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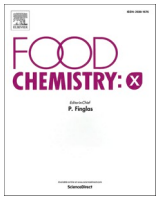

Effect of four-stage refinement with fortified measures to inhibit oil oxidation and purification on the quality of anchovy oil

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ABSTRACT

Traditional refining processes of crude anchovy oil uses high temperatures. In order to inhibit oil oxidation, intensified measures should be taken. The impact of traditional four-stage refinement on anchovy oil quality have not yet been systematically revealed. This study proposed improved four-stage refining process to refine anchovy oil, and the improved methods could efficiently reduce the risk of oil oxidation. Deacidification could remove some kinds of heavy metal ions but not transition metal ions. Decolorization could remove large amounts of volatile compounds, but it could not effectively improve the sensory quality of oil while deodorization did. The refined efficiency of prior stage greatly influenced that of the latter process. After refinement, purification applying multiple adsorbents could further improve oil quality, but greatly decreased oil oxidation stability. This study provided a reference to the refinement of fish oil and develop progressive refining process inspired by the purification.

1. Introduction

The ω-3 polyunsaturated fatty acids (PUFAs), especially EPA and DHA, have a wide range of benefits for human health, such as immunomodulatory properties, anti-inflammation, anti-cancer and anti-apoptotic effects. Fish oil is an important source of EPA and DHA, and has been applied in many foods to strengthen the nutritional efficacy (Ganesan et al., 2014; Marsol-Vall et al., 2021). However , EPA and DHA are easily oxidized, producing unpleasant odors such as fishy, rancid, etc. (Miyashita et al., 2018), which limits the application of fish oil in fortified foods (Chang & Lee, 2020; Chen et al., 2016; Güner et al., 2019).

Crude fish oil generally contains large amounts of impurities, such as phospholipid, free fatty acids, protein, water, minerals, organic contaminants etc., which prevent crude fish oil from being directly consumed by humans. The refining process of fish oil, which mainly includes degumming, deacidification, decolorization, and deodorization (Marsol-Vall et al., 2021), is an important approach to remove these impurities and improve the quality of fish oil (Yi et al., 2023). Degumming is an important method for removing phospholipids, proteins, and other colloidal impurities, which is also beneficial to implement subsequent refining processes. Colloidal impurities can combine with water and form colloidal particles which possess the adsorption capacity for free fatty acids and oxidation products. (Liu et al., 2021). During deacidification, the free fatty acids react with NaOH to generate the soapstocks which also have the ability to adsorb oxidation products, etc. (Huang & Sathivel, 2010). Decolorization process employs adsorbent, such as activated clay and activated carbon, to remove the pigments, oxidation products, etc. Deodorization is conducted under high-temperature and high-vacuum conditions. Parts of oxidation products can be distilled and enter into deodorizing distillate along with water vapor (Crexi et al., 2009; Miyashita et al., 2018).

The traditional refining process of fish oil is similar to that of vegetable oil, both of which usually applies high-temperature condition. However, vegetable oil usually shows more stable oxidation stability. During the degumming and deacidification of fish oil, the operation temperatures are usually set at 65–70 °C (Chakraborty & Joseph, 2015; Simat et al., 2019; Soldo et al., 2019), and due to the direct exposure of fish oil to air, it is easy to cause oxidation of fish oil. During the decolorization stage, the process temperature is usually set at 70–90 °C to achieve better adsorption effect for adsorbent (Crexi et al., 2010; García-Moreno et al., 2013; Wang, Huang, et al., 2021). During deodorization

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by steam distillation, high temperature conditions are necessary to support water vapor and volatile compounds to show a better volatilization effect. Typically, the temperature for deodorizing vegetable oil is usually set at 200–260 °C, which should decline to 160–200 °C for fish oil (Chew et al., 2016; Chew et al., 2017; Crexi et al., 2009; de Oliveira et al., 2016; Song et al., 2018). Therefore, stricter measures for inhibiting the oil oxidation should be taken during the refinement of fish oil.

In addition to the refining process, purification is another important method to remove the impurities in oil, which is usually applied to obtain relatively pure triglyceride (Wen et al., 2022). During the purification of oil, certain amounts of adsorbent fillers or their mixtures, such as activated carbon, celite, aluminum oxide, silica gel, etc., are packed into column chromatography. When oil passes through the column, large amounts of impurities are adsorbed and retained in column (Farhoosh et al., 2016; Mahdavianmehr et al., 2016; Shibata et al., 2015). The quality of purified fish oil, such as total oxidation level, metal element content, odor and volatile components, has been reported rarely. The purification can provide a reference for further refining process to obtain high quality of fish oil.

At present, there have been reports on the impact of refining on some quality indicators of fish oil (Guo et al., 2024; Zheng et al., 2024). However, the scopes of quality indicators studied can be further expanded to gain a more comprehensive understanding of the effects of refinement. The impact of each refinement stage on the quality of fish oil is not detailed and systematic enough. It is necessary to take fortified measures to inhibit the oil oxidation during the refinement of fish oil. This study aimed to reveal the effects of improved refining process and purification on the quality of anchovy oil. The improved refining process adopted the fortified measures including the application of nitrogen protection in degumming and deacidification, the improvement of vacuum in decolorization and the addition of antioxidant in oil during deodorization. In addition, the oxidative stabilities of fish oils from different refinement stages were explored. This study provided a reference for the improvement of the refining process of fish oil.

2. Materials and methods

2.1. Standards and reagents

Crude anchovy oil was provided by Zhoushan Xinnuojia Bioengineering Co., Ltd. (Zhoushan, China). Activated clay and Notit-8015 activated charcoal was purchased from Jiejingclay Co., Ltd. (Leping, Jiangxi, China) and Zhongji Chemicals Import & Export Co., Ltd. (Shanghai, China), respectively. $Na_2S_2O_3$-solution (0.100 mol/L) and *p*-anisidine were bought from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Deionized water was obtained from Millipore Milli-Q water purification system (Burlington, MA, USA). All other reagents of analytical grade used in this study were purchased from Sinopharm Chemical Reagent Co., Ltd. (Qingdao, China). Dihydromyricetin (DM, purity ≥98 %) used for deodorization was bought from Qingdao Mobio Biotech Co., Ltd. (Qingdao, China).

2.2. Refining of anchovy oil

Base on the traditional four-stage refining process (Durmaz & Gokmen, 2019; Liu et al., 2021; Wang, Wen, et al., 2021), the fortified measures to inhibit oil oxidation during the refinement were adopted in the new refining process. The detailed information was as follows.

