

Effects of Xucai Oligosaccharides on Fatty Acid and Volatile Off-Flavor Compound Composition in Tilapia (Postprint)

Authors: Wang Runping, Lu Fengxia, Jin Min, Di Wenjie, He Xiongfei, Xu Yongjian, Chan Zhuhua

Date: 2018-12-20T00:00:00+00:00

Abstract

To investigate the effects of *Gracilaria lemaneiformis* oligosaccharides on fatty acid composition and volatile fishy odor compounds in tilapia, this study prepared *Gracilaria lemaneiformis* oligosaccharides via biodegradation and formulated an oligosaccharide-supplemented diet containing 1% *Gracilaria lemaneiformis* oligosaccharides added to a basal diet. Tilapia with an initial body weight of (12.20 ± 0.23) g were fed either the basal diet (control group) or the oligosaccharide-supplemented diet (*Gracilaria lemaneiformis* oligosaccharide group) for 7 months, with three replicates per dietary treatment and 20 fish per replicate. Gas chromatography-mass spectrometry (GC-MS) analysis identified a total of 28 fatty acids (ranging from C12 to C24) in tilapia abdominal muscle. Compared with the control group, the *Gracilaria lemaneiformis* oligosaccharide group exhibited a reduced diversity of saturated fatty acids (SFAs). Additionally, the relative contents of C20:5n-3 (EPA), C22:6n-3 (DHA), and C20:4n-6 (ARA), as well as the DHA/EPA ratio, were significantly elevated in the abdominal muscle of the oligosaccharide group ($P < 0.05$). Headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) detected 31 volatile fishy odor compounds in the control group and 25 in the *Gracilaria lemaneiformis* oligosaccharide group. Ketones, amines, and aldehydes—volatile fishy odor compounds characterized by strong odor intensity and low sensory thresholds—exhibited significantly reduced relative contents in the oligosaccharide group compared with the control group ($P < 0.05$). In conclusion, dietary supplementation with *Gracilaria lemaneiformis* oligosaccharides effectively decreased SFA diversity, increased the contents of ARA, DHA, and EPA in polyunsaturated fatty acids (PUFA) and the DHA/EPA ratio, reduced the variety of volatile fishy odor compounds, and lowered the relative contents of ketones, amines, and aldehydes in tilapia abdominal muscle.

Full Text

Effects of *Gracilaria lemaneiformis* Oligosaccharides on Fatty Acid and Volatile Odor Substance Compositions of Tilapia (*Oreochromis mossambicus*)

WANG Runping¹, LU Fengxia², JIN Min², DI Wenjie², HE Xiongfei³, XU Yongjian¹, CHAN Zhuhua^{2*}

¹School of Marine Sciences, Ningbo University, Ningbo 315211, China

²Third Institute of Oceanography, State Oceanic Administration, Xiamen 361005, China

³Xiamen Hopegene Bioinformatics Technology Co., Ltd., Xiamen 361000, China

Abstract

To investigate the effects of *Gracilaria lemaneiformis* oligosaccharides on the fatty acid and volatile odor substance compositions of tilapia (*Oreochromis mossambicus*), we prepared *G. lemaneiformis* oligosaccharides via biological degradation and formulated an oligosaccharide-supplemented diet by adding 1% *G. lemaneiformis* oligosaccharides to a basal diet. Tilapias with an initial body weight of (12.20±0.23) g were fed either the basal diet (control group) or the oligosaccharide-supplemented diet (oligosaccharide group) for 7 months. Each dietary treatment consisted of three replicates with 20 fish per replicate. Gas chromatography-mass spectrometry (GC-MS) analysis identified 28 fatty acids in tilapia belly meat, ranging from C12 to C24. Compared with the control group, the oligosaccharide group exhibited significantly reduced diversity of saturated fatty acids (SFAs). In terms of fatty acid content, the relative contents of C20:5n-3 [eicosapentaenoic acid (EPA)], C22:6n-3 [docosahexaenoic acid (DHA)], and C20:4n-6 [arachidonic acid (ARA)], as well as the DHA/EPA ratio, were significantly elevated in the oligosaccharide group (P<0.05). Headspace solid-phase microextraction coupled with GC-MS (HS-SPME-GC-MS) detected 31 volatile odor compounds in the control group and 25 in the oligosaccharide group. The predominant odor-active compounds with low sensory thresholds included ketones, amines, and aldehydes, all of which showed significantly reduced relative contents in the oligosaccharide group (P<0.05). These results demonstrate that dietary supplementation with *G. lemaneiformis* oligosaccharides effectively reduces SFA diversity while increasing the contents of ARA, DHA, and EPA, as well as the DHA/EPA ratio, in tilapia belly meat. Furthermore, it reduces both the variety and relative abundance of volatile odor compounds, particularly ketones, amines, and aldehydes.

