LWT - Food Science and Technology 160 (2022) 113291

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Recombination of oxidized samples of DHA and purified sunflower oil reproduces the odor profile of impaired algae oil from *Schizochytrium* sp. and reveals the odor contribution of fatty acids other than DHA

Yun-Qi Wen [a,d], Chang-Hu Xue [a,b], Hong-Wei Zhang [c], Li-Li Xu [a], Xiao-Han Wang [a], Shi-Jie Bi [a], Qian-Qian Xue [a], Yong Xue [a], Zhao-Jie Li [a], Joaquín Velasco [d,*], Xiao-Ming Jiang [a,**]

[a] College of Food Science and Technology, Ocean University of China, No. 5 Yu Shan Road, Qingdao, Shandong Province, 266003, PR China
[b] Laboratory for Marine Drugs and Bioproducts, Pilot National Laboratory for Marine Science and Technology, Qingdao, Shandong Province, 266237, PR China
[c] Technology Center of Qingdao Customs District, No.83 Xinyue Road, Qingdao, Shandong Province, 266002, PR China
[d] Instituto de la Grasa, Consejo Superior de Investigaciones Científicas (CSIC), Ctra. de Utrera, km 1, Sevilla, 41013, Spain

ARTICLE INFO

Keywords:
Volatiles
Algae oil flavor
Sensory evaluation
DHA oxidation
Odorant synthetic effect

ABSTRACT

Algae oil from *Schizochytrium* sp. is one of the major natural sources of ω-3 polyunsaturated fatty acids (PUFAs), but its applications in foods are limited because of its unpleasant odor. The complex composition of algae oil originates an odor profile that differs from that of its major PUFA, i.e. docosahexaenoic acid (DHA). The aim of this work was to explore the specific role of DHA oxidation in the odor profile of algae oil. According to the fatty acid composition and odor characteristics, oxidized sunflower oil was selected and combined with oxidized DHA to simulate the odor profile of algae oil. The recombination oil showed similar odor characteristics to the algae oil sample indicating that DHA oxidation was key in the formation of the unpleasant odor in algae oil and that oxidation of fatty acids other than DHA also made a contribution. The constructed off-flavor of algae oil was a consequence of a synthetic effect between odorants from oxidized DHA and oxidized oleic, linoleic and linolenic acid. This study provided a reference for the source research of volatile substances producing fishy odor in algae oil.

1. Introduction

Eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3) are important structural components in synaptic membranes of the brain and retina and crucial for a correct function of heart and the cardiovascular system. They must be provided through diet for effective incorporation into cell membranes (Marsol-Vall, Aitta, Guo, & Yang, 2021). Fish oil and algae oil are the major natural sources of these two long-chain ω-3 PUFAs. They can be supplied as oil capsules or be included in diet through fortified foods (Jamshidi, Cao, Xiao, & Simal-Gandara, 2020; Marsol-Vall et al., 2021). However, the use of fish or algae oil is a challenging task as they are highly susceptible to lipid oxidation and readily develop fishy smells and rancidity (Chang & Lee, 2020; Chen et al., 2016; Güner, Yılmaz, & Yüceer, 2019).

Numerous studies have been conducted to disclose the complex mechanism involved in fishy odor in different foods. A variety of volatiles have been associated with fishy malodor and the specific compounds involved seem to depend on the type of food. It has been reported that high concentration of trimethylamine in milk from cows on standard diets showed fishy off-flavor (Lundén, Gustafsson, Imhof, Gauch, & Bosset, 2002). Matheis and Granvogl (2019) identified trimethylamine as the only odorant responsible for the fishy off-flavor in steam-treated rapeseed oil. Karahadian and Lindsay (1989) evaluated the compounds contributing to fishy flavors in fish oils, showing that (*E*,*Z*,*Z*)-2,4,7- and (*E*,*E*,*Z*)-2,4,7-decatrienal caused the distinct burnt/fishy flavors and (*Z*)-4-heptenal acted as a modifier of overall burnt/fishy flavors. Milo and Grosch (1993) investigated the odorant differences between freshly boiled trouts and trouts stored at −13 °C, showing that (*Z*)-3-hexenal and (*Z*,*Z*)-3,6-nonadienal substantially contributed to the fatty, fishy off-flavor in stored trouts. Venkateshwarlu, Let, Meyer, and Jacobsen (2004) reported that (*E*,*Z*)-2,6-nonadienal and 1-penten-3-one can be used as markers for fishy and metallic off-flavors in a milk

* Corresponding author.
** Corresponding author.
E-mail addresses: jvelasco@ig.csic.es (J. Velasco), jxm@ouc.edu.cn (X.-M. Jiang).

https://doi.org/10.1016/j.lwt.2022.113291
Received 16 September 2021; Received in revised form 21 February 2022; Accepted 25 February 2022
Available online 2 March 2022

emulsion. In addition, they found a compensatory effect of (*Z*)-4-heptenal and (*E*,*E*)-2,4-heptadienal, and a synergistic effect of (*E*,*Z*)-2,6-nonadienal and (Z)-4-heptenal in the formation of fishy off-flavor. Hexanal, nonanal and 1-octen-3-ol have been considered the main compounds contributing to the fishy flavor of silver carp products (Xue et al., 2021). The decrease of 1-octen-3-ol, 1-penten-3-ol, hexanal, heptanal, nonanal, decanal, (*E*)-2-octenal and 2,3-pentanedione contents in coating treated sea bass samples significantly lowered the fishy flavor (Li, Peng, Mei, & Xie, 2020). (*E*)-2-Octenal has been suggested to contribute to the unpleasant fishy and rancid off-flavors of sea bream, and heptanal, nonanal, hexanal or decanal presented a more characteristic fishy flavor (Parlapani, Verdos, Haroutounian, & Boziaris, 2015). The studies on algae oil or algae-oil containing foods are not numerous. In this respect, (*Z*)-heptenal, decanal, ethanone, and hexadecenoic acid have been related to fishy odors in algae oil-in-water nanoemulsions (Chen et al., 2016). Marsili and Laskonis (2014) reported odorant synergy effects of heptanal and (*E*,*Z*)-3,5-octadien-2-one causing the fishy malodors in algal oils.

Hammer and Schieberle (2013) tried to clarify the compounds responsible for fishy off-flavor using model ω-3 PUFAs, i.e. alpha-linolenic acid (ALA), EPA and DHA, which were oxidized by either copper(II) ions or lipoxygenase. They found that *trans*-4,5-epoxy-(*E*,*Z*)-2,7-decadienal, (*Z*)-1,5-octadien-3-one, (*E*,*E*,*Z*)-2,4,6-nonatrienal, (*Z*,*Z*)-2,5-octadienal, (*Z*,*Z*)-3,6-nonadienal, and (*Z*)-3-hexenal were the key odorants for fishy odor. Both the autoxidation and enzymatic oxidation of the three fatty acids produced the same odorants, but they exhibited different odor activities or flavor dilution factors depending on the fatty acid and/or the type of oxidation. These results suggested that a defined ratio of these odorants was necessary for fishy off-flavor.

DHA is by far the major PUFA in algae oil from *Schizochytrium* sp. presenting levels that can range between 35% and 45% (Marsili & Laskonis, 2014). Other PUFAs are present in much lower contents, such as EPA (0.47%) and ALA (0.06%) (Ryckebosch et al., 2013). Because of its greater susceptibility to lipid oxidation and its much higher levels, it is reasonable to think that oxidation of DHA is the cause of fishy off-flavor in algae oil. During the present research we found however that the odor profile produced by oxidation of DHA (standard) was different from that of oxidized algae oil. To the best of our knowledge, no study has been addressed to evaluate the contribution of fatty acids other than DHA to the sensory profile of algae oil.

The aim of this study was to determine the specific relationship between the flavor of oxidized DHA and the flavor of algae oil and whether fatty acids other than DHA make a significant contribution. Towards this aim, besides DHA and algae oil from *Schizochytrium* sp. three vegetable oils with different fatty acid compositions, namely, camellia oil, sunflower oil and linseed oil were also investigated. Apart from the absence of DHA or any other long-chain ω-3 PUFA, the selection of these vegetable oils were based upon their relative contents of oleic, linoleic and linolenic acids. Thus camellia and sunflower oils are rich in oleic (ω-9) and linoleic (ω-6) acid, respectively, whereas linseed oil contains substantial levels of linolenic acid (ω-3). The four oils were purified by column classic chromatography to mainly obtain their triacylglycerols (TAG) and thereby eliminate the influence of impurities such as trimethylamine. The purified oils and DHA were oxidized under controlled conditions to examine changes in odor profiles with the oxidation extent. The algae oil was compared to the vegetable oils and oxidized samples of DHA and sunflower oil were selected and combined to reproduce the off-flavor of algae oil. Such a combination allowed for determining the role of fatty acids other than DHA in the fishy malodor of algae oil.

