International Food Research Journal 31(5): 1202 - 1215 (October 2024)
Journal homepage: *http://www.ifrj.upm.edu.my*

Influence of traditional wooden barrel fermentation on fish: Comparative study on nutritional quality of muscles of fresh and stinky mandarin fish (*Siniperca chuatsi*)

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Article history

Received:
12 July 2023

Received in revised form:
19 July 2024

Accepted:
31 July 2024

Keywords

amino acids,
basic components,
fatty acids,
mineral composition,
stinky mandarin fish

Abstract

The present work examined the nutritional quality of stinky mandarin fish (*Siniperca chuatsi*), a Chinese fish product (*chòu guì yú*), by assessing its nutrients, amino acids, minerals, and fatty acids in comparison with those of fresh mandarin fish (raw material). Compared with the fresh fish, stinky mandarin fish muscle moisture content decreased significantly ($p < 0.05$) after salting. A total of 17 amino acids were detected in the stinky mandarin fish muscle, accounting for 15.56% of the total wet weight, with essential amino acid content accounting for 40.00% of total amino acids. The amino acid and free amino acid contents increased significantly ($p < 0.05$), from 5.90 to 6.12%, and from 33.33 to 468.08 mg/100 g, respectively. Although the fatty acid quality decreased sharply, the essential fatty acid content was relatively high. Furthermore, stinky mandarin fish was rich in minerals, with calcium and zinc contents of 52.48 and 1.21 mg/100 g, respectively. Therefore, stinky mandarin fish is highly edible due to its delicious taste and nutritional value. These findings would provide a basis for improving Chinese mandarin fish's industrial processing and nutritional quality.

DOI
https://doi.org/10.47836/ifrj.31.5.10

Introduction

Chinese perch (*Siniperca chuatsi*; family: Percichthyidae), also known as mandarin fish (Yang *et al*., 2017), is an important freshwater fish species with high economic value in Asian countries (Zhou *et al*., 2020). The mandarin fish is as famous as the Yellow River carp, Songjiang four-gill perch, and Xingkai Lake big white fish, thus known as one of China's "famous four freshwater fish." It is considered a first-class freshwater fish due to its white and tender meat, few spines, and delicious taste (Yang *et al*., 2014). Additionally, traditional Chinese medicine considers that mandarin fish nourishes *qi* and blood, and benefits the spleen and stomach. Therefore, the mandarin fish has an important economic value (Yang *et al*., 2021).

Due to the diversity of Chinese cuisines, mandarin fish can be cooked in various ways, such as steamed mandarin fish (Cantonese-style cuisine), squirrel-shaped mandarin fish (Su-style cuisine), and pickled fresh stinky mandarin fish (Hui-style cuisine). Among these, one of the most famous is the Anhui stinky mandarin fish (*i.e.*, Hui style). As a traditional Hui-style dish, it is prepared using fermented mandarin fish, by frying and stewing (Yang *et al*., 2019). Pickled fresh mandarin fish, commonly known as stinky mandarin fish or barrel fresh fish, is a type of fermented fish obtained by short-term pickling and fermentation, in which organic components, such as proteins and lipids, are degraded into amino acids, small peptides, free fatty acids, and volatile flavour components of small molecules. This could be caused by the fish's enzymes and/or complex microbial colonies (fermentation environment) (Dai *et al*., 2013a; Bao *et al*., 2018). This fermented product has a distinctive history, unique flavour (Li *et al*., 2013), garlic-clove-like texture, and delicious taste upon

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cooking (Zhou *et al*., 2022); thus, it is highly appreciated by consumers, and has become one of the traditional fermented freshwater fish products with Chinese characteristics (Yang *et al*., 2021). However, currently, the stinky mandarin fish on the market is primarily processed in traditional natural fermentation workshops. Its production capacity is limited, and cannot meet the growing consumer demand. On the other hand, although natural fermentation can induce delicious and unique flavours, there are other related problems, such as difficulty in flavour control, unstable product quality, and difficulties in judging the fermentation endpoint (Shen *et al*., 2021). Therefore, producers and researchers face challenges in promoting the industrialised and standardised processing of fermented mandarin fish, along with the associated necessary industrial upgrades and development (Xu *et al*., 2021).