Keywords: *Gracilaria lemaneiformis*; oligosaccharides; *Oreochromis mossambicus*; fatty acids; volatile odor substances

Tilapia (*Oreochromis mossambicus*), a tropical fish species native to Africa and the Middle East, is now widely cultivated in over 100 countries and regions worldwide. China introduced Mozambique tilapia from Southeast Asia in the early 1950s, and it has become one of the country's major processed and exported aquatic products, accounting for approximately 55% of global production [?]. Tilapia is highly valued for its nutritional quality, tender flesh, and affordability. With rising living standards, consumers increasingly demand higher fatty acid content and better flavor profiles in aquatic products, including tilapia. Unsaturated fatty acids, particularly n-3 polyunsaturated fatty acids (PUFAs), play crucial roles in immune regulation, cardiovascular health, anti-inflammatory responses, and anti-allergic functions [?].

However, some aquatic products develop unpleasant fishy odors that negatively affect consumer acceptance. Fish odor compounds are complex mixtures primarily composed of low-molecular-weight aldehydes, ketones, alcohols, and sulfur-containing volatile substances. Fishy odor formation involves multiple mechanisms: first, volatile organic compounds from the environment can adsorb onto fish surfaces during culture and storage through physical processes, intensifying inherent off-odors; second, under inappropriate conditions, microbial proliferation and enzymatic reactions in fish tissue can degrade precursor substances such as fatty acids, generating malodorous compounds [?].

Gracilaria lemaneiformis, a species of red algae, is rich in polysaccharides, proteins, cellulose, and minerals, making it a valuable resource for food, feed, and pharmaceutical applications. Agar degradation of *G. lemaneiformis* yields oligosaccharides composed of 2-10 monosaccharide units, including agaro-oligosaccharides and neoagaro-oligosaccharides. Agaro-oligosaccharides have 3,6-anhydro- α -L-galactose residues at their reducing ends, predominantly agarotriose, while neoagaro-oligosaccharides have α -D-galactose residues at their reducing ends, mainly neoagarobiose, neoagarotetraose, neoagarohexaose, and neoagaroctaose [?]. Previous studies have demonstrated that seaweed oligosaccharides possess antioxidant, antitumor, immunomodulatory, and anti-inflammatory activities, indicating significant development potential [?]. However, no reports have examined the effects of *G. lemaneiformis* oligosaccharides on the physiological characteristics of tilapia.

Therefore, this study prepared *G. lemaneiformis* oligosaccharides through biological degradation and investigated their effects on fatty acid composition and volatile odor substance profiles in tilapia using GC-MS and HS-SPME-GC-MS techniques, aiming to promote the application of *G. lemaneiformis* oligosaccharides in aquafeeds.

1.1 Experimental Materials and Instruments

Tilapia fry were purchased from Xiamen Nongjiafa Aquaculture Technology Co., Ltd. Basal feed was obtained from Xiamen Fuxing Biological Feed Co., Ltd. *Gracilaria lemaneiformis* was sourced from marine farms in Putian, Fujian.

Chromatography-grade methanol and fatty acid methyl ester standards were purchased from Sigma-Aldrich (USA). Oligosaccharide standards were obtained from Qingdao Bozhihuili Bio-Technology Co., Ltd. All other reagents were of analytical grade.

Instrumentation included a GCMS-QP2010 Plus gas chromatograph-mass spectrometer (Shimadzu, Japan), a 65 μ m PDMS/DVB SPME fiber (Supelco, USA), a PL4002 electronic balance (Mettler-Toledo, Shanghai), an XMTD-8222 thermostatic water bath (Shanghai Jinghong Experimental Equipment Co., Ltd.), a T10 high-speed homogenizer (IKA Group, Guangzhou), and a DIONEX ion chromatograph (Thermo Fisher Scientific, USA).

1.2 Preparation and Identification of *Gracilaria lemaneiformis* Oligosaccharides

Dried *G. lemaneiformis* powder was dissolved in seawater to prepare a 3% (w/v) degradation medium. *Flammeovirga pacifica* WPAGA1 bacteria in logarithmic growth phase (OD = 0.6–0.8) were inoculated into the medium at a 1:50 (v/v) ratio and cultured at 37°C with shaking at 200 rpm for 42 h [?]. After cultivation, the culture was centrifuged at 12,000 \times g for 20 min at 4°C. The supernatant was filtered through a 3 kDa membrane to remove water-soluble proteins and polysaccharides, then mixed with three volumes of absolute ethanol and stored at 4°C overnight for 8 h. Following centrifugation at 12,000 \times g for 10 min at 4°C, the supernatant was evaporated to remove ethanol and lyophilized to obtain *G. lemaneiformis* oligosaccharide powder.

Ion chromatography was used to identify the main components: oligosaccharide samples were dissolved in double-distilled water and separated on an IonPac column (250 mm \times 4 mm) using a mobile phase of 100 mmol/L NaOH and 150 mmol/L NaAc at a flow rate of 0.25 mL/min for 50 min. Eluted components were detected by conductivity and compared with oligosaccharide standards to determine the main constituents.