2. Materials and methods

2.1. Standards and reagents

Camellia oil, sunflower oil and linseed oil were all purchased from a local supermarket. Algae oil was provided by Qingdao Mingyuan Chemical Co., Ltd.. Activated clay and Notit-8015 activated charcoal was respectively purchased from Jiejingclay Co., Ltd. (Leping, Jiangxi, China) and Zhongji Chemicals Import & Export Co., Ltd. (Shanghai, China). A $Na_2S_2O_3$ solution (0.100 moL/L) and *p*-anisidine were acquired 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 were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Qingdao, China). DHA standard (purity >99%) and a fatty acid methyl ester (FAME) blend of 37 components were acquired from ANPEL Laboratory Technologies Inc. (Shanghai, China).

2.2. Oil purification

The oils were purified following the method by Shimajiri, Shiota, Hosokawa, and Miyashita (2013) with some modifications. A chromatographic column (50 × 4 cm i.d.) was packed sequentially with activated clay (20 g) and activated carbon (30 g). An amount of 50 g oil was passed through the column with n-hexane (1000 mL) for removing tocopherols and most pigments. Then the oil was further purified using a column (50 × 4 cm i.d.) packed with silica gel (200 g) and aluminum oxide (40 g). n-Hexane (500 mL) was used for elution followed by a mixture of n-hexane:chloroform (1:4, v/v). After 200 mL of this mixed solvent was eluted, other 1000 mL of the mixed solvent was used for the collection of the oil TAG. The solvent was removed at 35 °C for 60 min in a rotary evaporator. Furthermore, a nitrogen stream was also applied for 2 h to remove the remaining solvent at room temperature.

2.3. Oxidation of samples

An amount of 120 g of purified oil was placed into a wide-mouth brown glass bottle (1000 mL) and then stored in an oven at 60 °C. Aliquots of 20 g sample were taken out periodically. The sampling frequency depended on the oil stability. Thus the camellia and sunflower oils were sampled at 0.5, 1, 2, 4 and 6 days, linseed oil at 0.5, 1, 2 and 3 days, and algae oil at 0.5, 1, 1.5 and 2 days of storage. The samples were coded using letters, one per each type of oil, followed by a number representing the order of sampling. Zero was given to the fresh samples. As to DHA, 350 mg of this standard substance was weighed into a 10-mL brown glass bottle and also oxidized in the oven at 60 °C for 2 days. All samples were protected with nitrogen and preserved at −20 °C for not more than 1 day until analyses.

2.4. Peroxide value and anisidine value

The determinations of peroxide value and anisidine value were performed according to Wen et al. (2019).

2.5. Fatty acid composition analysis

The preparations of fatty acid methyl esters (FAMEs) were conducted according to Zhang et al. (2019) with some modification. About 20 mg of sample was weighed onto a tube and 10% H_2SO_4 in methanol was added. Then, the tube was filled with nitrogen and heated at 90 °C for 90 min applying shaking every 20 min. After cooling, 1 mL of n-hexane was added to extract the FAMEs. According to the method by Menegazzo, Petenuci, and Fonseca (2014), the FAMEs were analyzed by GC-MS using an Agilent 7890a chromatograph equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.20 μm). The initial temperature was set at 80 °C, then it was increased to 200 °C at 20 °C/min, and then to 280 °C at 5 °C/min. Finally, the temperature was increased to 300 °C at 10 °C/min and hold at 300 °C for 5 min. The mass spectrometer was operated in EI mode at 70 eV. The temperature of the ion source was set at 250 °C and the filament current was 25 μA.

2.6. Sensory assessment

The sensory evaluation was conducted by ten assessors (8 female/2 male, aged between 22 and 45 years) recruited from the Ocean University of China (Qingdao, China). Intensive training for accurately recognizing the aroma of samples was performed for a week before the evaluation. The flavor vocabulary and attributes of oils were referred to a previous study (Hammer & Schieberle, 2013). The attributes included fishy, frying, metallic, rancid, grassy and painty. The specific descriptions of each attribute as well as their references were selected according to the standard practice for sensory evaluation of edible oils and fats (E18, 2012). Detailed information is presented in Table 1S. About 1 g oil was weighed in 50 mL covered glass vessels and the sensory analyses were carried out in a sensory panel room kept at 21 ± 1 °C. The intensity of each odor attribute was evaluated on a 0–10 point scale with 0.5 steps. The flavor intensity represented by the score was: 0-not perceptible, 2-very slightly perceptible, 4-slightly perceptible, 6-considerably perceptible, 8-strongly perceptible and 10-very strongly perceptible. Every sample was tested three times by each panelist and a 1-min break was required between each test for sensory recovery.

2.7. HS-GC-IMS analyses of volatiles

Volatile analyses were performed on a GC-IMS system manufactured by Gesellschaft für Analytische Sensorsysteme mbH (G.A.S., Dortmund, Germany) according to Guo et al. (Guo et al., 2018) with some modifications. The GC was equipped with an autosampler (CTC Analytics AG, Zwingen, Switzerland) with a headspace sampling unit and a 1-mL gas-tight syringe (Gerstel GmbH, Mühlheim, Germany). An FS-SE-54-CB capillary column (15 m × 0.53 mm) was used for odorant separation. An amount of 1 g sample was transferred into a 20-mL vial and incubated at 40 °C for 30 min. After incubation, 0.5 mL of headspace was subsequently injected into the injector (80 °C) in splitless mode by a syringe heated at 50 °C. The analyses were run at 45 °C (isothermal conditions) using nitrogen (99.99% purity) at a programmed flow as follows: 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, and 100 mL/min for 5 min. The analytes were firstly separated onto the capillary column and then ionized in the IMS ionization chamber by a 3H ionization source (300 MBq activity) in a positive ion mode. The drift tube (9.8 cm) worked at a constant voltage (5 kV) at 45 °C with a nitrogen flow of 150 mL/min. For avoiding cross contamination, the syringe was automatically flushed with nitrogen (150 mL/min) for 0.5 min before and 3 min after each analysis.

2.8. Recombination of oxidized DHA and oxidized purified sunflower oil

The oxidized DHA and oxidized algae oil showed differences in odor. To explore these differences, oxidized DHA was recombined with oxidized sunflower oil and the recombinant was compared to oxidized algae oil. Specifically, 150 mg oxidized DHA was weighed into a brown glass bottle and blended with different proportions of oxidized sunflower oil (B5). The amount of B5 was gradually increased until the odor of the recombined oil was similar to that of stored algae oil (D4). Finally, a total amount of 0.6 g of B5 was selected. Then, the recombined oil (referred to as DHA&B5) was used for analysis.

2.9. Triangle test

Triangle tests were used as a multi-purpose sensory test to identify one different sample out of three. This test was employed to determine overall sensory differences between two kinds of products. Two samples were identical and one was different. These three samples were coded with individual and random three-digit numbers and presented to the panelists at one time. The panelist was requested to identify the odd sample and each panelist had a 33% chance of guessing correctly. Discriminative sensory analysis was conducted at the Sensory Lab located at the department of Food Science and Technology of the Ocean University of China. A total of 16 untrained participants did the triangle test. They were given random coded samples, i.e. the recombinant oil and D4. Detailed codes for each sample, serving orders, results of this test and the *p*-value were all according to ISO:4120 (*Available online:* https://www.iso.org/standard/76666.html) (Table 2S).

2.10. E-nose analysis

An E-nose instrument (ISENSO INTELLIGENT, China) was used for the analysis of odorants in the recombination oil and D4 (algae oil) to determine differences in volatile compounds between the two oils. The intelligent bionic olfactory (E-nose) system comprised fourteen metal oxide semiconductors (MOS) of different chemical composition and thickness combined with pattern recognition algorithms (Kachele, Zhang, Gao, & Adhikari, 2017). The operating parameters applied were as indicated next: cleaning time-120s, sampling time-60s, gas flow-1 L/min, initial responding value of MOS-less than 1.0. The samples were placed into a 10-mL headspace extraction vial at room temperature during the sampling of the e-nose.