Recently, Yang *et al*. (2019) optimised the process parameters of the natural fermentation of mandarin fish. They showed that when the added salt amounted to 6%, the fermentation temperature was 12°C, and after seven days of natural fermentation, the meat was in the shape of garlic cloves, and the smell was identifiably stinky mandarin fish. The texture of the traditional stinky mandarin fish and the volatile flavour substances were analysed, and the primary fermentation microorganisms were isolated and identified as *Bacillus thuringiensis* and *B. cereus* (Yang *et al*., 2014). Zhou *et al*. (2021) isolated a strain of *Lactobacillus sakei* from traditionally fermented mandarin fish. The strain had a certain salt tolerance limit (8% NaCl tolerance), and could significantly inhibit spoilage associated with *Escherichia coli* and *Staphylococcus aureus*. Fermentation using this strain improved the colour and texture of stinky mandarin fish and their aroma, and increased the floral substance content of the plant (*e.g*., limonene, α-terpineol, and linalool) but reduced malodorous agents (*e.g*., methyl mercaptan). Ke (2021) screened *L. casei*, *L. paracasei*, and *Pichia* spp. from stinky mandarin fish, and claimed that these strains played a key role in the production of aroma substances, and therefore, could reduce trimethylamine content in the product. Xu *et al*. (2022) found that adding 200 U/g of papain effectively increased the levels of free amino acids (FAAs) in fermented mandarin fish, and decreased biogenic amine content, but this increased the degree of fat oxidation. Mixed strains (*Pediococcus pentosaceus*, *L. sakei*, and *S. meatus*, with a mass ratio of 1:1:3) were used to ferment mandarin fish at 22°C for four days, and the amino nitrogen content of the fish was similar to that of naturally fermented products, but the indicators of fat oxidation and freshness were significantly improved (Wu *et al*., 2022). In summary, the majority of recent studies on stinky mandarin fish have focused on the isolation and identification of strains, optimisation of the fermentation process, and determination of physical and chemical indicators. However, there are few related studies on the analysis and evaluation of nutritional quality.

While promoting the industrialisation of traditional fermented fish products, attention should also be given to the nutritional value and health benefits associated with the products. It is necessary to develop a modernised yet traditional food industry, with a green and healthy concept. Therefore, to increase knowledge regarding the nutritional quality of stinky mandarin fish, the present work used traditional stinky mandarin fish from the southern Anhui Province of China as raw material, and a corresponding batch of fresh mandarin fish as the control. The basic nutritional components, hydrolysed protein amino acids, FAAs, fatty acids, and mineral elements were determined, and the nutritional quality was evaluated and compared, before and after traditional fermentation in wooden barrels, to provide the basis for establishing the quality standard, modern processing and utilisation, and improving the nutritional quality of this traditional short-term fermented fish, *i.e*., the stinky mandarin fish.

Materials and methods

Materials and reagents

Fresh mandarin fish and stinky mandarin fish were procured from Anhui Huangshan Huichu Food Co., Ltd. (Huangshan, Anhui, China) and Huangshan Wanxin Huisan Food Supply Chain Co. Ltd. (Huangshan, Anhui, China), and stinky mandarin fish were prepared by traditional fermentation for 7 - 8 d in the Huangshan area of southern Anhui. The preparation procedure for the traditional stinky mandarin fish is shown in Figure 1. Samples were stored at -20°C after vacuum sealing for further use (approximately 3 w). The *n*-hexane used was of chromatographic grade; the sulfosalicylic, nitric, and perchloric acids were of superior grade (GR); and the other reagents were of analytical grade (AR).

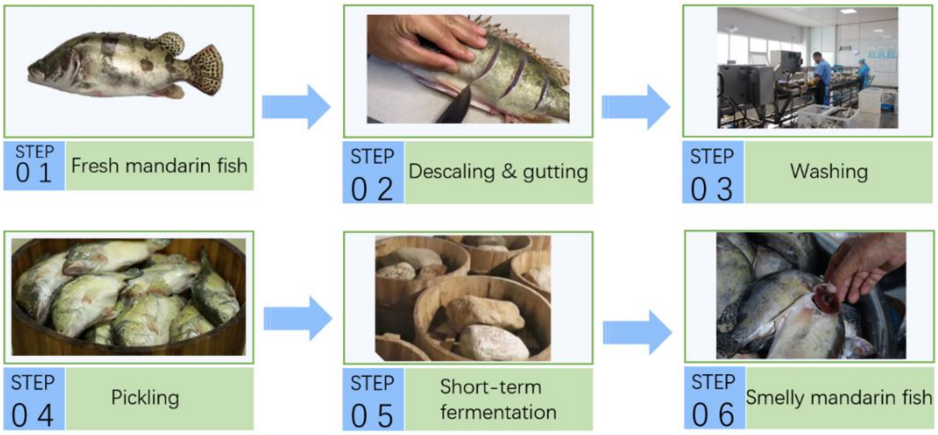

Figure 1. Traditional stinky mandarin fish preparation process.