1.3 Tilapia Feeding Trial Design

Oligosaccharide-supplemented diet preparation: Basal freshwater fish pellet feed was ground and passed through an 80-mesh sieve. *G. lemaneiformis* oligosaccharide powder was added at 1% (w/w), thoroughly mixed, hydrated with appropriate water, formed into strips, pelleted, dried at 40°C, cooled, and stored in sealed bags.

Control diet: Basal feed was ground and sieved without any additives, then processed identically to produce pellets of uniform size.

Culture management: The experiment was conducted in concrete tanks (1 m \times 1 m) disinfected with quicklime and potassium permanganate before use. After acclimation on basal feed for 3 months, healthy tilapias with uniform size (initial weight: 12.20 \pm 0.23 g) were randomly allocated to two groups (three

replicates per group, 20 fish per replicate). Culture conditions included water depth of 80–100 cm, temperature of $29\pm 2^{\circ}\text{C}$, pH of 7.5 ± 0.1 , continuous aeration, and natural photoperiod. Fish were fed the control or oligosaccharide-supplemented diet at 4% body weight daily, divided into two feedings at 09:00 and 17:00. Uneaten feed and feces were removed 1 h after feeding. One-third of the water volume was replaced every 3 days, with complete water exchange weekly to maintain water quality. The feeding trial lasted 7 months.

1.4 Fatty Acid Composition Analysis

Total lipid extraction and methylation [?]: At the end of the trial, six tilapias per group were sacrificed and 8 g of fresh belly meat was collected on ice. After washing with phosphate-buffered saline (PBS) and mincing, samples were homogenized in 300 mL of chloroform-methanol (2:1, v/v) and sonicated twice on ice for 30 min each, followed by 24 h extraction. The mixture was filtered, and the chloroform layer was collected in a separatory funnel and concentrated by vacuum centrifugation to obtain total fatty acids. For methylation, 10 mg of fatty acid sample was dissolved in 1 mL of 10% sulfuric acid-methanol solution and incubated at 60°C for 15 min. After cooling, 1 mL of n-hexane was added, vortexed, and the upper layer was collected for GC-MS analysis.

GC-MS conditions [?]: DB-Wax capillary column ($60\text{ m} \times 0.25\text{ mm} \times 0.25\text{ m}$); helium carrier gas at 1.0 mL/min; constant pressure of 40 kPa. Injector and detector temperatures were 250°C . Oven temperature program: initial 50°C held for 1 min, ramped to 180°C at $20^{\circ}\text{C}/\text{min}$, then to 230°C at $3^{\circ}\text{C}/\text{min}$, held for 15 min. GC/MS interface and ion source temperatures were 250°C and 230°C , respectively. Electron ionization (EI) energy was 70 eV, with mass scan range of m/z 50–500 and scan rate of 2 Hz.

1.5 Volatile Odor Substance Analysis

HS-SPME-GC-MS was used to detect volatile odor compounds in tilapia meat after different dietary treatments [?].

Sample preparation and solid-phase microextraction: Two grams of minced, washed belly meat was mixed with 8 mL of 0.1 g/mL NaCl solution, homogenized, and transferred to a 15 mL headspace vial. The SPME needle was inserted through the septum, and extraction was performed at 50°C for 30 min. The fiber was immediately desorbed in the GC injector at 250°C for 10 min.

GC-MS conditions: DB-5MS capillary column ($30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ m}$); helium carrier gas at 1.0 mL/min; splitless injection. Injector temperature was 250°C . Oven program: initial 40°C held for 3 min, ramped to 250°C at $8^{\circ}\text{C}/\text{min}$, held for 10 min. GC/MS interface and ion source temperatures were 270°C and 230°C , respectively. EI energy was 70 eV, with mass scan range of m/z 35–350 and scan rate of 2 Hz.

1.6 Statistical Analysis

Mass spectral data were automatically searched against the NIST05 and Wiley libraries using Shimadzu software. Only compounds with similarity scores >80% were reported. Relative contents were determined by peak area normalization [?]. Data were organized using Excel 2007 and analyzed using SPSS 22.0. Independent-sample t-tests [?] were used to compare component contents between treatments.

2.1 Preparation and Identification of *Gracilaria lemaneiformis* Oligosaccharides

Ion chromatography analysis revealed that the oligosaccharides prepared by microbial fermentation consisted primarily of neoagaro-oligosaccharides (neoagarobiose, neoagarotetraose, neoagarohexaose) and agaro-oligosaccharides (agarotriose) [Figure 1: see original paper]. The chromatographic peaks of the prepared oligosaccharides matched those of standard compounds [Figure 1: see original paper]-A, and their chemical structures are illustrated in [Figure 1: see original paper]-B.