2.11. Statistical analysis

Each oil was oxidized in a single bottle and only one aliquot was taken in each sampling. The oil samples were all analyzed in triplicate and results were provided as mean ± standard deviation (n = 3). For the aim of this study replication of the oxidation assays was found to be unnecessary. Apart from removal of impurities, the purification of oils was also useful to eliminate normal differences in quality between the oils. The recombination experiments were carried out using six replications. LAV software version 2.2.1 was used for HS-GC-IMS data collection and fingerprint drawing. The HS-GC-IMS data were first visually checked by color for filtering out abnormal samples. Then, the data were normalized by using the SIMCA software (Version 16.0, Umetrics AB, Umeå, Sweden), and the normalized data were scaled using the type of Par. Unsupervised principal component analysis (PCA) was used to discriminate the samples from different groups and supervised orthogonal partial least squares discriminant analysis (OPLS-DA) was applied to identify the substances that caused subtle differences in odor in the recombination experiments. The OPLS-DA was selected because it can screen out differential variables between groups more accurately than PLS-DA, especially when differences between groups are small and those within groups are large (Xu et al., 2020). SPSS 20.0 software (SPSS Inc, Chicago, IL, USA) was also used for the statistical analysis of experimental data and the mean values were compared applying one-way ANOVA, followed by Dunnett's test. The statistical tests were considered to be significant at *p*-values less than 0.05. Origin 2017 (Northampton, MA, USA) was used for Fig. drawing.

3. Results and discussion

3.1. Oxidation of purified oils

Four types of oils containing different fatty acid compositions, namely, camellia oil, sunflower oil, linseed oil and algae oil, were purified by column chromatography to obtain their TAGs. Then these were stored at 60 °C to different oxidation extents and characterized.

3.1.1. Peroxide value (POV) and p-anisidine value (AnV)

As expected by the differences in their fatty acid compositions (Tables 3S–6S), the four oils reached different oxidation extents at the end of the assay. Algae oil showed the fastest growth rate of POV, followed by linseed oil, sunflower oil and camellia oil. As an example, the POV of camellia oil and sunflower oil increased respectively to 5.1 and 34.1 mmol/kg on the sixth day of storage, whereas the linseed oil

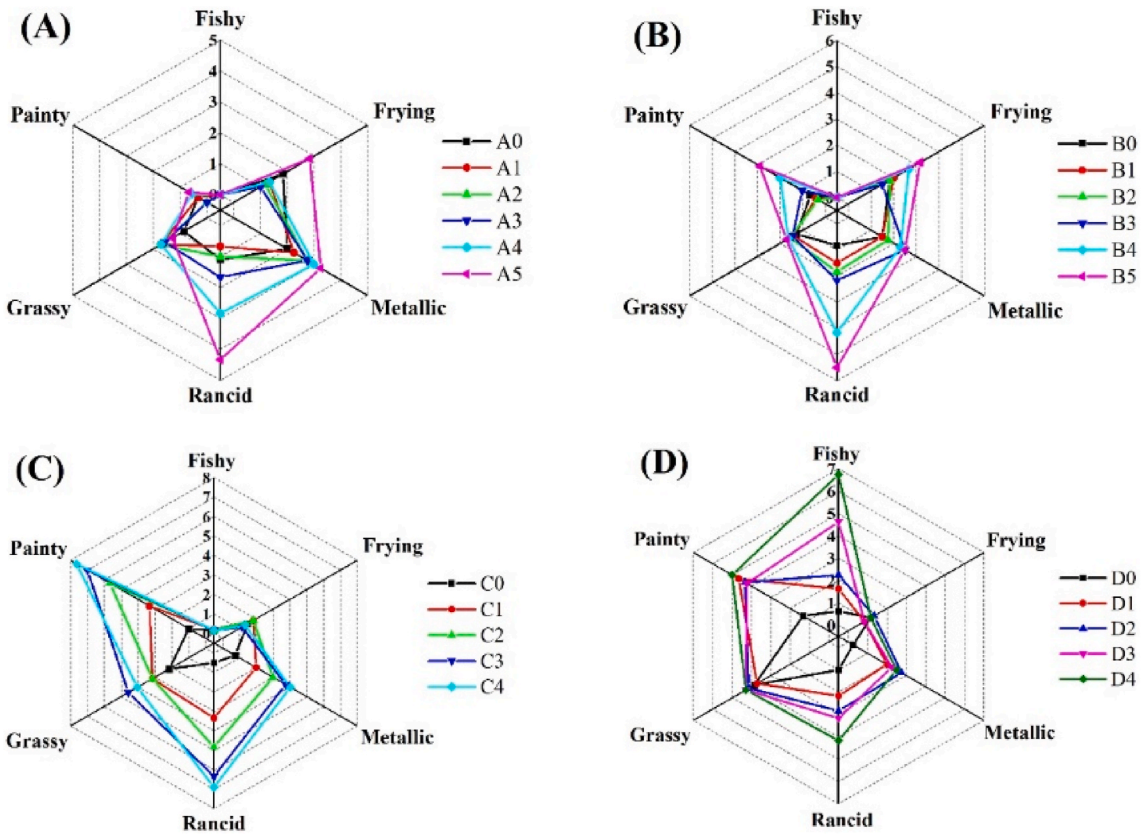

Fig. 1. Sensory odor profiles of camellia oil (**A**), sunflower oil (**B**), linseed oil (**C**) and algae oil (**D**) during storage at 60 °C. Numbers in sample codes refer to sampling order during the storage assay. The intensity values for each odor attribute were: 0-not perceptible, 2-very slightly perceptible, 4-slightly perceptible, 6-considerably perceptible, 8-strongly perceptible and 10-very strongly perceptible.

showed 134 mmol/kg on the third day and the algae oil 156 mmol/kg in 2 days of storage (Fig. 1S). Similarly, the AnV of camellia oil and sunflower oil increased slowly, whereas linseed oil and algae oil exhibited values as high as 25.9 and 101.7, respectively, after 2 days of incubation (Fig. 2S). Therefore, the purified algae oil presented the lowest oxidative stability followed by the linseed, sunflower and camellia oil. In a similar study on purified oils, namely, fish, echium, linseed and soybean oil, results showed that the fish oil was the least stable oil, followed by echium, linseed and soybean oil (Shibata, Uemura, Hosokawa, & Miyashita, 2015). As reported elsewhere, DHA has shown the greatest oxidative susceptibility among typical PUFAs, followed by EPA, α-linolenic acid and methyl linoleate (Miyashita, Uemura, & Hosokawa, 2018). DHA was the major PUFA in the algae oil (35.3%) of the present study, whereas α-linolenic acid was in linseed oil (22.1%). Therefore, the low oxidative stability of algae oil and linseed oil can be attributed to their respectively high contents of these two PUFAs.

3.1.2. Changes in fatty acid composition

Changes in the fatty acid composition during oxidation are given in Tables 3S–6S. Camellia oil showed very small but significant ($p < 0.05$) losses of C18:1ω9 and no significant changes were observed for C18:2ω6, which can be attributed to its very low global oxidation extent at the end of the oxidation assay as shown by the low POV (5.1 mmol/kg oil). The sunflower oil exhibited significant losses of C18:1ω9, C18:2ω6 and C18:3ω3. The losses of C18:1ω9 and C18:2ω6 were comparable between each other and the very low content of C18:3ω3 eventually dropped below the detection limit. In linseed oil, C18:3ω3 decreased from 22.10% to 17.43%, whereas C18:1ω9 and C18:2ω6 changed respectively from 17.86% to 16.62% and from 33.49% to 31.40%, i.e. proportionally to a lower extent compared to C18:3ω3. As to the algae oil, DHA (C22:6ω3) significantly dropped from 35.31% to 31.61% and the low contents of C18:3ω3 and C20:5ω3 also decreased from 0.09% to 0.03% and from 0.29% to 0.19%, respectively. Changes observed in C18:1ω9 were not significant and significant losses of C18:2ω6 were only detected at the end of the oxidation assay. In summary, the oxidation of fatty acids was dominated by DHA in the algae oil and by α-linolenic acid in the linseed oil. The oxidation of the sunflower oil was dominated by oleic and linoleic acid, and linolenic acid also participated in oxidation. Therefore, concomitant oxidation of unsaturated fatty acids occurred in the oils. Even though differences in the oxidation rates between fatty acids are considerably high when tested separately, their relative oxidation in blends depends on their relative proportions. Oxidation of those more stable can be influenced by the oxidation products of the more reactive fatty acids (Morales, Marmesat, Dobarganes, Márquez-Ruiz, & Velasco, 2012).

