. https://doi.org/10.1139/o59-099
13. García-Moreno PJ, Morales-Medina R, Pérez-Gálvez R et al (2014) Optimisation of oil extraction from sardine (*Sardina pilchardus*) by hydraulic pressing. Int J Food Sci Technol 49:2167–2175. https://doi.org/10.1111/ijfs.12527
14. Guo T, Hao Q, Nan Z et al (2022) Green extraction and separation of *Dendranthema indicum* essential oil by supercritical carbon dioxide extraction combined with molecular distillation. J Clean Prod 376:134208. https://doi.org/10.1016/j.jclepro.2022.134208
15. Zhang Y, Sun Q, Liu S et al (2021) Extraction of fish oil from fish heads using ultra-high pressure pre-treatment prior to enzymatic hydrolysis. Innov Food Sci Emerg Technol 70:102670. https://doi.org/10.1016/j.ifset.2021.102670

16. Rosenthal A, Pyle DL, Niranjan K (1996) Aqueous and enzymatic processes for edible oil extraction. Enzyme Microb Technol 19:402–420. https://doi.org/10.1016/S0141-0229(96)80004-F
17. Ivanovs K, Blumberga D (2017) Extraction of fish oil using green extraction methods: a short review. Energy Procedia 128:477–483. https://doi.org/10.1016/j.egypro.2017.09.033
18. Soldo B, Šimat V, Vlahović J et al (2019) High quality oil extracted from sardine by-products as an alternative to whole sardines: production and refining. Eur J Lipid Sci Technol 121:1800513. https://doi.org/10.1002/ejlt.201800513
19. Wu TH, Bechtel PJ (2008) Salmon by-product storage and oil extraction. Food Chem 111:868–871. https://doi.org/10.1016/j.foodchem.2008.04.064
20. Abd El-Samee LD, El-Wardany I, Abdel-Fattah SA et al (2019) Dietary omega-3 and antioxidants improve long-chain omega-3 and lipid oxidation of broiler meat. Bull Natl Res Cent 43:45. https://doi.org/10.1186/s42269-019-0085-y
21. Iqbal S, Bhanger MI (2007) Stabilization of sunflower oil by garlic extract during accelerated storage. Food Chem 100:246–254. https://doi.org/10.1016/j.foodchem.2005.09.049
22. Yang MH, Lin HJ, Choong YM (2002) A rapid gas chromatographic method for direct determination of BHA, BHT and TBHQ in edible oils and fats. Food Res Int 35:627–633. https://doi.org/10.1016/S0963-9969(01)00164-8
23. Branen AL (1975) Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. J Am Oil Chem Soc 52:59–63. https://doi.org/10.1007/BF02901825
24. Martínez ML, Penci MC, Ixtaina V et al (2013) Effect of natural and synthetic antioxidants on the oxidative stability of walnut oil under different storage conditions. LWT Food Sci Technol 51:44–50. https://doi.org/10.1016/j.lwt.2012.10.021
25. Cheng KW, Shi JJ, Ou SY et al (2010) Effects of fruit extracts on the formation of acrylamide in model reactions and fried potato crisps. J Agric Food Chem 58:309–312. https://doi.org/10.1021/jf902529v
26. Boskou D (2011) Chap 8: Nonnutrient antioxidants and stability of frying oils. In: Boskou D, Elmadfa (eds) Frying of food: oxidation, nutritent and non-nutrient antioxidants, biologically active compounds, and high temperatures, 2nd edn. CRC Press, Boca Raton, pp 197–224. https://doi.org/10.1201/b10437-9
27. Kim H, Mai TTH (2020) Variations in total phenols, total anthocyanins, and antioxidant activity levels in black chokeberry (*Aronia melanocarpa*) fruits subjected to dry and moist heat treatments. Korean J Food Sci Technol 52:503–509. https://doi.org/10.9721/KJFST.2020.52.5.503
28. Hwang SJ, Yoon WB, Lee OH et al (2014) Radical-scavenging-linked antioxidant activities of extracts from black chokeberry and blueberry cultivated in Korea. Food Chem 146:71–77. https://doi.org/10.1016/j.foodchem.2013.09.035