Sample pre-treatment

After thawing for 10 h at 4°C, various parts of fresh and stinky mandarin fish from the same batch were collected, chopped, and mixed evenly. The prepared samples were stored at -20°C for 1 w to determine the basic nutritional components, amino acid profiles, fatty acids, and mineral contents.

Determination of basic nutritional components and pH

The fresh and stinky mandarin fish meat samples were analysed for basic nutritional components following the National Food Safety Standards of China. These measurements consisted of GB 5009.3-2016 for moisture content using a DHG-9123J air drying oven (Shanghai Sanfa Scientific Instrument Co., Ltd., Shanghai, China), GB 5009.4-2016 for ash content, GB 5009.5-2016 for crude protein content using a K9840 automatic Kjeldahl nitrogen analyser (Haineng Instrument Co., Ltd., Jinan, Shandong, China), GB 5009.6-2016 for crude fat, and GB5009.237-2016 for pH.

Amino acid composition of hydrolysed protein

Samples (0.1 g each) of freeze-dried fresh and stinky mandarin fish meat were accurately weighed and transferred to 10-mL ampoule bottles. After adding 5 mL of 6-mol/L HCl, and filling the ampoule with nitrogen, the ampoule was quickly sealed using an alcohol torch. The sealed samples were hydrolysed in an oven at 130°C for 3.5 h, and then transferred to a 100-mL volumetric flask for washing with distilled water three times. After fixing the volume, 1 mL of hydrolysate was taken and freeze-dried for 6 h. The residue was dissolved in 1 mL of 0.02-mol/L HCl, and filtered with a 0.22-μm microporous membrane for further analysis. Amino acid compositions were determined using an L-8900 amino acid automatic analyser (Hitachi, Ltd., Tokyo, Japan).

Free amino acid composition

The FAA composition analysis of the two meat samples used 2.0 g of freeze-dried sample. The minced meat was combined with 4% sulfosalicylic acid until reaching a volume of 10 mL. Next, the mixture was centrifuged at 12,000 rpm for 10 min, and the supernatant was filtered using a 0.22-μm microporous membrane for further analysis.

Fatty acid composition determination

The sample was methylated following the methods of Wu *et al*. (2019) with minor modifications. Crude fat was extracted using the Soxhlet extraction method, with 0.5 g of crude fat weighed and placed in a stoppered test tube, followed by the addition of 4 mL of 1-mol/L KOH-CH_3OH. The mixture was then saponified in a water bath at 60°C for approximately 30 min until the oil droplets completely disappeared. After cooling, 40 mL of 12.5% H_2SO_4-CH_3OH was added, and the resulting solution was esterified at 60°C for 5 min. The esterified samples were mixed with 45 mL of distilled water and 3 mL of *n*-hexane. The solution was left to stand for 10 min, and the lower aqueous phase was separated. The residual liquid was added to 4 mL of saturated aqueous sodium chloride solution, and washed three times. After removing the supernatant, 1.0 g of anhydrous sodium sulphate was added, and the solution was centrifuged at 4,000 – 5,000 rpm for 10 min. The supernatant was collected and filtered using 0.22-µm microporous membrane for gas chromatography-mass spectrometry (GC-MS). Fatty acid composition was determined using a QP-2010 GC-MS instrument (Shimadzu Corporation, Kyoto, Japan).

Mineral content determination

The freeze-dried fish meat was weighed (1.0 g) and placed in a 100-mL beaker. After adding 12 mL of nitric acid and 6 mL of perchloric acid, the mixture was digested by heating until the solution was clear. The solution was transferred to a 100-mL volumetric flask, and the residue was washed three times using deionised water. The mixture volume was increased to 100 mL for analysis. The mineral content was measured using an inductively coupled plasma emission spectrometer, and the test conditions consisted of the following: carrier gas, 0.8 L/min; auxiliary gas, 0.5 L/min; cooling gas, 15.0 L/min; high-frequency generator power, 1.3 kW; and sample lift, 1.0 mL/min (Cheng *et al*., 2021).