3.1.3. Sensory evaluation and analysis of volatile substances

Sensory odor profiles of the oils with different storage time are depicted in Fig. 1. The camellia oil elicited rancid smell that gradually increased to a score of 4.3 after 6 days of storage and therefore it was certainly perceptible. In addition, light frying and metallic odors with scores of 2.8 and 3.3, respectively, were also detected. The sunflower oil showed clear rancid smell (score of 5.5) after 6 days of storage. Unlike the camellia oil, a painty smell was also perceived (score of 2.9) at the end of the assay. The linseed oil, which was stored for only 3 days, presented strong painty smell with a score of 7.6. Additionally, distinct odor of rancid and grassy were also detected, but not fishy. The smell of algae oil was unacceptable after 2 days of storage and the fishy smell was the most prominent. The score of the fishy smell increased rapidly from 0.63 to 6.75. At the same time, painty, grassy and rancid smells were clearly perceived, reaching values of 5.00, 4.29 and 4.17, respectively. Therefore, the marked differences in the fatty acid compositions of the

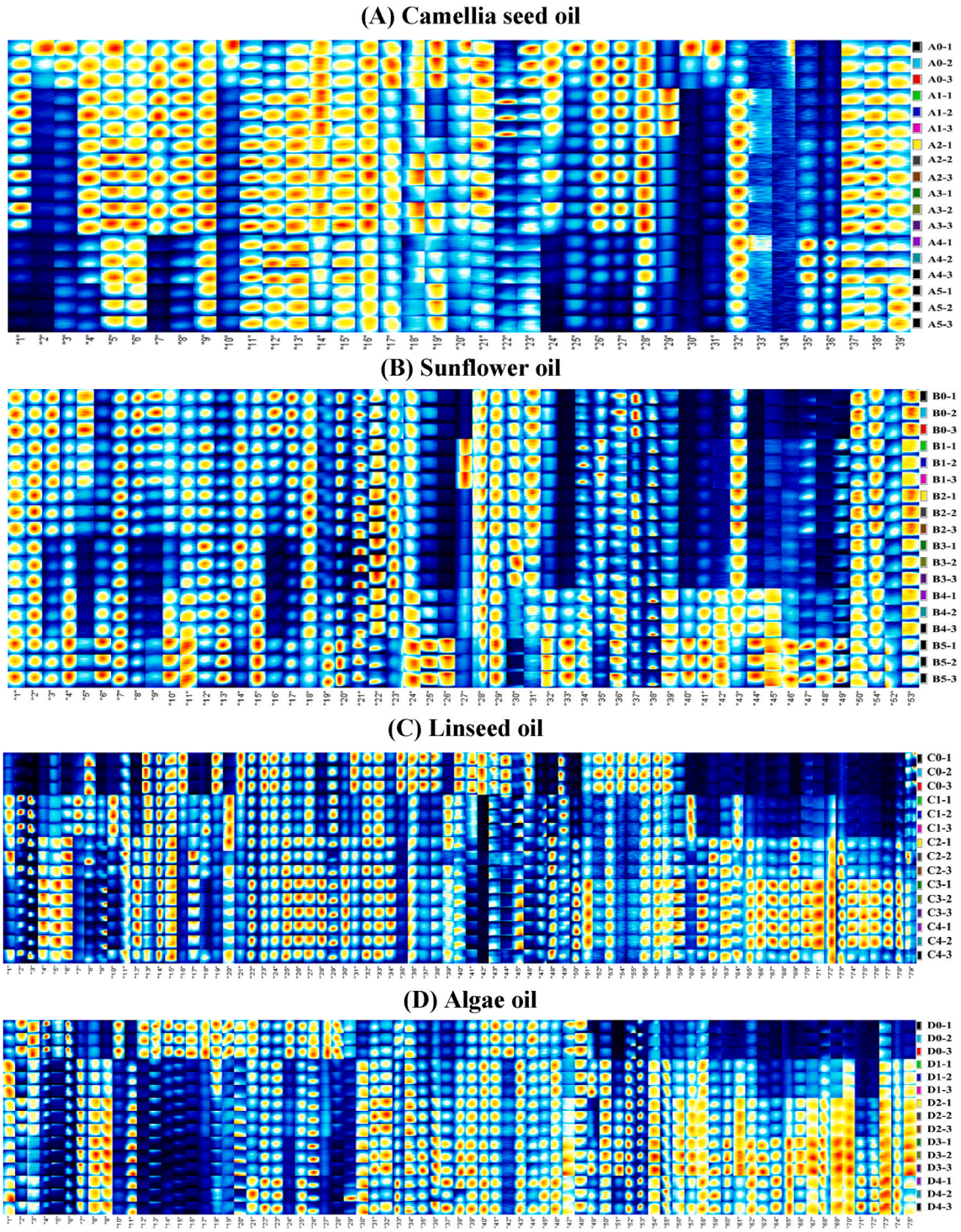

Fig. 2. HS-GC-IMS fingerprints of volatile substances in the oils with different storage periods. Analyses were performed in triplicate. Numbers in sample codes refer to sampling order during the storage assay followed by replication order.

four oils gave rise to different odor deterioration rates and odor profiles that changed with the incubation time.

The volatile substances in the four oils were detected along the oxidation time using HS-GC-IMS. The fingerprints of volatile substances are shown in Fig. 2. Each point represents a volatile compound and color provides information on the content of the volatile in such a way that different colors express different contents. During the oxidation of camellia oil and sunflower oil, 39 and 53 compounds were detected, respectively. In a recent report 30 volatile compounds have been found in camellia oil and hydrocarbons and aldehydes were the predominant compounds detected (He, Wu, Zhou, & Chen, 2021). Linseed oil and algae oil exhibited as many as 79 and 75 volatiles, respectively. The volatile compounds of algae oil showed large changes in the type and content during the storage (Fig. 2D). For example, the concentrations of No. 71 and 72 compounds gradually increased during storage, while the concentrations of No. 51 and 63 substances first increased and then decreased, which may be due to a decomposition that was greater than formation in late storage.

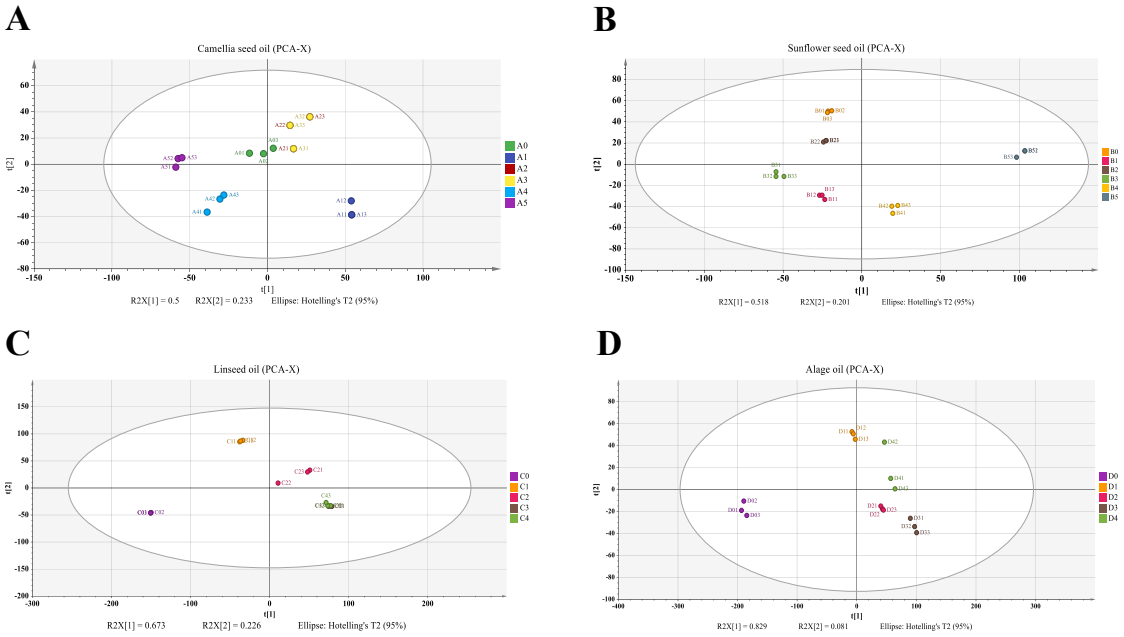

Fig. 3. Score plots of the PCA of volatile compounds in the oils with different storage periods. Numbers in sample codes refer to sampling order during the storage assay followed by replication order.