2.2.1. Degumming

Approximately 200 g of crude anchovy oil was weighed into a flask. Then, 10 g of water and 0.2 g of phosphoric acid (85 %, *v/v*) were added. The flask was filled with nitrogen and sealed with a rubber stopper. The mixture was hold at 65 °C and stirred at 500 rpm for 30 min. After cooling to room temperature, the mixture was centrifuged at 6000 ×*g* for 10 min and the supernatant was collected to obtain degummed oil.

2.2.2. Neutralization

The degummed oil was transfer into a flask followed by adding NaOH solution (3.65 % *w/w*, with 0.45 % of excess as for oil mass). The flask was filled with nitrogen and sealed, then kept at 65 °C with magnetic stirring at 500 rpm for 40 min. After cooling, the mixture was centrifuged and the upper layer was repeatedly washed using hot water at 90 °C until pH-neutral oil was obtained. During the wash, nitrogen was continuously introduced into the equipment. The residual water in neutralized oil was removed by centrifugation and hold at 80 °C for 10 min with high vacuum (300 Pa).

2.2.3. Decolorization

The prepared neutralized oil was bleached using a mixed adsorbent (5 % of activated earth and 0.5 % activated aluminum oxide). The decolorization was conducted at 80 °C for 40 min with 300 Pa of system residual pressure. The bleached oil was collected after centrifugation and filtration applying a Buchner funnel with a pre-layer of diatomaceous earth.

2.2.4. Deodorization

According to our previous study (Wen et al., 2019), dihydromyricetin presented the best antioxidant activity among selected nine antioxidants when used in anchovy oil. Dihydromyricetin with 0.04 % of additive amount (*w/w*) was added to the bleached oil before deodorization. Then, the bleached oil was loaded into a deodorization equipment system. The oil was hold at 180 °C and agitated by steam (with 4 % of usage amount) for 60 min. The vacuum of deodorizing system was maintained at 100 Pa.

2.3. Purification of deodorized oil

The purification method of deodorized oil referred to Wen et al. (2022) with some modifications. A chromatographic column was packed with 20 g of activated clay and 30 g of activated carbon. An amount of 60 g oil was passed through the column using 1000 mL of *n*-hexane. The obtained oil was then purified through a column packed with silica gel and aluminum oxide. *n*-Hexane (500 mL) and a mixture of *n*-hexane: chloroform (1:4, *v/v*) were used for elution. The solvent was removed by a rotary evaporator. Then, the remaining solvent was removed through a nitrogen stream for 2 h at 25 °C.

2.4. Analytical methodology

2.4.1. Acid value (AV), peroxide value (POV), anisidine value (n-PV)

The acid value analysis was according to China National Standard GB/T 5009.229–2016. Briefly, 1 g of oil sample was dissolved in ethanol/water (95:5, *v/v*), and the following titration used 0.1 M KOH solution. Phenolphthalein was used as indicator. The determination of POV, n-PV and the calculation of total oxidation value (Totox) of oil sample were according to Wen et al. (2019). As to POV, briefly, about 2 g of oil sample was weighed into an iodine flask, then 30 mL chloroform: acetic acid solvent (3:2, *v/v*) and 1 mL saturated potassium iodide solution were added successively. After reacting in dark for 5 min, 100 mL distilled water was added. The titration was conducted using 0.0100 mol/L $Na_2S_2O_3$ solution and 3 mL 0.2 % starch solution to judge the endpoint. As to n-PV, about 2 g of oil was dissolved in isooctane. *p*-Anisidine reagent was used for the reaction in the darkness for 8 min and the absorbance was measured at 350 nm. Totox was calculated through using POV and p-AV and according to the equation: Totox = 2POV + n-PV.

2.4.2. Insoluble impurities, moisture, color and decolorization rate

The insoluble impurities analysis referred to Gila et al. (2020) with some modifications. A total of 20 g oil sample was dissolved in 200 mL *n*-hexane. This mixture was passed through a filter paper. If necessary, more *n*-hexane can be used. The filter paper was dried in an oven at 103

°C, and then weighed for calculation. The moisture analysis also referred to Gila et al. (2020).

The color of oil was usually detected by Lovibond method and this analysis referred to our previous study (Wang, Huang, et al., 2021). The scale of red value was from 0 to 70 and yellow value from 0 to 70. For evaluated the decolorization rate, the absorbance of anchovy oil was scanned from 400 to 650 nm at first. The maximum absorption peak was found at 448 nm, then the absorbances of anchovy oil before and after decolorization were measured at 448 nm. The decolorization rate was calculated according to the formula: DR = (A0-A1) × 100 %/A0. Among them, DR is decolorization rate. A0 is the absorbance of anchovy oil before decolorization and A1 is the absorbance of anchovy oil after decolorization.

2.4.3. Metal element contents

The determination of metal element contents in anchovy oil using microwave digestion apparatus and ICP-MS referred to Wang et al. (2019). Microwave digestion method can ionize oil sample with short time and achieve good digestion effect. Metal ions including Cr, Mn, Fe, Cu, etc. were determined by ICP-MS and calculated using an external calibration method.

2.4.4. Fatty acid composition analysis

The preparations of fatty acid methyl esters: 20 mg of sample and 2 mL of 10 % H_2SO_4 in methanol were mixed in a tube, and then the tube was filled with nitrogen followed by heating at 90 °C for 90 min and shaking every 20 min. After cooling to room temperature, 1 mL of *n*-hexane was used to extract the fatty acid methyl esters. The fatty acid methyl esters were analyzed by GC–MS (Agilent 7890a chromatograph coupled with an HP-5MS capillary column (30 m × 0.25 mm × 0.20 μm)). The oven temperature was with an initial value of 80 °C, then increased to 200 °C at 20 °C/min, and then to 280 °C at 5 °C/min, finally increased to 300 °C at 10 °C/min and kept at 300 °C for 5 min. The MS was performed in EI mode at 70 eV. The ion source temperature was set at 250 °C, and the filament current was 25 μA.