29. CODEX Alimentarius Commission (2017) Standard for fish oils. CXS 329-2017
30. Stevanato N, da Silva C (2019) Radish seed oil: ultrasound-assisted extraction using ethanol as solvent and assessment of its potential for ester production. Ind Crops Prod 132:283–291. https://doi.org/10.1016/j.indcrop.2019.02.032
31. Junaid PM, Dar AH, Dash KK et al (2022) Advances in seed oil extraction using ultrasound assisted technology: a comprehensive review. J Food Process Eng 46:14192. https://doi.org/10.1111/jfpe.14192
32. Naveenkumar R, Baskar G (2022) Ultrasonic assisted extraction of oil from castor seeds: optimization using response surface methodology, extraction kinetics and characterization. Energy Sources Part A Recover Util Environ Eff 44:2497–2508. https://doi.org/10.1080/15567036.2019.1650136
33. Komartin RS, Stroescu M, Chira N et al (2021) Optimization of oil extraction from *Lallemantia iberica* seeds using ultrasound-assisted extraction. J Food Meas Charact 15:2010–2020. https://doi.org/10.1007/s11694-020-00790-w
34. Shamspur T, Mohamadi M, Mostafavi A (2012) The effects of onion and salt treatments on essential oil content and composition of *Rosa damascena* Mill. Ind Crops Prod 37:451–456. https://doi.org/10.1016/j.indcrop.2011.07.019
35. AOAC (2000) Method 965.33. In: Horwitz W (ed) Official methods of analysis of the AOAC International, 17th edn. AOAC International, Gaithersburg
36. Seaborn GT, Gooch JA, Van Dolah FM et al (eds) (1993) Biomedical test materials program: analytical methods for the quality assurance of fish oil, 2nd edn. NOAA Tech Memo NMFS-SEFSC-329
37. Garvey R, Fogel G (1996) Estimating water content in oils: moisture in solution, emulsified water, and free water. In: Proceedings of a joint conference technology showcase: integrated monitoring, diagnostics and failure prevention, Mobile, Alamba, April 22–26
38. Nechev JT, Edvinsen GK, Eilertsen KE (2021) Fatty acid composition of the lipids from Atlantic salmon—comparison of two extraction methods without halogenated solvents. Foods 10:73. https://doi.org/10.3390/foods10010073
39. Dash DR, Pathak SS, Pradhan RC (2021) Extraction of oil from *Terminalia chebula* kernel by using ultrasound technology: influence of process parameters on extraction kinetics. Ind Crops Prod 171:113893. https://doi.org/10.1016/j.indcrop.2021.113893
40. Shalmashi A (2009) Ultrasound-assisted extraction of oil from tea seeds. J Food Lipids 16:465–474. https://doi.org/10.1111/j.1745-4522.2009.01159.x
41. Hernández-Santos B, Rodríguez-Miranda J, Herman-Lara E et al (2016) Effect of oil extraction assisted by ultrasound on the physicochemical properties and fatty acid profile of pumpkin seed oil (*Cucurbita pepo*). Ultrason Sonochem 31:429–436. https://doi.org/10.1016/j.ultsonch.2016.01.029
42. Savic Gajic IM, Savic IM (2023) Optimization of ultrasound procedure for the sustainable production of oil from plum seeds. Carbon Resour Convers 6:20–26. https://doi.org/10.1016/j.crcon.2022.10.003
43. Thilakarathna RCN, Siow LF, Tang TK et al (2023) A review on application of ultrasound and ultrasound assisted technology for seed oil extraction. J Food Sci Technol 60:1222–1236. https://doi.org/10.1007/s13197-022-05359-7
44. Gutte KB, Sahoo AK, Ranveer RC (2015) Effect of ultrasonic treatment on extraction and fatty acid profile of flaxseed oil. OCL Oilseeds Fats Crop Lipids 22:D606. https://doi.org/10.1051/ocl/2015038