2.2 Effects of *Gracilaria lemaneiformis* Oligosaccharides on Fatty Acid Composition

Tilapias were fed the basal diet supplemented with 1% *G. lemaneiformis* oligosaccharides for 7 months. GC-MS analysis of fatty acid profiles in belly meat is presented in .

A total of 28 fatty acids (C12-C24) were identified in both groups, with saturated fatty acids (SFAs) predominating, followed by polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs). The oligosaccharide group showed reduced SFA diversity (6 types) compared with the control group (12 types), specifically lacking C12:0, C13:0, C21:0, C22:0, C23:0, and C24:0. No differences were observed in unsaturated fatty acid diversity.

SFAs exhibited the highest relative content in both groups, primarily C16:0 followed by C18:0. The oligosaccharide group showed significantly lower C14:0 content ($P < 0.05$), though most other SFAs remained unaffected ($P > 0.05$). Notably, dietary supplementation significantly increased the relative contents of multiple PUFAs ($P < 0.05$), including n-3 PUFAs (EPA, DHA) and n-6 PUFA (ARA). The DHA/EPA ratio and ARA content were significantly elevated, while C18:2n-6 content was significantly reduced ($P < 0.05$).

2.3 Effects of *Gracilaria lemaneiformis* Oligosaccharides on Volatile Odor Substance Composition

HS-SPME-GC-MS detected 31 volatile odor compounds in the control group and 25 in the oligosaccharide group . The major components were alkanes, acids, and ketones, accounting for 64.5% of total detected compounds. Overall,

the oligosaccharide group showed significantly reduced relative contents of most volatile odor compounds ($P < 0.05$).

The control group contained 10 alkanes and 1 alkene, while the oligosaccharide group contained 8 alkanes and 1 alkene. 1-Chlorododecane was the most abundant alkane in both groups. Three major alkanes (dodecane, 2,6,10,15-tetramethylheptadecane, and tetracosane) showed significantly reduced relative contents in the oligosaccharide group ($P < 0.05$).

Six acid compounds were detected in the control group and five in the oligosaccharide group, with benzoic acid being the most abundant. Benzoic acid content was lower in the oligosaccharide group but not significantly ($P > 0.05$). Four ketones were identified in the control group and three in the oligosaccharide group. The most abundant ketone in the control group, 5-isopropyl-2,4-imidazolidinedione, was undetectable in the oligosaccharide group. Two other ketones (2,2,3-trimethyl-1-phenyl-3-butyldiene-1-one and 4,4,5,6-tetramethyl-1,3-oxadiazin-2-thione) showed significantly reduced relative contents ($P < 0.05$).

Additional volatile compounds included aldehydes, amines, phenols, and esters. Both groups contained two aldehydes (pentanal and heptadecyl aldehyde). Phenolic compounds (2,4-di-tert-butylphenol, 2,6-di-tert-butyl-4-propionylphenol) and aldehydes showed significantly lower relative contents in the oligosaccharide group ($P < 0.05$). The amine compound N,N-dimethyldecylamine, detected at 9.35% relative content in the control group, was absent in the oligosaccharide group.

3.1 Preparation and Identification of *Gracilaria lemaneiformis* Oligosaccharides

Current methods for preparing seaweed oligosaccharides primarily include acid hydrolysis [?] and biological degradation [?]. Biological degradation offers several advantages over acid hydrolysis, including lower cost, environmental friendliness, simpler procedures, and higher efficiency. Moreover, biological degradation yields diverse oligosaccharide types with promising applications. In this study, we used the agar-degrading deep-sea bacterium *Flammeovirga pacifica* WPAGA1 [?, ?] to biologically degrade *G. lemaneiformis* for feed supplementation.

Before exploring the biological functions of *G. lemaneiformis* oligosaccharides, we analyzed their composition using ion chromatography. The *F. pacifica* WPAGA1 strain degraded *G. lemaneiformis* to produce neoagaro-oligosaccharides and agaro-oligosaccharides. According to literature reports, agaro-oligosaccharides and neoagaro-oligosaccharides are generated by -agarase or -agarase cleavage of -1,3 or -1,4 glycosidic bonds in agar molecules [?]. Numerous studies have reported various bioactivities of oligosaccharides, including antitumor, anti-inflammatory, and antioxidant effects [?]. However, no studies have investigated the effects of dietary *G. lemaneiformis* oligosaccharides on

fish meat quality and flavor. This study is the first to supplement tilapia feed with *G. lemaneiformis* oligosaccharides and analyze their effects on fatty acid and volatile odor substance compositions, providing new insights for oligosaccharide feed development.