2.5. HS-GC-IMS analyses

The analyses of volatile odorants from anchovy oil samples were performed through a GC-IMS system referring to our previous study (Wen et al., 2023). Briefly, A GC-IMS system (G.A.S., Dortmund, Germany) equipped with a headspace sampling unit (Gerstel GmbH, Mülheim, Germany), an autosampler (CTC Analytics AG, Zwingen, Switzerland), and an FS-SE-54-CB capillary column (0.25 μm, 15 m × 0.53 mm, Agilent Technologies, CA, USA) was used. A total of 1 g sample was weighed into a 20 mL headspace vial that was incubated at 40 °C for 30 min. Then, 0.5 mL gas was taken and injected into the injection port heated at 80 °C. The volatiles were separated through the capillary column at isothermal conditions (45 °C). The flow program was 2 mL/min for 5 min, 5 mL/min for 5 min, 10 mL/min for 5 min, 20 mL/min for 5 min, 50 mL/min for 5 min, 100 mL/min for 5 min. The volatiles were first separated in the capillary column. The second separation occurred in the drift tube (9.8 cm) working at a constant voltage (5 kV) at 45 °C with a nitrogen flow of 150 mL/min.

2.6. Sensory evaluation

The sensory evaluation was conducted by panelist recruited from Ocean university of China (Qingdao, China), constituting by ten persons (8 female/2 male; aged between 22 and 45 years). The flavor vocabulary and attributes of oils were produced after a discussion among the panelists, which included fishy, rancid, grassy, soapy, metallic and sweet/biscuit-like. The detailed information was presented in Table 1. The intensity of each odor attribute was evaluated on a 0–5 point scale with 0.5 steps. The flavor intensity represented by the score was shown as below: 0-not perceptible, 1-very slightly perceptible, 2-slightly perceptible, 3-considerably perceptible, 4-strongly perceptible and 5-very strongly perceptible.

Table 1
Descriptions, definitions and references for the odor attributes indicated by the panelists for anchovy oil.

Odor attributes	definitions	References
Fishy	an aromatic reminiscent of cod liver oil	cod liver oil diluted in good-quality soybean oil
Rancid	an aromatic reminiscent of odor or flavor of highly oxidized oils containing high amount of linolenic acid such as sunflower, cottonseed, or peanut	Good quality cottonseed oil aged for four days at 60 °C or until a peroxide value of approximately 5.0 is reached
Grassy	an aromatic reminiscent of the green character of mowed grass	Crude soybean oil from non-heat-treated soybeans diluted in good-quality soybean oil
Soapy	an aromatic reminiscent of unscented soap	Ivory brand unscented soap flakes
Metallic	an aromatic associated with metal coins	0.01 % ferrous sulfate diluted in distilled, filtered water
Sweet/biscuit-like	an aromatic reminiscent of shortbread biscuits	Shortbread biscuits

2.7. Storage of anchovy oils

A total of 10 g of crude anchovy oil was placed into a brown glass bottle (10 mL), followed by shake. The glass bottle was placed in a 60 °C oven for storage. Samplings were performed periodically and the samples were analyzed of POV. The storage experiments of degummed oil, neutralized oil, bleached oil, deodorized oil and purification oil were same to that of crude anchovy oil.

2.8. Statistical analysis

LAV software version 2.2.1 and GC × IMS Library Search were applied for the data collection, fingerprint drawing and qualitative analysis. All experiments were performed in triplicate, and the results were shown as mean ± standard deviation ($n = 3$). SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis and the mean values were compared with one-way ANOVA, followed by Dunnett's test. The statistical tests were considered to be significant at p-values less of 0.05. Origin 2017 (Northampton, MA, USA) was used for the figure drawing.

3. Results and discussion

3.1. Physicochemical indexes

The change of AV, POV, n-PV and Totox of anchovy oil during the refining process and purification were shown in the Fig. 1. After the neutralization, the AV had a significant decrease from 0.76 mg KOH/g of crude oil to 0.08 mg KOH/g of neutralized oil (Fig. 1A). However, the AV increased slightly after decolorization (0.09 mg KOH/g) and deodorization (0.11 mg KOH/g). The AV showed a substantial reduction after the purification, indicating it could massively remove free fatty acids from deodorized oil. The POV decreased continuously during the refinement and purification (Fig. 1B). The decolorization, deodorization and purification could significantly reduce the contents of primary oxidation products, and the removal rates of them were 42.0 %, 42.3 % and 70.3 %, respectively. Finally, the POV of deodorized oil and purified oil were 4.28 and 1.27 mmol/kg, respectively. As regard to n-PV in Fig. 1C, neutralized oil had the highest value of 35.4. The adsorbent used in decolorization possessed a good adsorption effect on the secondary oxidation products, resulting in the high removal rate of 24.5 %. The deodorization process showed a certain ability to remove secondary