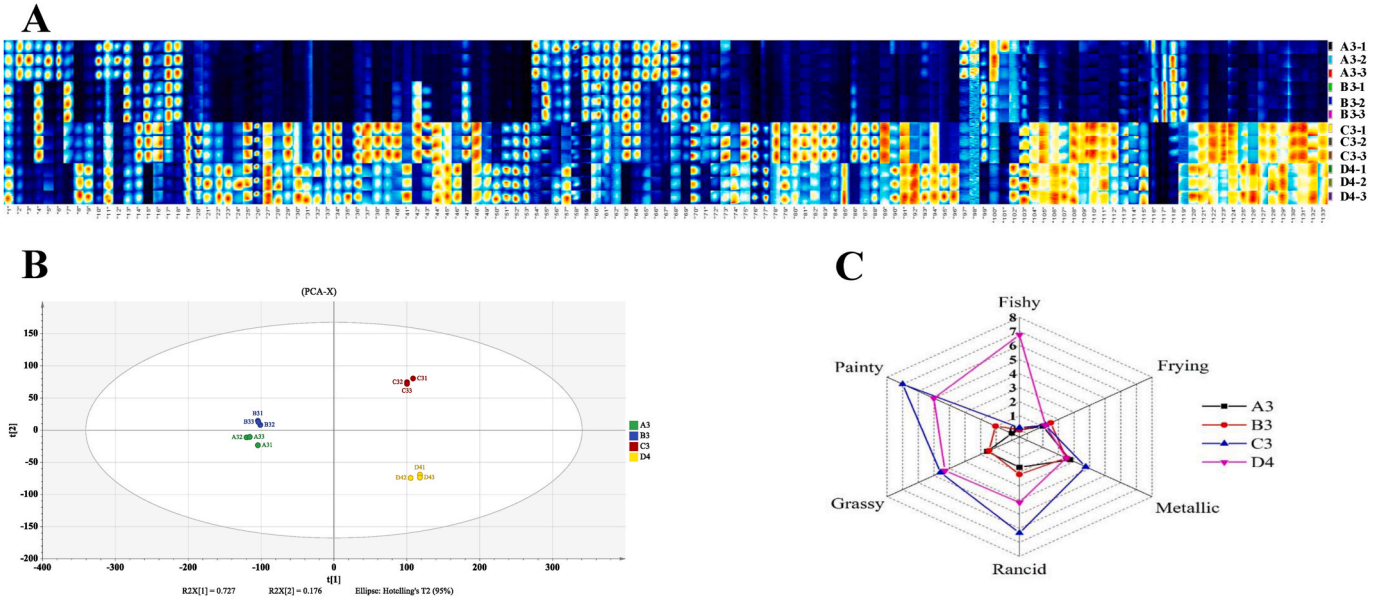

Fig. 4. Comparison of camellia oil (A3), sunflower oil (B3), linseed oil (C3) and algae oil (D3) stored for 2 days at 60 °C. HS-GC-IMS fingerprints of volatile substances (**A**), score plots of the PCA (**B**) and sensory odor profiles (**C**). Numbers in sample codes refer to sampling order during the storage assay followed by replication order.

An unsupervised PCA model was applied to identify similarities in the underlying patterns in the data. The number of dimensions could be reduced in the PCA model, and then similarities between different classes could be primary assessed (Xu et al., 2020). The PCA analysis of the four oils at different storage stages is shown in Fig. 3. The D0 and D4 samples in the algae oil were clearly separated between each other, indicating that their volatile compositions were quite different (Fig. 3D). On the contrary, the D2 and D3 samples were close between each other and therefore showed similarities. From Figs. 2 and 3 it can be drawn that the type and contents of the flavor compounds changed during storage providing differences in odor along storage in each oil.

3.2. Sensory profile and volatile compounds of the oils with the same storage time

In order to compare the sensory profile and volatile compounds of the four oils with the same storage time, samples stored for 2 days were selected (A3, B3, C3 and D4). The fingerprints of the volatile substances of these samples included 133 compounds (Fig. 4A), which was larger than the number of volatiles in the four oils in Fig. 2. This fact was owing to the large differences shown by A3 and B3 compared to C3 and D4. As it can be observed, C3 and D4 presented large differences in the concentrations of a number of volatiles. PCA also provided clear differences

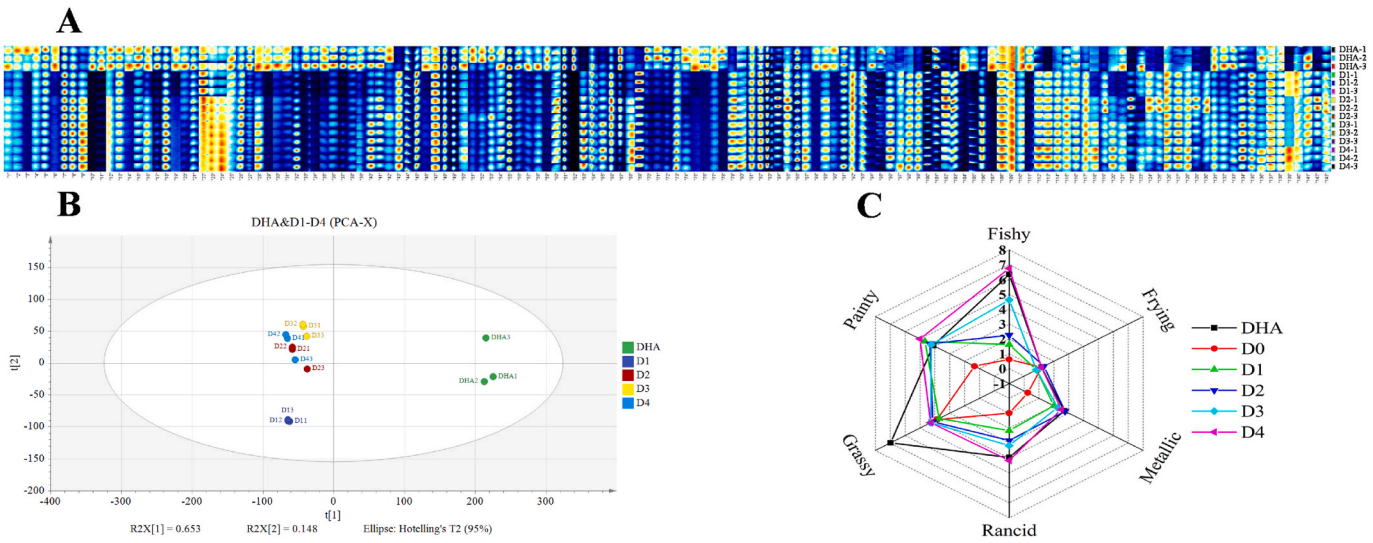

Fig. 5. Comparison between oxidized DHA (DHA) and algae oil stored at 60 °C for different periods (from D0 to D4). HS-GC-IMS fingerprints of volatile substances (**A**), score plots of the PCA (**B**) and sensory odor profiles (**C**). Numbers in sample codes refer to sampling order during the storage assay followed by replication order.

in the volatiles between the four oils (Fig. 4B). The differences between A3 and B3 were small, while large differences were found between C3 (linseed oil TAG) and D4 (algae oil TAG). As shown in Fig. 4C, the fishy smell of D4 was by far much more intense compared to the other three samples. When compared to D4, C3 had the stronger smells of painty and rancid. Therefore, the volatile substances of the four samples stored for the same time were different and so were their flavors. As Shibata et al. (2015) reported, differences in the fatty acid composition of TAG model systems resulted in considerable differences in secondary oxidation products (volatile compounds). The number of bis-allylic sites in a PUFA determines its reactivity and that of its hydroperoxides in such a way that the more the bis-allylic sites, the more number of secondary products will form (Miyashita et al., 2018; Wen et al., 2019). In summary, after 2 days of storage, the camellia oil and sunflower oil showed small flavor deterioration, while the linseed oil showed a strong odor of painty and rancid, and the algae oil exhibited a strong smell of fishy and painty.

3.3. Evaluation of sensory profile and volatile compounds of oxidized DHA and stored algae oil

As outlined above, the fatty acid composition of stored samples had an important effect on its odor profile. In order to explore the effect of DHA oxidation on the odor of algae oil, the volatile compounds of oxidized DHA and algae oil were analyzed by HS-GC-IMS and PCA was applied to analyze differences (Fig. 5). As shown by both analyses, the volatile compounds of DHA and algae oil exhibited large differences in the types and concentrations (Fig. 5A and B). Sensory data showed however similarities between DHA and D4 (Fig. 5C), i.e. the most oxidized algae oil sample, but with significant differences in the grassy odor, whose scores were 6.98 and 4.29 for DHA and D4, respectively. Therefore, the odor profiles of algae oil with different oxidation extents were all different from that of oxidized DHA. These results suggested that although oxidation of DHA was a key factor in the odor profile of algae oil, DHA alone was not able to form the characteristic off-flavor of algae oil. In this respect, Hammer and Schieberle (2013) studied the oxidation of DHA to reveal the compounds responsible for fishy odor. A set of odorants generated from autoxidized DHA elicited an intense fishy odor but a slight smell of green. Compared to the present study, these authors employed milder storage conditions (25 °C) that could have resulted in such a low oxidation of DHA to produce small amounts of the compounds responsible for the green odor.