3.2 Effects of *Gracilaria lemaneiformis* Oligosaccharides on Fatty Acid Composition

C14:0 and C18:0 are common saturated fatty acids in tilapia. In this study, C16:0 was the predominant SFA in belly meat of both groups, consistent with findings in other freshwater fish species [?, ?]. The high contents of C16:0 and C18:0 indicate that these fatty acids serve as major energy sources in many tilapia tissues [?]. C14:0 positively correlates with hepatic cholesterol synthesis [?], and its significant reduction in the oligosaccharide group suggests that *G. lemaneiformis* oligosaccharides may help lower cholesterol levels in tilapia.

PUFAs and MUFAs, containing unsaturated bonds, are susceptible to free radical attack, which affects cell membrane structure, fluidity, and physiological status [?]. Gao [?] reported that agaro-oligosaccharides exhibited DPPH· scavenging activity with an IC₅₀ value of 0.89 mg/mL and could inhibit superoxide anion production by paraquat while enhancing paraquat tolerance in *Drosophila*, particularly in females. Chen et al. [?] found that agaro-oligosaccharides with polymerization degrees of 2, 4, 6, 8, and 10 effectively scavenged superoxide anion radicals (O⁻·), hydroxyl radicals (·OH), and DPPH·. Liu [?] demonstrated that *G. lemaneiformis* oligosaccharides significantly enhanced the activities of glutathione peroxidase, superoxide dismutase, alkaline phosphatase, and catalase, as well as total antioxidant capacity in black seabream liver. Zhou et al. [?] suggested that highly unsaturated fatty acids (HUFAs) may reduce lipid content in carp hepatopancreas by decreasing lipid synthesis or inhibiting lipid transport. Therefore, oligosaccharides may exert antioxidant effects by directly scavenging O⁻·, ·OH, and DPPH· or by enhancing antioxidant enzyme activities [?], thereby protecting PUFAs and MUFAs, regulating hepatic fatty acid synthesis, and influencing fish fatty acid composition.

ARA is an important unsaturated fatty acid in aquatic animals, particularly tilapia, serving as a key component in ovaries, sperm, and oocytes, and significantly affecting fertilization rates, yolk sac volume, and hatching rates [?]. Tian et al. [?] found that ARA significantly reduced the expression of key lipogenic genes such as peroxisome proliferator-activated receptor (PPAR) and fatty acid synthase (FAS) in adipose tissue and hepatopancreas, affecting lipid metabolism at the transcriptional level in grass carp. DHA and EPA are essential for fish growth, survival, and normal physiological functions, and their ratio significantly impacts these parameters and immune function [?]. Wu et al. [?] reported that high DHA/EPA ratios (2.0-3.0) enhanced head kidney leukocyte phagocytosis and respiratory burst activity in grouper compared with low ratios (0.3-0.7). Belayev et al. [?] and Rossmesl et al. [?] demonstrated anti-inflammatory and lipid-lowering effects of DHA derivatives, such as 10,7s-

docosatrienes, which inhibit polymorphonuclear leukocyte infiltration and pro-inflammatory gene expression while providing neuroprotection [?]. In this study, dietary *G. lemaneiformis* oligosaccharides significantly increased ARA content and the DHA/EPA ratio, suggesting they may substantially influence fish immunity and tissue function by modulating these PUFA levels.

PUFAs provide numerous health benefits for humans, including hypolipidemic, antiplatelet, hypotensive, antitumor, and immunomodulatory effects, significantly reducing cardiovascular disease incidence [?], and are crucial for maintaining normal brain structure and function [?]. Brain DHA content directly affects membrane properties, synapse formation, and plasticity, thereby influencing learning and memory [?]. MUFAs regulate lipid metabolism, reduce low-density lipoprotein cholesterol oxidation susceptibility, protect vascular endothelium, and decrease thrombotic status [?]. Tilapia is an important freshwater aquaculture species and advantageous export product in China, serving as a major source of protein and unsaturated fatty acids [?]. The significant increase in ARA, DHA, and EPA contents demonstrates that *G. lemaneiformis* oligosaccharides can enhance the nutritional value of tilapia.

3.3 Effects of *Gracilaria lemaneiformis* Oligosaccharides on Volatile Odor Substance Composition

Cai et al. [?] reported that volatile components were more abundant in rainbow trout belly meat than in dorsal meat; therefore, we analyzed belly meat from both tilapia groups. The predominant volatile odor compounds in tilapia belly meat were alkanes, aldehydes, and ketones, accounting for 49.39%, 1.77%, and 7.62% in the control group, and 52.0%, 0.95%, and 2.73% in the oligosaccharide group, respectively. Thus, fishy odor in tilapia may primarily originate from volatile alkanes, aldehydes, and ketones [?].

Alkanes are generated through homolytic cleavage of fatty acid alkoxy radicals, have relatively high sensory thresholds, and are considered to contribute modestly to fish flavor [?]. 1-Chlorododecane was the most abundant alkane in both groups. Chlorinated alkanes have been reported in fish oil volatile extracts and described as having grassy odors [?], and may contribute to the characteristic odor of rabbit meat [?]. Whether chlorinated alkanes are important components of tilapia odor requires further investigation.