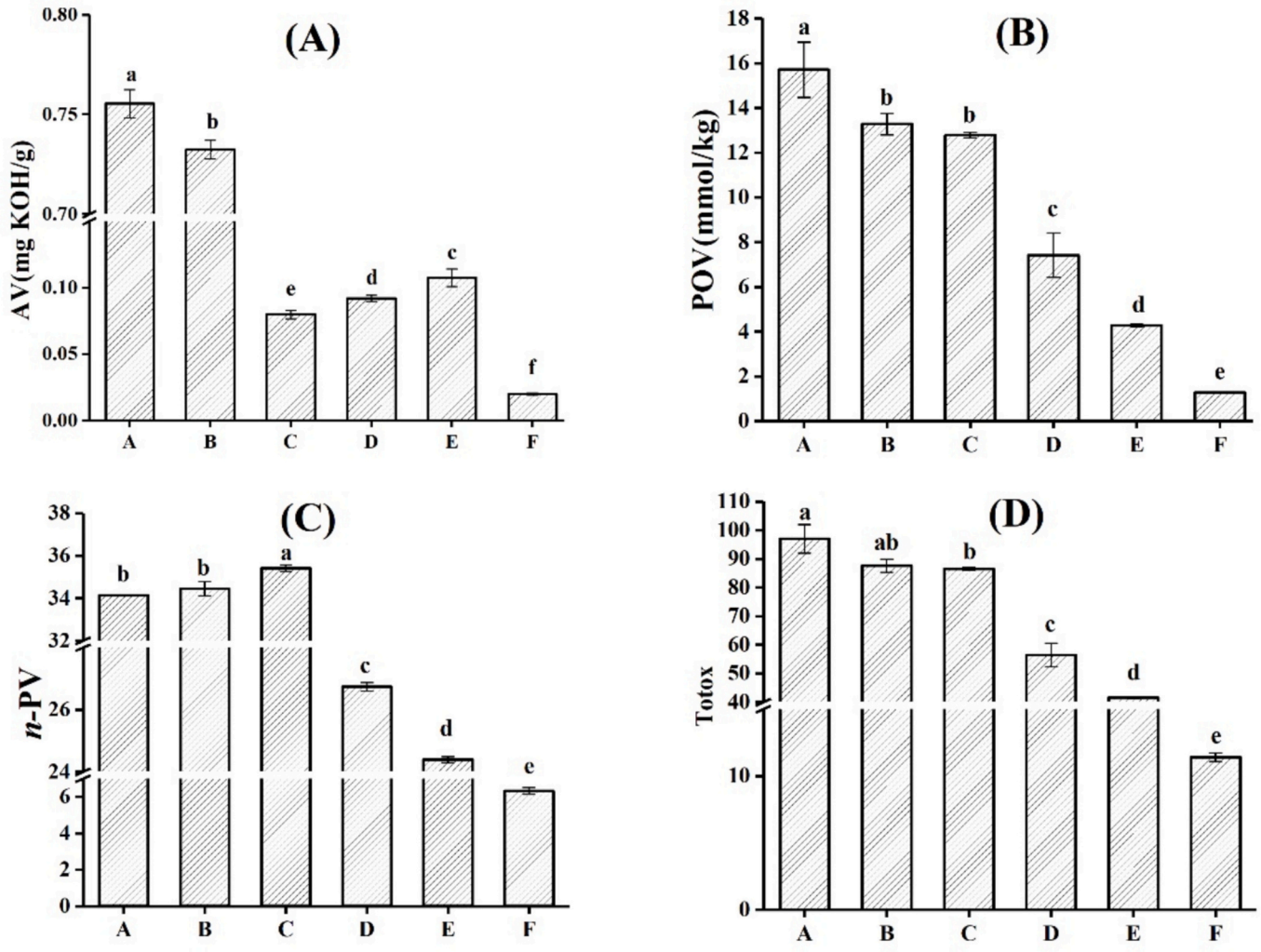

Fig. 1. The AV, POV, n-PV and Totox of anchovy oils during the refining process and purification. A, B, C, D, E and F represented the crude oil, degummed oil, deacidified oil, decolorized oil, deodorized oil, purified oil, respectively. Different letters in the same figure indicated statistically significant difference ($p < 0.05$).

oxidation products. The purification could remove large amounts of secondary oxidation products, resulting in the n-PV of purified oil was 6.3. Fig. 1D depicted the Totox changes during refining process and purification. The Totox continuous declined, indicating decolorization, deodorization and purification were efficient processes in removing the oxidation products of anchovy oil.

After the neutralization, the n-PV of anchovy oil increased. As this process was conducted under the protection by nitrogen, the possible reason was that the produced amount of secondary oxidation products from the decomposition of primary oxidation products, was more than the removed amount through the adsorption by soapstock. Simat et al. (2019) reported the POV change during the refinement of tuna by-products oil, showing that the POV increased from 1.2 mmol/kg of crude oil to 1.9 mmol/kg of deodorized oil. Menegazzo et al. (2014) performed the chemical refinement on Nile tilapia oil and hybrid sorubim oil, showing that the POV increased from 0.4 mmol/kg of crude oil to 2.8 mmol/kg of deodorized oil for Nile tilapia oil, and increased from 0.1 mmol/kg of crude oil to 2.7 mmol/kg of deodorized oil for hybrid sorubim oil. Considering their refining process without the strict measures to inhibit oil oxidation, the POV increase confirmed fish oil oxidation occurred. Similar phenomenon was observed during the refinement of fish oils conducted by de Oliveira et al. (2016), Song et al. (2018) and Huang and Sathivel (2010). Therefore, it was better to take more measures to inhibit fish oil oxidation during its refining process.

The impact of modified four-stage refinement process on the insoluble impurity contents and moisture content, the color and decolorization rate of anchovy oils was evaluated. As shown in Table 2, the insoluble impurity contents gradually decreased during the refinement, and degumming had the highest removal rate of 41.7 %. After the purification, the insoluble impurities were almost completely removed. The moisture content also gradually decreased during the refinement. Due to the application of high temperature in deodorization, the

Table 2
The contents of insoluble impurities and moisture, the color of anchovy oils during the refining and purification process.

Samples	Insoluble impurities (%)	Moisture (%)	Color	
			Red value	Yellow value
A	0.12 ± 0.01^{a}	0.43 ± 0.03^{a}	10.1 ± 1.0^{a}	35.3 ± 1.3^{a}
B	0.07 ± 0.00^{b}	0.14 ± 0.01^{b}	7.0 ± 0.2^{b}	28.4 ± 1.7^{b}
C	0.05 ± 0.00^{c}	0.09 ± 0.01^{c}	3.3 ± 0.3^{c}	25.5 ± 0.8^{c}
D	0.03 ± 0.00^{d}	0.07 ± 0.00^{d}	2.5 ± 0.2^{d}	14.8 ± 1.1^{d}
E	0.02 ± 0.00^{d}	0.04 ± 0.00^{e}	1.5 ± 0.0^{e}	10.6 ± 0.5^{e}
F	ND*	0.01 ± 0.00^{f}	0.5 ± 0.0^{f}	1.5 ± 0.0^{f}

A, B, C, D, E and F represented the crude oil, degummed oil, deacidified oil, decolorized oil, deodorized oil, purified oil, respectively. Different letters indicated statistically significant difference ($p < 0.05$). *Not detected.

removal rate of moisture was highest for deodorization (42.9 %). During the purification, multiple adsorbents were used, resulting that water (polar compound) was largely removed and with the final content of 0.01 % in purified oil. The crude anchovy oil showed brownish red color and its red and yellow values were 10.1 and 35.3, respectively. The red value showed a large decrease after deacidification, which might due to some reactions occurred between pigment and alkali. Decolorization could effectively remove pigments from oil and the decolorized oil showed faint yellow with the red and yellow values of 2.5 and 14.8, respectively. Meanwhile, the decolorization rate of this process was 45.3 %. The purification had a significant influence on the oil color, and the purified oil only had a pale yellow with the red and yellow values of 0.5 and 1.5, respectively.