3.4. Recombination of oxidized DHA and oxidized sunflower oil

The differences found in the odor profiles and volatile components between the DHA and algae samples suggested that oxidation of other fatty acids in the oil might be involved. In order to verify this hypothesis, oxidized DHA was recombined with oxidized sunflower oil to simulate

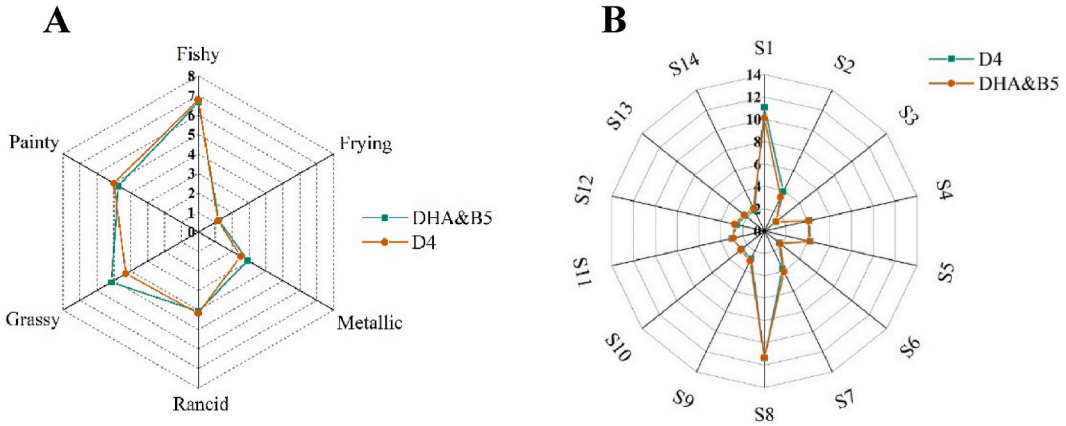

Fig. 6. Sensory odor profiles (**A**) and electronic nose analysis (**B**) of the recombination oil (DHA&B5) and algae oil stored for 2 days at 60 °C (D4).

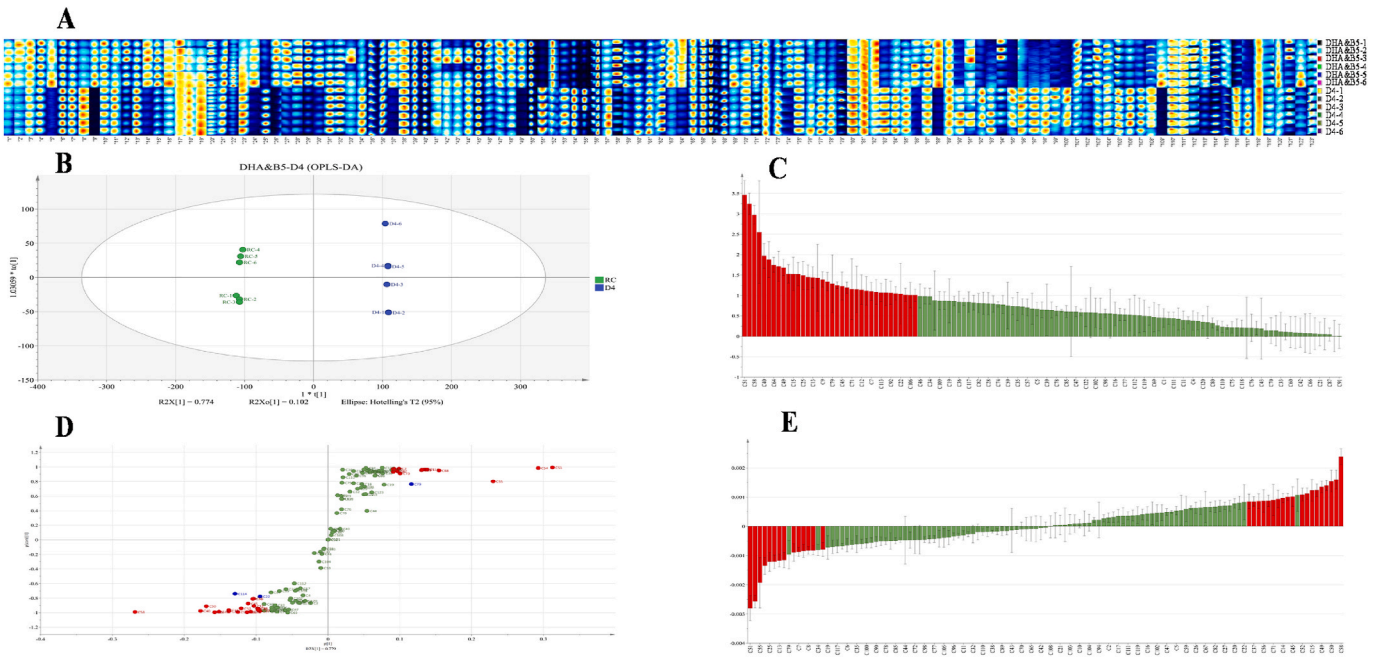

Fig. 7. HS-GC-MS fingerprints of the recombination oil (DHA&B5) and oxidized algae oil (D4) (**A**). Score plots of the OPLS-DA model ($R^2X = 0.779$, $R^2Y = 0.994$, $Q^2 = 0.987$) (**B**). Variable importance in the projection (VIP) plot of the independent OPLS-DA model (**C**). S-plot of the OPLS-DA model (**D**). Loading plot with jack-knifed confidence intervals (**E**).

the odor profile of algae oil. The sunflower oil was selected because the fatty acid composition of the recombination oil was similar to that of algae oil. Due to the differences in flavor found between oxidized DHA and oxidized sunflower oil, their combination could also help determine the role of oxidized DHA in the recombination oil. B4 and B5 samples were chosen for the recombination. Results showed that the recombination with B5 resulted in a more similar odor profile to that of oxidized algae oil (data not shown). Therefore, B5 was the oil selected and the recombination oil (DHA&B5) was compared to the more oxidized algae sample (D4). Sensory analysis showed quite similar odor profiles, with very slight differences in the grassy intensity (Fig. 6A). When compared to DHA, the grassy score of the recombination oil was lower (5.15 versus 6.98), whereas the fishy and painty odors were slightly enhanced. These results suggested synergistic effects between odorants of the two combined samples, i.e. DHA-derived volatiles and odor compounds produced from the fatty acids of sunflower oil.

Flavor reconstitution experiments can be considered a useful approach to determine the volatiles responsible for flavor in foods. Utz et al. (2021) successfully constructed the odor of dairy model systems with eight flavor-active compounds through aroma recombination. In addition, ten key aroma-active compounds identified in black garlic were recombined to successfully simulate its aroma profile (Yang, Song, Wang, & Jing, 2019). Similarly, Xu et al. (2021) were also successful in simulating the odor of French fries and frying soybean oil with key aroma-active compounds using aroma recombination experiments. The recombination of compounds presenting different odors can produce the odor of a given sample through a synthetic effect of odorants. Besides, a masking effect between odor compounds can also be involved. In this regard, camelina oil has shown a masking effect on the 'fishy' odor when blending with fish oils (Eidhin & O'Beirne, 2010). Therefore, the results of the present study suggested that the mixture of odorants in oxidized DHA and oxidized sunflower oil may have produced a synthetic effect. For determining which chemicals are responsible for fishy malodors, Marsili and Laskonis (2014) recombined heptanal and (*E*,*Z*)-3,5-octadien-2-one, showing that odorant synergy effects of these two compounds caused the fishy malodors in algae oils. A defined ratio of *trans*-4,5-epoxy-(*E*,*Z*)-2,7-decadienal, (*Z*)-1,5-octadien-3-one, (*Z*)-3-hexenal, (*Z*,*Z*)-2,5-octadienal, (*Z*,*Z*)-3,6-nonadienal, and (*E*,*E*,*Z*)-2,4,6-nonatrienal has been reported to be necessary to generate the fishy off-flavor in standard PUFAs, which might also be related to odorant synergistic effects (Hammer & Schieberle, 2013).