Aldehydes and ketones, as secondary lipid oxidation products, are primary contributors to characteristic fish odors, particularly fishy smells [?]. Ketones are important components of fish flavor, generally imparting fatty and burnt notes [?], and many enones can interact with aldehydes to intensify fishy odors [?]. Low-molecular-weight aldehydes often produce fishy, musty odors at low concentrations; for example, pentanal has a strong pungent odor [?]. These compounds have low olfactory thresholds and are readily perceived even at trace levels, significantly impacting fish flavor [?]. In this study, three ketones (5-isopropyl-2,4-imidazolidinedione, 2,2,3-trimethyl-1-phenyl-3-butyldiene-1-one, and 4,4,5,6-

tetramethyl-1,3-oxadiazin-2-thione) showed significantly reduced relative contents in the oligosaccharide group, with 5-isopropyl-2,4-imidazolidinedione being undetectable, while the other two ketones were reduced to 46.4% and 42.9% of control levels, respectively. Aldehyde contents (pentanal and heptadecyl aldehyde) were also significantly lower in the oligosaccharide group. Many researchers attribute enhanced fishy odor to degradation of unstable lipid oxidation intermediates into small-molecular-weight aldehydes and ketones [?]. Numerous studies have demonstrated that *G. lemaneiformis* oligosaccharides scavenge peroxide free radicals and maintain redox homeostasis [?]. We propose that *G. lemaneiformis* oligosaccharides reduce aldehyde and ketone formation by directly scavenging radicals and oxides or enhancing antioxidant enzyme activity, thereby decomposing peroxides, interrupting peroxidation chains, and preventing lipid oxidation.

Postmortem spoilage odors in fish primarily result from decomposition of nitrogenous substances, producing amines such as ammonia, trimethylamine, and histamine [?]. The amine compound N,N-dimethyldecylamine was detected at 9.35% relative content in the control group but was absent in the oligosaccharide group. Most amines have low sensory thresholds and fishy odors that contribute to spoilage characteristics [?]; therefore, reduced amine content can improve overall tilapia flavor.

4 Conclusion

In summary, feed prepared with *G. lemaneiformis* oligosaccharides produced by deep-sea degrading bacterium *Flammeovirga pacifica* WPAGA1 effectively reduced SFA diversity and increased the contents of ARA, DHA, and EPA, as well as the DHA/EPA ratio, in tilapia belly meat. Additionally, it reduced the variety and relative abundance of volatile odor compounds, particularly ketones, amines, and aldehydes with low sensory thresholds and strong odors.

References

- [1] 包特力根白乙. 中国罗非鱼养殖产业发展及市场前景 [J]. 安徽农业科学,2014,42(33):11956-11958.
- [2] FUKUI M,KANG K S,OKADA K,et al.EPA,an omega-3 fatty acid,induces apoptosis in human pancreatic cancer cells:role of ROS accumulation,caspase-8 activation,and autophagy induction[J].Journal of Cellular Biochemistry,2013,114(1):192-203.
- [3] 王新颖, 黎介寿. -3 多不饱和脂肪酸影响炎症和免疫功能的基础研究 [J]. 肠外与肠内营养,2007,14(1):54-58.
- [4] 王国超. 罗非鱼腥味物质成分检测及脱除方法的研究 [D]. 硕士学位论文. 青岛: 中国海洋大学,2012.
- [5] 刘美英, 梅建凤, 易喻, 等. 琼胶寡糖生物活性的研究进展 [J]. 药物生物技术,2008,15(6):493-496.