45. Aitta E, Marsol-Vall A, Damerau A et al (2021) Enzyme-assisted extraction of fish oil from whole fish and by-products of Baltic herring (*Clupea harengus membras*). Foods 10:1811. https://doi.org/10.3390/foods10081811
46. Šližyte R, Daukšas E, Falch E et al (2005) Yield and composition of different fractions obtained after enzymatic hydrolysis of cod (*Gadus morhua*) by-products. Process Biochem 40:1415–1424. https://doi.org/10.1016/j.procbio.2004.06.033
47. Chemat F, Rombaut N, Sicaire AG et al (2017) Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason Sonochem 34:540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035
48. Lou Z, Wang H, Zhang M et al (2010) Improved extraction of oil from chickpea under ultrasound in a dynamic system. J Food Eng 98:13–18. https://doi.org/10.1016/j.jfoodeng.2009.11.015
49. Tiwari BK (2015) Ultrasound: a clean, green extraction technology. TrAC Trends Anal Chem 71:100–109. https://doi.org/10.1016/j.trac.2015.04.013
50. Werman MJ, Neeman I (1987) Avocado oil production and chemical characteristics. J Am Oil Chem Soc 64:229–232. https://doi.org/10.1007/BF02542007
51. Liu L, Yu X, Zhao Z et al (2017) Efficient salt-aided aqueous extraction of bitter almond oil. J Sci Food Agric 97:3814–3821. https://doi.org/10.1002/jsfa.8245
52. Tran TH, Ha LK, Nguyen DC et al (2019) The study on extraction process and analysis of components in essential oils of black pepper (*Piper nigrum* L.) seeds harvested in Gia Lai Province, Vietnam. Processes 7:56. https://doi.org/10.3390/pr7020056
53. Chu BS, Baharin BS, Man YBC et al (2005) Comparison of selected adsorbents for adsorption and desorption of vitamin E from palm fatty acid distillate. J Food Lipids 12:23–33. https://doi.org/10.1111/j.1745-4522.2005.00003.x
54. Yin F, Sun X, Zheng W et al (2021) Screening of highly effective mixed natural antioxidants to improve the oxidative stability of microalgal DHA-rich oil. RSC Adv 11:4991–4999. https://doi.org/10.1039/d0ra10312h
55. Gülçin I, Huyut Z, Elmastaş M et al (2010) Radical scavenging and antioxidant activity of tannic acid. Arab J Chem 3:43–53. https://doi.org/10.1016/j.arabjc.2009.12.008

56. Asnaashari M, Farhoosh R, Sharif A (2014) Antioxidant activity of gallic acid and methyl gallate in triacylglycerols of Kilka fish oil and its oil-in-water emulsion. Food Chem 159:439–444. https://doi.org/10.1016/j.foodchem.2014.03.038
57. Denev P, Číž M, Kratchanova M et al (2019) Black chokeberry (*Aronia melanocarpa*) polyphenols reveal different antioxidant, antimicrobial and neutrophil-modulating activities. Food Chem 284:108–117. https://doi.org/10.1016/j.foodchem.2019.01.108
58. Guillén MD, Uriarte PS (2012) Aldehydes contained in edible oils of a very different nature after prolonged heating at frying temperature: presence of toxic oxygenated α,β unsaturated aldehydes. Food Chem 131:915–926. https://doi.org/10.1016/j.foodchem.2011.09.079
59. Adams TB, Gavin CL, Taylor SV et al (2008) The FEMA GRAS assessment of α,β-unsaturated aldehydes and related substances used as flavor ingredients. Food Chem Toxicol 49:2935–2967. https://doi.org/10.1016/j.fct.2008.06.082
60. Simopoulos AP (2008) The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease. Asia Pac J Clin Nutr 17(Suppl 1):131–134
61. Pigott GM, Tucker BW (1990) Seafood: effects of technology on nutrition, 1st edn. Marcel Dekker, Inc, New York, pp 221–228
62. Jobling M, Leknes O (2010) Cod liver oil: feed oil influences on fatty acid composition. Aquac Int 18:223–230. https://doi.org/10.1007/s10499-008-9238-y

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