In order to further verify that the smell of the recombination oil was similar to that of oxidized algae oil (D4), a triangle test and an electronic nose analysis were performed. Results showed that only 5 out of 16 panelists provided correct answers ($p > 0.05$) in the triangle test (Roessler, Pangborn, Sidel, & Stone, 1978), indicating that the recombined oil did not have an aroma significantly different from that of D4 (Table 2S). Accordingly, differences between the recombined oil and D4 could not be distinguished, verifying the smell similarity between the two oils. Fig. 6B illustrates the results of the electronic nose analysis. Fourteen metal oxide semiconductors (MOS) could provide selectivity towards 14 kinds of volatile compounds. The recombination oil and D4 showed similarities in all of these 14 kinds of volatiles. Overall, these results also indicated that synergistic effects of odorants might have occurred between the odorants of oxidized DHA and oxidized sunflower oil to produce a similar odor profile to that of algae oil.

3.5. *Identification of differential volatile compounds between the recombination oil and oxidized algae oil (D4)*

Although the odor of the recombination oil was similar to that of algae oil (D4), small differences in the volatile compounds were observed (Fig. 7A and B). Therefore, it was necessary to explore the compounds that might have been involved in the subtle differences in the odor profiles. An orthogonal partial least squares discriminant analysis (OPLS-DA) was applied. This is a supervised chemometric method for data mining, which could overcome the limitations of factors such as environment and systematic errors affecting data accuracy in actual operation. OPLS-DA provides good discrimination ability for distinguishing different groups, especially those with large intergroup differences (Xu et al., 2020). The score plot of the OPLS-DA model applied in the present study is shown in Fig. 7B, where the R^2X, R^2Y and Q^2 values were 0.779, 0.994, and 0.987, respectively, representing high fit goodness and prediction ability. The robustness of the OPLS-DA

Table 1
Information on the identified differential volatile compounds between the recombined oil and D4.

Compound	CAS	Formula	MW [a]	RI [b]	RT [c]	DT [d]	Odor type	Odor threshold [e] (mg/kg) (medium)	Fold change [f]
Methyl decanoate	C110-42-9	$C_{11}H_{22}O_2$	186.3	1562	1138.23	1.565	Winey, oily, fruity	0.0043–0.0088 (water)	0.25
Propanoic acid ethyl ester	C105-37-3	$C_5H_{10}O_2$	102.1	980.4	168.50	1.4544	Fruity	0.1 (deodorized olive oil)	0.32
2-Ethylfuran	C3208-16-0	C_6H_8O	96.1	960	159.02	1.0432	Chemical	8 (cottonseed oil)	5.06
2-Methylpropanal	C78-84-2	C_4H_8O	72.1	817.2	106.502	1.3581	Herbal, green, malty	0.043 (vegetable oil)	3.31
Ethyl heptanoate	C106-30-9	$C_9H_{18}O_2$	158.2	1099	261.98	1.4038	Fruity	0.002 (water)	0.39

[a] Molecular weight.
[b] Retention index.
[c] Retention time from GC-IMS.
[d] Drift time from GC-IMS.
[e] Taken from van Gemert (2011).
[f] Taken from OPLS-DA analysis.

model was evaluated using permutation tests. The results were shown in Fig. 3S. The R^2 and Q^2 of 200 times permutation tests were 0.052 and −0.514, respectively. According to Qu et al. (2018) and Song et al. (2021), the negative value obtained for Q^2 (−0.514) was indicative of sufficient ruggedness. Differential volatile compounds were identified from results of Fig. 7C, D and E that show the filtration parameters. According to other studies (Jiang et al., 2021; Song et al., 2021), values higher than 1.0 for the variable importance in the projection (VIP) of PLS-DA (Fig. 7C) and values higher than 0.8 for |p(corr)| in the S-plot (Fig. 7D) were selected. In addition, those samples with jack-knifed confidence intervals including zero were removed (Fig. 7E). Statistical significance was characterized by a fold change of 2.5 (ratio >2.5 or < 0.4) and $p < 0.05$ (Jiang et al., 2021; Song et al., 2021). With these selected parameters, 5 flavor compounds were finally filtered out. The information on the identified differential volatile compounds between the recombination oil and D4 is shown in Table 1. The fold change value represented a multiple of the difference in the volatile compound concentration between the two oils. The concentration of 2-ethylfuran showed the largest difference in concentration between the two oils. However, the odor threshold of this compound is high (8 mg/kg in cottonseed oil), leading to a small influence on the odor. Methyl decanoate and ethyl heptanoate also showed high values of fold change, but their odor thresholds have only been reported in water (van Gemert, 2011).

4. Conclusions

A recombination between oxidized samples of DHA and sunflower oil was adopted to simulate the odor profile of algae oil, which was the first time to study the formation mechanism of algae oil flavor from the perspective of fatty acid oxidation. The recombination oil showed a similar odor profile to that of oxidized algae oil and revealed that oxidation of DHA is key in the flavor deterioration of algae oil and that oxidation of oleic, linoleic and linolenic acid are also involved. The constructed off-flavor of algae oil was a consequence of a synthetic effect between odorants from oxidized DHA and oxidized oleic, linoleic and linolenic acid. The analytical approach of the present study has demonstrated its capability of identifying key volatiles and also differential volatile components between samples with similar odor profiles. Thus, it can be an essential tool in further studies aimed at improving the odor quality of algae oil by removing specific volatiles in a targeted fashion.

CRediT authorship contribution statement

Yun-Qi Wen: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Chang-Hu Xue:** Funding acquisition. **Hong-Wei Zhang:** Formal analysis. **Li-Li Xu:** Visualization. **Xiao-Han Wang:** Visualization. **Shi-Jie Bi:** Data curation. **Qian-Qian Xue:** Data curation. **Yong Xue:** Conceptualization. **Zhao-Jie Li:** Conceptualization. **Joaquín Velasco:** Writing – review & editing, Visualization, Supervision. **Xiao-Ming Jiang:** Conceptualization, Supervision, Project administration.

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.

Acknowledgements

This work was supported by grants from the National Key R&D Program of China (Grant No. 2018YFC0311201 and 2018YFC1406806).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113291.

References

Chang, H. J., & Lee, J. H. (2020). Emulsification and oxidation stabilities of DAG-rich algae oil-in-water emulsions prepared with the selected emulsifiers. *Journal of the Science of Food and Agriculture, 100*(1), 287–294. https://doi.org/10.1002/jsfa.10037

Chen, X. W., Chen, Y. J., Wang, J. M., Guo, J., Yin, S. W., & Yang, X. Q. (2016). Phytosterol structured algae oil nanoemulsions and powders: Improving antioxidant and flavor properties. *Food & Function, 7*(9), 3641–4118. https://doi.org/10.1039/c6fo00449k

E18, A. C.. (2012). *Standard practice for sensory evaluation of edible oils and fats*. ASTM International.

Eidhin, D. N., & O'Beirne, D. (2010). Oxidative stability and acceptability of camelina oil blended with selected fish oils. *European Journal of Lipid Science and Technology, 112*(8), 878–886. https://doi.org/10.1002/ejlt.200900243

van Gemert, L. J. (2011). *Odour Thresholds. Compilations of odour threshold values in air, water and other media* (2nd ed.). Oliemans Punter & Partners BV.

Güner, M., Yılmaz, E., & Yüceer, Y. (2019). Off-odor removal from fish oil by adsorbent treatment with selected metal-organic frameworks. *Flavour and Fragrance Journal, 34*(3), 163–174. https://doi.org/10.1002/ffj.3489

Guo, Y., Chen, D., Dong, Y., Ju, H., Wu, C., & Lin, S. (2018). Characteristic volatiles fingerprints and changes of volatile compounds in fresh and dried *Tricholoma matsutake* Singer by HS-GC-IMS and HS-SPME-GC-MS. *Journal of Chromatography B, 1099*, 46–55. https://doi.org/10.1016/j.jchromb.2018.09.011

Hammer, M., & Schieberle, P. (2013). Model studies on the key aroma compounds formed by an oxidative degradation of ω-3 fatty acids initiated by either copper(II) ions or lipoxygenase. *Journal of Agricultural and Food Chemistry, 61*(46), 10891–10900. https://doi.org/10.1021/jf403827p

He, J., Wu, X., Zhou, Y., & Chen, J. (2021). Effects of different preheat treatments on volatile compounds of camellia (*Camellia oleifera Abel.*) seed oil and formation mechanism of key aroma compounds. *Journal of Food Biochemistry, 45*(3), Article e13649. https://doi.org/10.1111/jfbc.13649

Jamshidi, A., Cao, H., Xiao, J., & Simal-Gandara, J. (2020). Advantages of techniques to fortify food products with the benefits of fish oil. *Food Research International, 137*, Article 109353. https://doi.org/10.1016/j.foodres.2020.109353