- [6] 高超. 龙须菜降解产物的活性研究及降解菌 *Flammeovirga pacifica* 中硫酸酯酶基因的克隆表达 [D]. 硕士学位论文. 厦门: 国家海洋局第三海洋研究所, 2013.
- [7] XU H, FU Y Y, YANG N, et al. *Flammeovirga pacifica* sp. nov. isolated from deep-sea sediment [J]. International Journal Systematic Evolutionary Microbiology, 2012, 62(4): 937-941.
- [8] 刘胜龙. 深海菌株 *Flammeovirga pacifica* 酶解龙须菜产糖的条件优化及相关基因的克隆表达 [D]. 硕士学位论文. 厦门: 集美大学, 2014.
- [9] 刘海凤. 龙须菜海藻寡糖纯化及其对昆明鼠酒精肝损伤保护作用的研究 [D]. 硕士学位论文. 厦门: 厦门大学, 2016.
- [10] 樊燕, 孙晨阳, 王博, 等. GC/MS 分析俄罗斯鲟鱼不同部位脂肪酸组成 [J]. 现代食品科技, 2015, 31(1): 231-235.
- [11] 楼乔明, 王玉明, 杨文鸽, 等. 南极磷虾粉脂质及脂肪酸组成分析 [J]. 水产学报, 2012, 36(8): 1256-1262.
- [12] 江健, 王锡昌, 陈西瑶. 顶空固相微萃取与 GC-MS 联用法分析淡水鱼肉气味成分 [J]. 现代食品科技, 2006, 22(2): 219-222.
- [13] 陈俊卿, 王锡昌. 固相微萃取与气质联用法分析鱼肉中气味成分 [J]. 现代食品科技, 2004, 20(3): 117-118.
- [14] DOMÍNGUEZ R, GÓMEZ M, FONSECA S, et al. Effect of different cooking methods on lipid oxidation formation volatile compounds foal meat [J]. Meat Science, 2014, 97(2): 223-230.
- [15] 查如琴. 基于 SPSS 的双总体 (未知, n 30) 配对样本 t 检验与独立样本 t 检验 [J]. 读与写: 教育教学刊, 2016(7): 44-45.
- [16] CHEN H M, YAN X J, PENG Z, et al. Antioxidant activity and hepatoprotective potential of agaro-oligosaccharides in vitro and in vivo [J]. Nutrition Journal, 2006, 5(1): 31.
- [17] CHEN X L, HOU Y P, JIN M, et al. Expression and characterization of a novel thermostable and pH-stable α -agarase from deep-sea bacterium *Flammeovirga* OC4 [J]. Journal of Agricultural and Food Chemistry, 2016, 64(38): 7251-7258.
- [18] HOU Y P, CHEN X L, CHAN Z H, et al. Expression and characterization of a thermostable and pH-stable α -agarase encoded by a new gene from *Flammeovirga pacifica* WPAGA1 [J]. Process Biochemistry, 2015, 50(7): 1068-1075.
- [19] GAO B L, JIN M, LI L, et al. Genome sequencing reveals complex polysaccharide-degrading ability of novel deep-sea bacterium *Flammeovirga pacifica* WPAGA1 [J]. Frontiers in Microbiology, 2017, 8: 600.
- [20] 王淑茹, 王丁刚. 茶叶多糖的抗凝血及抗血栓作用 [J]. 中草药, 1992, 23(5): 254-256.
- [21] 付学鹏, 杨晓杰. 蒲公英多糖的提取及含量测定 [J]. 现代食品科技, 2007, 23(5): 37-39.
- [22] 麻艳群, 黄凯, 陈涛, 等. 饲料磷脂水平对巴丁鱼体组织脂肪酸组成的影响 [J]. 水产科学, 2015(8): 476-484.

- [23] FRANKS J S,WARREN J R,BUCHANAN M V.Age and growth of cobia,*Rachycentron canadum*,from the northeastern Gulf of Mexico[J].Fishery Bulletin,1999,97(3):459-471.
- [24] 陈洁文. 高血压患者血清磷脂脂肪酸谱与血脂的相关性研究 [D]. 硕士学位论文. 杭州: 浙江大学,2006.
- [25] 吉红, 田晶晶. 高不饱和脂肪酸 (HUFAs) 在淡水鱼类中的营养作用研究进展 [J]. 水产学报,2014,38(9):1650-1665.
- [26] 陈海敏, 严小军, 王峰, 等. 琼胶寡糖抑制血管形成作用的研究 [J]. 营养学报,2007,29(4):405-407,410.
- [27] 刘燕. 龙须菜的发酵及发酵龙须菜对黑鲷生长、免疫和抗氧化能力的影响 [D]. 硕士学位论文. 广州: 华南农业大学,2016.
- [28] 周继术, 吉红, 王建华, 等. 鱼油对鲤生长及脂质代谢的影响 [J]. 中国海洋大学学报: 自然科学版,2008,38(2):275-280.
- [29] 韩涛, 王骥腾, 王勇, 等. 饲料中不同水平鱼蛋白水解物对军曹鱼稚鱼生长及体组成的影响 [J]. 水生生物学报,2010,34(1):94-100.
- [30] TIAN J J,JI H,OKU H,et al.Effects of dietary arachidonic acid (ARA) on lipid metabolism health status juvenile grass carp,*Ctenopharyngodon idellus*[J].Aquaculture,2014,430:57-65.
- [31] 谭肖英. 黄颡鱼脂类营养生理研究 [D]. 博士学位论文. 武汉: 华中农业大学,2012.
- [32] WU F C,TING Y Y,CHEN H Y.Docosahexaenoic acid is superior to eicosapentaenoic acid as the essential fatty acid for growth of grouper,*Epinephelus malabaricus*[J].The Journal of Nutrition,2002,132(1):72-79.
- [33] BELAYEV L,MARCHESELLI V L,KHOUTOROVA L,et al.Docosahexaenoic acid complexed albumin elicits high-grade ischemic neuroprotection[J].Stroke,2005,36(1):118-123.
- [34] ROSSMEISL M,JELENIK T,JILKOVA Z,et al.Prevention and reversal of obesity and glucose intolerance in mice by DHA derivatives[J].Obesity,2009,17(5):1023-1031.
- [35] MARCHESELLI V L,HONG S,LUKIW W J,et al.Novel Docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration pro-inflammatory expression[J].Journal of Biological Chemistry,2003,278(44):43807-43817.
- [36] 任衍开. 多不饱和脂肪酸的研究进展 [J]. 健康导报: 医学版,2015,20(4):270.
- [37] KURATKO C N,BARRETT E C,NELSON E B,et al.The relationship of docosahexaenoic (DHA) learning behavior healthy children:a review[J].Nutrients,2013,5(7):2777-2810.
- [38] HENNEBELLE M,CHAMPEIL-POTOKAR G,LAVIALLE M,et al.Omega-3 polyunsaturated fatty acids and chronic stress-induced modulations