3.2. Metal element contents

Eight metal elements were analyzed of their contents in anchovy oils from different refinement stages. Cr, Mn, Fe, Cu and Zn belong to transition metal elements, which could catalyze the reaction of oil oxidation and decrease the oxidative stability of fish oil. As, Cd and Pb were heavy metal elements which were harmful to human health. Fig. 2A-E showed the transition metal element contents of anchovy oils. Fe had a high content of 7096.8 μg/kg in crude anchovy oil and gradually decrease to 1381.3 μg/kg in deodorized oil. Purification showed unsignificant influence on Fe content. On the contrary, decolorization showed a high removal rate of Fe as much as 44.9 %. For Cr, Mn and Cu, the refining process could remove them by a certain amount. The refinement and purification showed limited influence on Zn content. For these four transition metal elements, their contents were 171.0 μg/kg (Cr), 82.9 μg/kg (Mn), 68.1 μg/kg (Cu) and 4619.5 μg/kg (Zn) after the

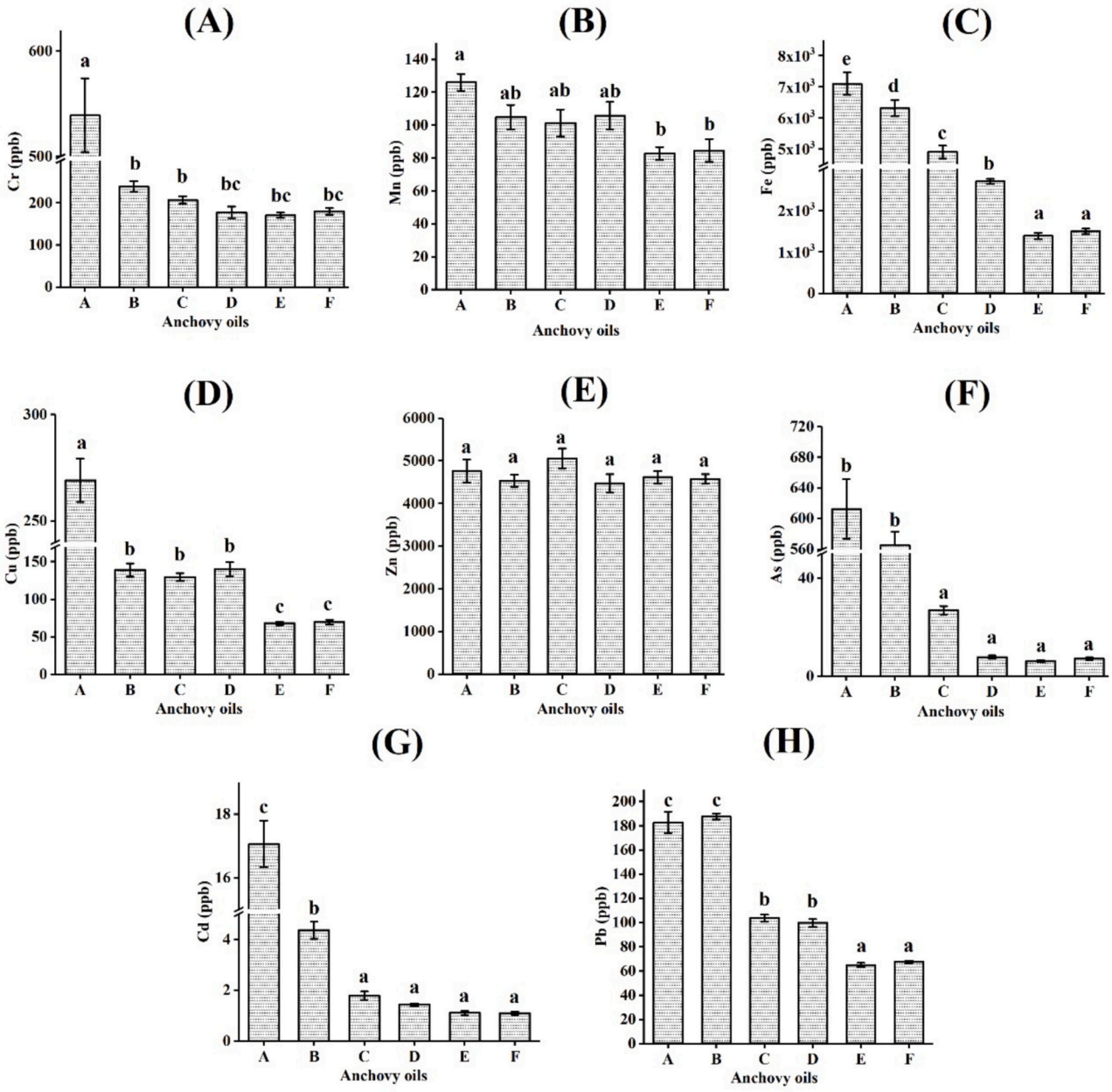

Fig. 2. The metal element contents of anchovy oils during the refining process and purification. A, B, C, D, E and F represented the crude oil, degummed oil, deacidified oil, decolorized oil, deodorized oil, purified oil, respectively. Different letters in the same figure indicated statistically significant difference ($p < 0.05$).

purification. Fig. 2F-H showed the As, Cd and Pb contents, respectively, and they all continually decreased during refinement. Finally, their contents in deodorized oil were 6.1, 1.1 and 65.1 μg/kg, respectively. The neutralization exerted the most serious influence on removing these heavy metal elements. However, purification was powerless on the removal of these metal elements. Vaisali et al. (2015) reported that the mucilaginous substances formed during degumming could remove portion of trace metals and bleaching was an important process to remove trace metals. Charanyaa et al. (2017) studied the Cu and Fe contents in Indian Sardine oils from various refinement stages, reporting that the Cu and Fe contents respectively decreased from 606 to 445 μg/kg and from 2062 to 1266 μg/kg after degumming process. Finally, the Cu content in bleached oil was 100 μg/kg and Fe was not found in bleached oil. According to this study, all of degumming, neutralization and decolorization could show significant influence on the metal contents of anchovy oil.

3.3. Fatty acid profiles

As shown in Fig. 3, the fatty acids profile of oil samples from six stages presented the difference. Additionally, the contents of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) also had a certain degree of difference. After the purification, the contents of EPA, DHA and PUFA increased and the content of MUFA had a decrease. The contents of EPA and DHA ranged from 5.8 % to 6.5 % and from 11.0 % to 13.1 %, respectively. The significance of the difference was further evaluated and provided in Table 3. The difference of EPA contents between these six oil samples was not significant and it was same to the DHA contents. The contents of SFA, MUFA and PUFA showed unsignificant differences between crude oil and deodorized oil. However, the content of PUFA had a significant increase during the purification.

During the refinement of tuna by-product oil, the EPA, DHA and MUFA contents decreased, while SFA and PUFA contents increased. Inconsistently, during the refinement of tuna liver oil, the EPA, SFA and MUFA contents decreased, while DHA and PUFA contents increased (Simat et al., 2019). After deodorization, the EPA content of deodorized oil was similar to bleached oil, while the DHA content of deodorized oil was higher than that of bleached oil (Crexi et al., 2009). With respect to Nile tilapia oil, EPA, DHA and PUFA contents increased after refinement. As regard to hybrid sorubim oil, DHA, MUFA and PUFA contents showed an improvement, while the EPA content was unchanged (Menegazzo et al., 2014). The fatty acid contents of fish oil were influenced by various factors during the refining process, such as the fatty acid composition of crude oil, the applied process parameter. In this study, the refining process didn't have significant influence on the unsaturated fatty acid compositions.