Jiang, B., Hu, L., Zhang, X., Zhang, H., Zhang, F., Chen, L., … Jiang, X. (2021). Uncovering proteome variations of differently heat-treated sea cucumber (*Apostichopus japonicus*) by label-free mass spectrometry. *Food Chemistry, 344*, Article 128575. https://doi.org/10.1016/j.foodchem.2020.128575

Kachele, R., Zhang, M., Gao, Z., & Adhikari, B. (2017). Effect of vacuum packaging on the shelf-life of silver carp (*Hypophthalmichthys molitrix*) fillets stored at 4 °C. *LWT-Food Science and Technology, 80*, 163–168. https://doi.org/10.1016/j.lwt.2017.02.012

Karahadian, C., & Lindsay, R. C. (1989). Evaluation of compounds contributing characterizing fishy flavors in fish oils. *Journal of the American Oil Chemists' Society, 66*(7), 953–960. https://doi.org/10.1007/BF02682616

Li, P., Peng, Y., Mei, J., & Xie, J. (2020). Effects of microencapsulated eugenol emulsions on microbiological, chemical and organoleptic qualities of farmed Japanese sea bass (*Lateolabrax japonicus*) during cold storage. *LWT-Food Science and Technology, 118*, Article 108831. https://doi.org/10.1016/j.lwt.2019.108831

Lundén, A., Gustafsson, V., Imhof, M., Gauch, R., & Bosset, J. O. (2002). High trimethylamine concentration in milk from cows on standard diets is expressed as fishy off-flavour. *Journal of Dairy Research, 69*(3), 383–390. https://doi.org/10.1017/S002202990200568X

Marsili, R. T., & Laskonis, C. R. (2014). Odorant synergy effects as the cause of fishy malodors in algal marine oils. *Journal of Agricultural and Food Chemistry, 62*(40), 9676–9682. https://doi.org/10.1021/jf502252q

Marsol-Vall, A., Aitta, E., Guo, Z., & Yang, B. (2021). Green technologies for production of oils rich in n-3 polyunsaturated fatty acids from aquatic sources. *Critical Reviews in Food Science and Nutrition*, 1–21. https://doi.org/10.1080/10408398.2020.1861426

Menegazzo, M. L., Petenuci, M. E., & Fonseca, G. G. (2014). Production and characterization of crude and refined oils obtained from the co-products of Nile tilapia and hybrid sorubim processing. *Food Chemistry, 157*, 100–104. https://doi.org/10.1016/j.foodchem.2014.01.121

Milo, C., & Grosch, W. (1993). Changes in the odorants of boiled trout (*Salmo fario*) as affected by the storage of the raw material. *Journal of Agricultural and Food Chemistry, 41*(11), 2076–2081. https://doi.org/10.1021/jf00035a048

Miyashita, K., Uemura, M., & Hosokawa, M. (2018). Effective prevention of oxidative deterioration of fish oil: Focus on flavor deterioration. *Annual Review of Food Science and Technology, 9*, 209–226. https://doi.org/10.1146/annurev-food-030117-012320

Morales, A., Marmesat, S., Dobarganes, M. C., Márquez-Ruiz, G., & Velasco, J. (2012). formation of hydroperoxy-, keto- and hydroxy-dienes in FAME from oils: Influence of temperature and addition of α-tocopherol. *Journal of the American Oil Chemists' Society, 89*, 675–684. https://doi.org/10.1007/s11746-011-1961-2

Parlapani, F. F., Verdos, G. I., Haroutounian, S. A., & Boziaris, I. S. (2015). The dynamics of *Pseudomonas* and volatilome during the spoilage of gutted sea bream stored at 2 °C. *Food Control, 55*, 257–265. https://doi.org/10.1016/j.foodcont.2015.03.004

Qu, W., Nie, C., Zhao, J., Ou, X., Zhang, Y., Yang, S., et al. (2018). Microbiome-metabolomics analysis of the impacts of long-term dietary advanced-glycation-end-product consumption on C57BL/6 mouse fecal microbiota and metabolites. *Journal of Agricultural and Food Chemistry, 66*(33), 8864–8875. https://doi.org/10.1021/acs.jafc.8b01466

Roessler, E., Pangborn, R., Sidel, J., & Stone, H. (1978). Expanded statistical tables for estimating significance in paired—preference, paired–difference, duo–trio and triangle tests. *Journal of Food Science, 43*(3), 940–943. https://doi.org/10.1111/j.1365-2621.1978.tb02458.x

Ryckebosch, E., Bruneel, C., Termote-Verhalle, R., Lemahieu, C., Muylaert, K., Van Durme, J., … Foubert, I. (2013). Stability of omega-3 LC-PUFA-rich photoautotrophic microalgal oils compared to commercially available omega-3 LC-PUFA oils. *Journal of Agricultural and Food Chemistry, 61*(42), 10145–10155. https://doi.org/10.1021/jf402296s

Shibata, A., Uemura, M., Hosokawa, M., & Miyashita, K. (2015). formation of acrolein in the autoxidation of triacylglycerols with different fatty acid compositions. *Journal of the American Oil Chemists' Society, 92*(11–12), 1661–1670. https://doi.org/10.1007/s11746-015-2732-2

Shimajiri, J., Shiota, M., Hosokawa, M., & Miyashita, K. (2013). Synergistic antioxidant activity of milk sphingomyeline and its sphingoid base with α-tocopherol on fish oil triacylglycerol. *Journal of Agricultural and Food Chemistry, 61*(33), 7969–7975. https://doi.org/10.1021/jf401788j

Song, X., Wang, G., Zhu, L., Zheng, F., Ji, J., Sun, J., … Sun, B. (2021). Comparison of two cooked vegetable aroma compounds, dimethyl disulfide and methional, in Chinese Baijiu by a sensory-guided approach and chemometrics. *LWT-Food Science and Technology, 146*, Article 111427. https://doi.org/10.1016/j.lwt.2021.111427

Utz, F., Kreissl, J., Stark, T. D., Schmid, C., Tanger, C., Kulozik, U., … Dawid, C. (2021). Sensomics-assisted flavor decoding of dairy model systems and flavor reconstitution experiments. *Journal of Agricultural and Food Chemistry, 69*(23), 6588–6600. https://doi.org/10.1021/acs.jafc.1c02165

Venkateshwarlu, G., Let, M. B., Meyer, A. S., & Jacobsen, C. (2004). Modeling the sensory impact of defined combinations of volatile lipidoxidation products on fishy and metallic off-flavors. *Journal of Agricultural and Food Chemistry, 52*(6), 1635–1641. https://doi.org/10.1021/jf0351321

Wen, Y. Q., Xue, C. H., Xu, L. L., Wang, X. H., Bi, S. J., Xue, Q. Q., … Jiang, X. M. (2019). Application of plackett-burman design in screening of natural antioxidants suitable for anchovy oil. *Antioxidants, 8*(12), 627–642. https://doi.org/10.3390/antiox8120627

Xue, C., You, J., Zhang, H., Xiong, S., Yin, T., & Huang, Q. (2021). Capacity of myofibrillar protein to adsorb characteristic fishy-odor compounds: Effects of concentration, temperature, ionic strength, pH and yeast glucan addition. *Food Chemistry, 363*, Article 130304. https://doi.org/10.1016/j.foodchem.2021.130304

Xu, L., Mei, X., Chang, J., Wu, G., Zhang, H., Jin, Q., et al. (2021). Comparative characterization of key odorants of French fries and oils at the break-in, optimum, and degrading frying stages. *Food Chemistry*. , Article 130581. https://doi.org/10.1016/j.foodchem.2021.130581

Xu, L. L., Zhang, H. W., Zhang, X. M., Lin, H., Guo, Y. M., Yu, C., … Li, Z. X. (2020). Natural shrimp (*Litopenaeus vannamei*) tropomyosin shows higher allergic properties than recombinant ones as compared through SWATH-MS-based proteomics and immunological response. *Journal of Agricultural and Food Chemistry, 68*(41), 11553–11567. https://doi.org/10.1021/acs.jafc.0c03840

Yang, P., Song, H., Wang, L., & Jing, H. (2019). Characterization of key aroma-active compounds in black garlic by sensory-directed flavor analysis. *Journal of Agricultural and Food Chemistry, 67*(28), 7926–7934. https://doi.org/10.1021/acs.jafc.9b03269

Zhang, L. Y., Ding, L., Shi, H. H., Xu, J., Xue, C. H., Zhang, T. T., et al. (2019). Eicosapentaenoic acid in the form of phospholipids exerts superior anti-atherosclerosis effects to its triglyceride form in ApoE(-/-) mice. *Food & Function, 10*(7), 4177–4188. https://doi.org/10.1039/c9fo00868c