of glutamatergic neurotransmission in the hippocampus[J].Nutrition Reviews,2014,72(2):99-112.

[39] 史敬, 马依彤.n-3 多不饱和脂肪酸对心血管疾病的临床应用 [J]. 心血管病学进展,2016,37(3):278-282.

[40] 王玮, 丁建乐, 房金岑. 罗非鱼产业标准化现状及分析 [J]. 上海海洋大学学报,2012,21(6):976-981.

[41] 蔡原, 刘哲, 宋明伟, 等. 虹鳟不同部位鱼肉挥发性风味物质组成比较 [J]. 食品科学,2011,32(16):269-273.

[42] 卢春霞, 翁丽萍, 王宏海, 等. 3 种网箱养殖鱼类的主体风味成分分析 [J]. 食品与发酵工业,2010(10):163-169.

[43] TOLDRA F.Proteolysis and lipolysis in flavour development of dry-cured meat products[J].Meat Science,1998,49(Suppl.1):S101-S110.

[44] UMEDA M,MANABE Y,UCHIMIYA H.Phosphorylation of the C2 Subunit of the Proteasome in Rice (*Oryza Sativa* L.)[J].FEBS Letters,1997,403(3):313-317.

[45] 姜颖. 兔肉腥味物质的鉴定及其形成机理初探 & 人胎肝磷酸化蛋白质表达谱的构建 [D]. 博士学位论文. 南京: 南京农业大学,2003.

[46] IGLESIAS J,MEDINA I.Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle[J].Journal of Chromatography A,2008,1192(1):9-16.

[47] 裘迪红, 欧昌荣, 苏秀榕, 等. 植物乳杆菌发酵草鱼肉挥发性成分的变化规律 [J]. 食品科学,2015,36(20):174-180.

[48] 杨华, 娄永江, 杨震峰.GC-MS 法分析养殖大黄鱼脱腥前后挥发性成分的变化 [J]. 中国食品学报,2008,8(3):147-151.

[49] 杜国伟, 夏文水. 鲢鱼糜脱腥前后及贮藏过程中挥发性成分的变化 [J]. 食品工业科技,2007,28(9):76-80.

[50] 冯倩倩. 罗非鱼腥味形成机理及脱除技术研究 [D]. 硕士学位论文. 广州: 华南理工大学,2013.

[51] VARLET V,PROST C,SEROT T.Volatile aldehydes smoked fish:analysis methods,occurrence mechanisms formation[J].Food Chemistry,2007,105(4):1536-1556.

[52] THIANILAKUL Y,BENJAKUL S,RICHARDS M P.Changes in heme proteins and lipids associated with off-odour of seabass (*Lates calcarifer*) and red tilapia (*Oreochromis mossambicus*×*O.niloticus*) during iced storage[J].Food Chemistry,2010,121(4):1109-1119.

[53] 王国超, 李来好, 郝淑贤, 等. 水产品腥味物质形成机理及相关检测分析技术的研究进展 [J]. 食品工业科技,2012,33(5):401-404.

[54] ENOKI T,TOMINAGA T,TAKASHIMA F,et al.Anti-tumor-promoting activities of agaro-oligosaccharides two-stage mouse carcinogenesis[J].Biological Pharmaceutical Bulletin,2012,35(7):1145-1149.

[55] 侯艳平.*Flammeovirga pacifica* WPAGA1 中琼胶酶基因的克隆表达、酶学性质分析及降解产物的初步研究 [D]. 硕士研究生论文. 厦门: 国家海洋局第三海洋研究所,2015.

[56] CHUNG H Y,YEUNG C W,KIM J S,et al.Static headspace analysis-olfactometry (SHA-O) of odor impact components in salted-dried white herring (*Ilisha elongata*)[J].Food Chemistry,2007,104(2):842-851.

[57] 郭大钧, 奚印慈. 测定鱼类鲜度指标之二——三甲胺 [J]. 中国水产,1983(12):23-23.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv –Machine translation. Verify with original.