Table 3
The fatty acid contents of anchovy oils during the refining and purification processes.

Samples	EPA	DHA	SFA	MUFA	PUFA
A	6.06 ± 0.23^{a}	11.46 ± 0.98^{a}	38.87 ± 1.12^{a}	37.49 ± 2.17^{a}	23.63 ± 1.76^{ab}
B	5.89 ± 0.13^{a}	11.20 ± 0.88^{a}	39.79 ± 2.01^{a}	37.70 ± 2.64^{a}	22.51 ± 0.84^{a}
C	6.15 ± 0.24^{a}	12.21 ± 0.91^{a}	38.34 ± 1.90^{a}	37.40 ± 1.44^{a}	24.26 ± 0.83^{b}
D	5.83 ± 0.38^{a}	10.98 ± 0.29^{a}	40.34 ± 2.78^{a}	38.59 ± 0.93^{a}	21.07 ± 0.19^{a}
E	5.92 ± 0.11^{a}	11.57 ± 0.88^{a}	39.16 ± 1.26^{a}	38.02 ± 1.62^{a}	22.82 ± 0.98^{a}
F	6.50 ± 0.51^{a}	13.12 ± 0.90^{ab}	38.67 ± 2.99^{a}	36.71 ± 1.32^{ab}	24.62 ± 1.84^{b}

A, B, C, D, E and F represented the crude oil, degummed oil, deacidified oil, decolorized oil, deodorized oil, purified oil, respectively. Different letters indicated statistically significant difference ($p < 0.05$).

3.4. Sensory quality

The sensory qualities of six oil samples were evaluated and the scores were shown in Fig. 4. The crude anchovy oil emitted strong fishy and rancid odor with the scores of 4.2 and 4.4, respectively. After the degumming and deacidification, both of fishy and rancid odor were still unacceptable with the scores of 3.8. The metallic odor was not effectively eliminated after the deacidification with the score of 2.7 for deacidified oil. Besides, the deacidified oil could be scented of soapy odor, which was caused by the residually slight soapstock. After the decolorization, the fishy, rancid, metailic and soapy odor were significantly impaired with the scores of 2.8, 2.2, 1.2 and 1.7 for bleached oils, respectively. After the deodorization, the sensory quality of anchovy oil was significantly improved, only with very slightly perceptible fishy and metailic odor. However, the grassy odor was slightly perceptible with

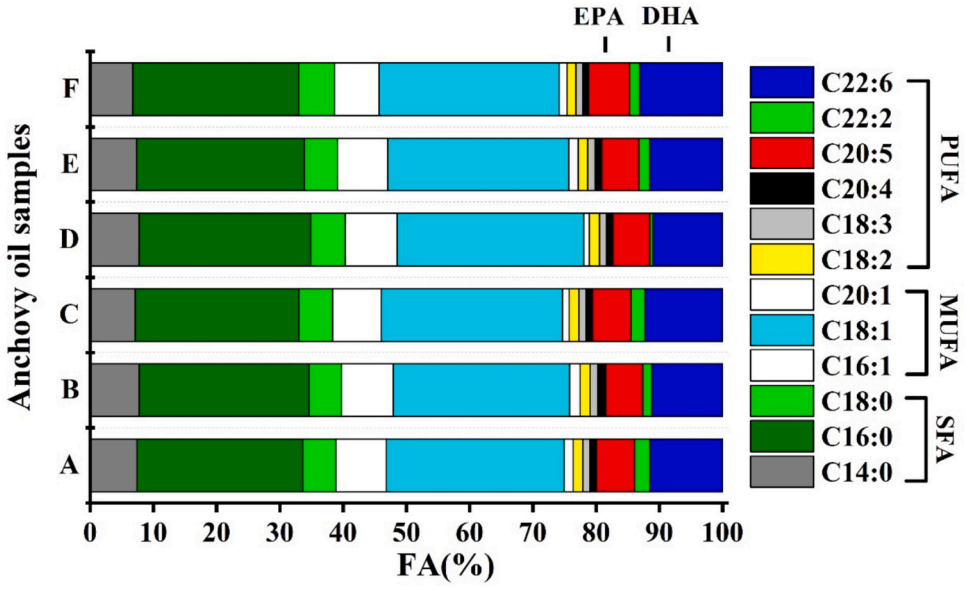

Fig. 3. The fatty acid profiles of anchovy oils during the refining process and purification. A, B, C, D, E and F represented the crude oil, degummed oil, deacidified oil, decolorized oil, deodorized oil, purified oil, respectively.

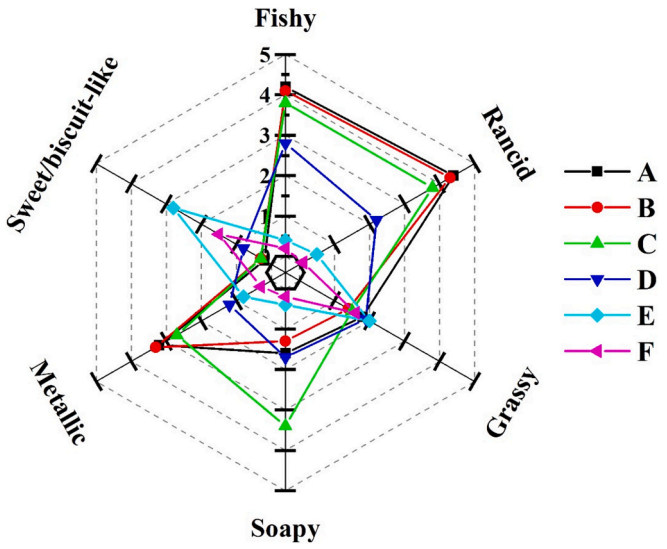

Fig. 4. The sensory scores of anchovy oils during the refining process and purification. A, B, C, D, E and F represented the crude oil, degummed oil, deacidified oil, decolorized oil, deodorized oil, purified oil, respectively.

the score of 2.0. Interestingly, there was a significant enhancement of biscuit-like odor, which might be related to application of dihydromyricetin in anchovy oil during deodorization. After the purification, the sensory quality was further improved and the purified oil only emitted slightly perceptible grassy and biscuit-like odor.

According to our best knowledge, the study on the effect of four-stage refining process on the sensory quality of fish oil was scarce. de Oliveira et al. (2016) evaluated the effect of deodorization on the sensory attributes of tuna by-product oils, indicating that fishy, frying and rancid odor were the predominant sensory attributes of refined fish oil and the operation temperatures of 160 °C for 1 h and 200 °C for 1 h were suggested for the deodorization of tuna by-product oil. The effects of various deodorization methods on the sensory attributes of fish oil were evaluated by Song et al. (2018). In terms of steam distillation, the removal of off-flavors was unsatisfactory, and the sensory score of fishy for deodorized fish oil was high. The fish oil treated by activated clay showed lower score of fishy than that of the sample treated by steam distillation. However, according to our study, the deodorization had a better removal effect on fishy odor than that of bleaching process. This study used bleached oil as raw material for deodorization process. Otherwise, the study by Song et al. (2018) used deacidified oil as raw material. Decolorization was an important refining process in the removal of impurities in oil, and it could not only reduce the pigments in fish oil, but also remove peroxides, metal ions and other trace contaminants. These impurities contained in deacidified oil might show adverse effect on the deodorization process.

3.5. Volatile compounds

The volatile compounds of anchovy oil samples from different process stages were analyzed by HS-GC-IMS and the fingerprint of these compounds was drawn as Fig. 5A. A total of 56 volatiles were found. The volatile profiles showed continuous changes during the refinement, especially during the decolorization and deodorization, which had drastic changes. As shown in Fig. 5B, PCA model was established to further evaluate the volatile profile differences between these six oil samples. The R^2 of 0.986 and Q^2 of 0.960 indicated high fit goodness and prediction ability for the current PCA model. In the coordinate system, the crude oil and degummed oil located far away from other oil samples, implying the volatile compositions of these two oils had large difference with those of other oils. Deacidified oil, decolorized oil and deodorized oil were distributed in second, fourth and first quadrant, respectively, indicating their volatile compositions were also diverse. The distance between decolorized oil and purified oil was close, indicating the similarity between them. It was worth noting that the volatile compositions of anchovy oil showed large change after the decolorization, especially in aldehyde, ketone, hydrocarbon and some of other type compounds (Fig. 5A). Additionally, the deodorization further removed large amounts of these compounds. However, the alcohol compounds including 2-octanol, 2-ethylhexanol, menth-1-en-8-ol, nerol and aldehydes including (*Z,Z*)-3,6-nonadienal, (*Z*)-2-nonenal (*E,Z*)-2,6-nonadienal were difficult to be removed during the refinement.

The volatile compound compositions of tuna by-product oil and tuna liver oil including crude oil, bleached oil and deodorized oil were analyzed. Sixteen components including one ester, five alcohols, three hydrocarbons and six aldehydes were identified, and their compositions or proportions showed significant changes during the refinement (Simat et al., 2019). Similarly, sixteen volatile components were found in sardine oil and sardine by-products oil, and these volatile compound compositions also changed significantly during their refining processes (Soldo et al., 2019).

Considering the sensory scores of anchovy oils (Fig. 4), decolorization could significantly decrease the scores of fishy, rancid and soapy odors, which was consistent to that the decolorization could significantly reduce the contents of the volatile compounds in deacidified oil (Fig. 5). Deodorization could effectively remove large amounts of volatile compounds, resulting in greatly improving the sensory quality of anchovy oil. Furan compounds were important flavor compounds in foods (He et al., 2021; Wang, Wen, et al., 2021). A total of 5 furan compounds were detected in crude anchovy oil and removed in large quantities by deodorization process. (*Z,Z*)-3,6-Nonadienal could emit the green odor of fish oil (Sullivan & Budge, 2012), and its content almost didn't change during the four-stage refinement and purification. (*E,Z*)-3,5-octadien-2-one was considered as a key compound participating in the formation of fishy odor (Marsili & Laskonis, 2014), which also always existed in anchovy oil during these four refinement stages.

3.6. Oxidative stability

For evaluating the oxidative stabilities of six oil samples, their POV were monitored during the storages in oven. The POV changes of these stored anchovy oils were showed in Fig. 6. Although the crude oil possessed a higher initial POV, the increase rate of its POV was lower than that of deacidified oil and decolorized oil. The POV of deacidified oil and decolorized oil exceeded the POV of crude oil only after two days of storage. Contrastively, the POV of degummed oil and deodorized oil were always lower than that of crude oil during the storage. Obviously, the POV of purified oil showed a fast increase and its POV reached 372.6 mmol/kg only after eight days of storage. Interestingly, the POV increase of decolorized oil was kept at a very slow rate, which might be related to the retained dihydromyricetin in decolorized oil (the dihydromyricetin was added to decolorized oil before the deodorization). Additionally, high temperature condition and sufficient agitation by steam adopted during the deodorization process made it easier for dihydromyricetin to donate hydrogen atoms to the existed and generated free radicals to break free-radical chain reaction (Miyashita et al., 2018). Therefore, the addition of dihydromyricetin in decolorized oil before the deodorization could reduce the adverse effect of high temperature on anchovy oil.

The oxidative stabilities of stored oils were influenced by many factors, such as oxidation product contents, fatty acid compositions, endogenous antioxidant contents, metal ion contents, pigment contents (Xu et al., 2022). Yoon and Kim (1994) reported refinement could remove 34 % of tocopherols and 51 % of oryzanols in rice bran oils, and the oxidation stability gradually decreased at the following order: crude oil, degummed oil, bleached oil and deacidified oil. The oxidation stability of deodorized oil was almost same to that of bleached oil. Durmaz

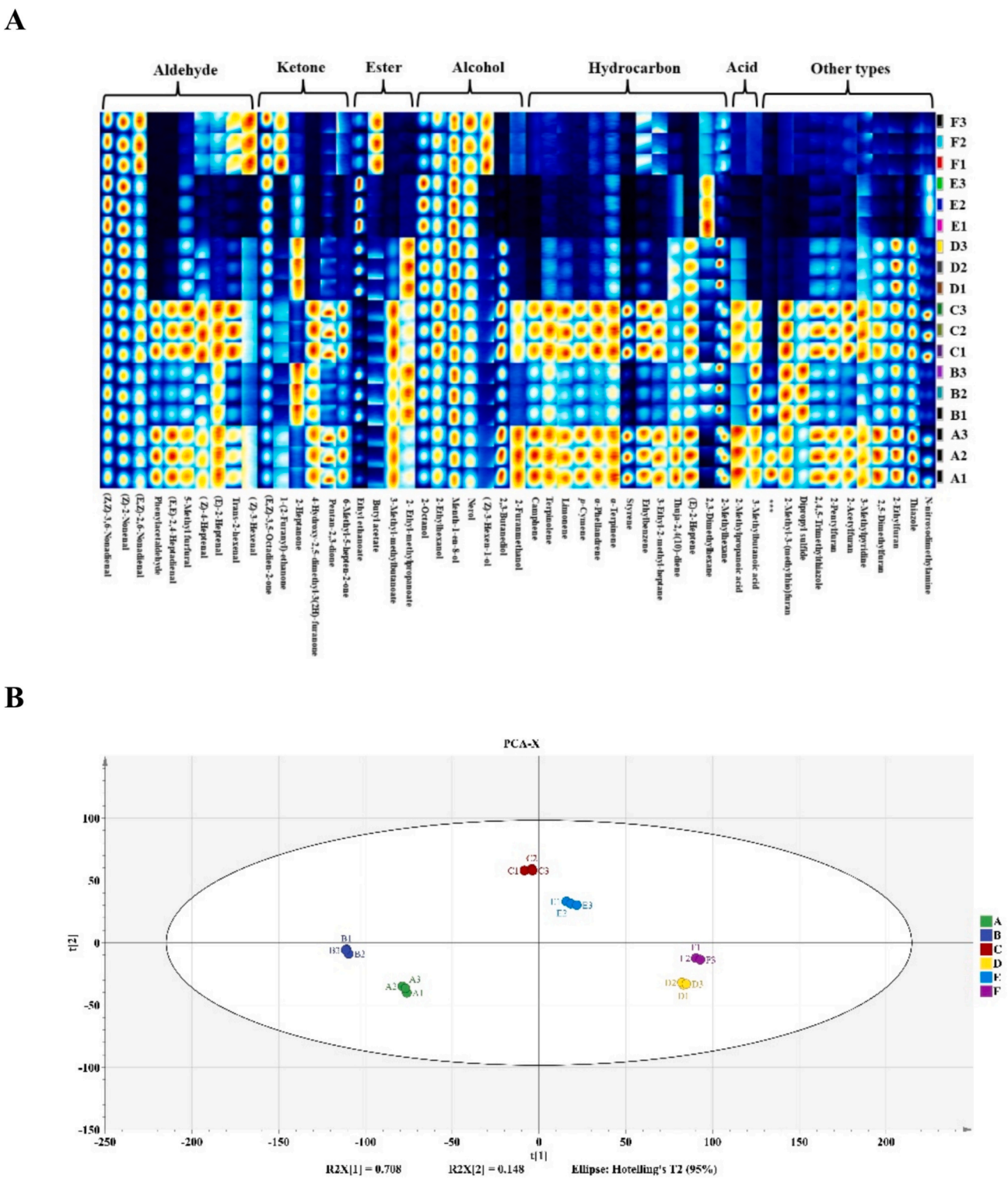

Fig. 5. HS-GC-IMS fingerprint of the volatile compounds of anchovy oils from the refining and purification process (A); The score plots of the PCA model (B) (R^2 = 0.986, Q^2 = 0.960).

and Gokmen (2019) pointed that deacidified hazelnut oil possessed a better oxidative stability than that of crude hazelnut oil. Deodorized oil presented a worse oxidative stability than that of bleached oil which might due to the loss of tocopherols during deodorization. In this study, the components accelerating the oil oxidation, such as metal ions, pigments, were removed during degumming. In addition, the components inhibiting the oil oxidation, such as tocopherols, were partly retained, resulting the oxidative stability of degummed oil was better than that of crude oil. This situation might be opposite during deacidification and decolorization, i.e. a large amount of antioxidant components was removed and only a small amount of prooxidative components were removed.

4. Conclusions

The fortified measures to inhibit oil oxidation included application of

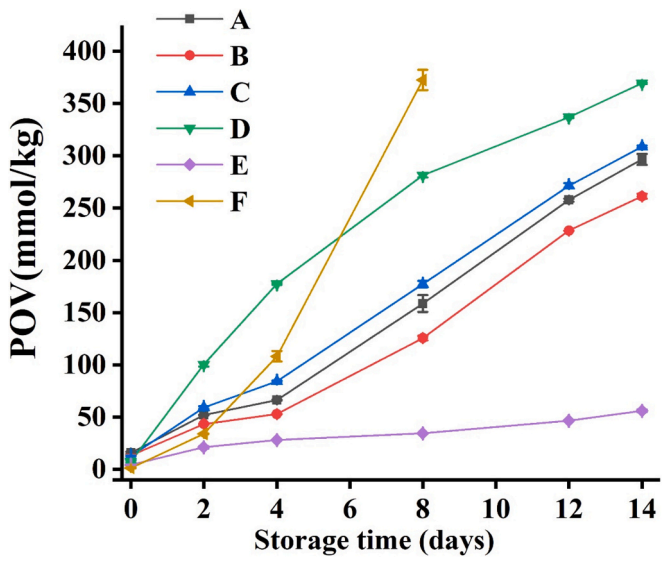

Fig. 6. The POV changes of fish oils during storage. A, B, C, D, E and F represented the crude oil, degummed oil, deacidified oil, decolorized oil, deodorized oil, purified oil, respectively.

nitrogen protection during degumming and deacidification, improving vacuum during decolorization and using dihydromyricetin during deodorization, which could efficiently reduce the risk of oil oxidation, and were beneficial to remove oxidation products and volatile compounds. During the refinement, the deacidification had a good removal effect on heavy metal ions, but a limited removal effect on transition metal ions. Decolorization could remove oxidation products and large amounts of volatile compounds, but its improvement on sensory quality was quite limited. Based on decolorization, the deodorization could effectively remove volatiles and improve the sensory quality of bleached oil. The refined result of previous refinement stage had an impact on that of subsequent refinement stage, hence combining four refining stages together was conducive to improving the refinement effect. During the deodorization, applying dihydromyricetin could inhabit oil oxidation and improve the oxidation stability of refined anchovy oil. Purification could effectively reduce oxidation product content, remove volatile compounds and improve the sensory quality, but greatly decrease the oxidation stability of anchovy oil.

CRediT authorship contribution statement

Li Li Xu: Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Ya Nan Xu:** Data curation. **Wei Jia Liu:** Data curation. **Chang Hu Xue:** Funding acquisition, Conceptualization. **Tong Cheng Xu:** Supervision, Project administration, Conceptualization. **Yun Qi Wen:** Visualization, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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