Nutritional quality evaluation

To evaluate the protein quality of fresh and stinky mandarin fish meat, indexes including amino acid score (AAS), chemical score (CS), branched-chain amino acid/aromatic amino acid (BCAA/AAA), and essential amino acid index (EAAI) were calculated using Eq. 1-4 (Wang *et al*., 2021a; Yin *et al*., 2022):

$$AAS = \frac{amino\ acid\ content\ in\ meat\ (mg/100\ g)}{amino\ acid\ content\ in\ FAO/WHO\ reference\ (mg/100g)} \quad \text{(Eq. 1)}$$

$$CS = \frac{amino\ acid\ content\ in\ meat\ (mg/100g)}{amino\ acid\ content\ in\ egg\ protein\ (mg/100g)} \quad \text{(Eq. 2)}$$

$$BCAA/AAA = \frac{Branched\ chain\ amino\ acid\ content\ (Val+Leu+Ile, mg/100g)}{Aromatic\ chain\ amino\ acid\ content\ (Phe+Tyr, mg/100g)} \quad \text{(Eq. 3)}$$

$$EAAI = \sqrt[n]{\frac{100a}{a_e} \times \frac{100b}{b_e} \times \cdots \times \frac{100g}{g_e}} \quad \text{(Eq. 4)}$$

where, n = number of essential amino acids compared, a - g = concentrations of essential amino acids (EAAs) (mg/100 g), and a_e - g_e = EAA concentrations in the egg proteins model proposed by the Institute of Nutrition and Food Hygiene of the Chinese Academy of Preventive Medicine.

Statistical analysis

SPSS Statistics for Windows (version 19.0; IBM Corp., Armonk, NY, USA) was used for statistical analyses. Duncan's test with $p < 0.05$ was conducted. Additionally, data were expressed as mean ± standard deviation.

Results

Basic nutritional components of stinky mandarin fish

As presented in Table 1, the moisture, crude protein, crude fat, and ash contents of the stinky mandarin fish were 76.71 ± 1.54, 17.69 ± 1.60, 2.34 ± 0.42, and 3.28 ± 0.22%, respectively. Compared with fresh mandarin fish, the moisture content of stinky mandarin fish decreased significantly ($p <$ 0.05), whereas the crude fat and ash contents increased significantly ($p < 0.05$), and the crude protein content increased slightly ($p > 0.05$). During the marinating process, the addition of salt and other spices resulted in a significant decrease in the moisture content of mandarin fish meat, and thus, a relative increase in the contents of crude protein, crude fat, and ash (Czerner and Yeannes, 2013). Table 1 also lists the basic muscle components of conventional freshwater fish species, including several fish with the highest yield in freshwater culture in China (Xu *et al*., 2014). Compared with previous reports of other common freshwater fish, the crude protein content in the meat of the stinky mandarin fish (17.69%) was relatively high; although lower than that of grass carp (18.75%) (Yang and Xia, 2012), it was significantly higher than that of yellow catfish (15.37%) (Huang *et al*., 1999), and slightly higher than that of herring (17.08%), bighead carp (17.00%), silver carp (17.42%) (Yang and Xia, 2012), and Yellow River carp (17.48%) (Zhu *et al*., 2000). Its crude fat content was also relatively high, reaching 2.34%, which was significantly higher than that of herring (1.50%), grass carp (1.07%), silver carp (1.08%), bighead carp (1.12%) (Yang and Xia, 2012), and yellow catfish (1.61%) (Huang *et al*., 1999), but lower than that of Yellow River carp (3.47%) (Zhu *et al*., 2000). The ash content of the stinky mandarin fish was significantly higher than that of other freshwater fish, reaching 3.28%. Furthermore, after fermentation, the pH of the mandarin fish decreased from 6.86 ± 0.02 (fresh mandarin fish) to 6.69 ± 0.14 (stinky mandarin fish). Thus, the basic nutrient index of fermented mandarin fish was higher than that of other common freshwater fish.

Table 1. Proximate composition of stinky mandarin fish muscle and other fish (%, wet weight).

Type	Moisture	Crude protein	Total lipid	Total ash
Stinky mandarin fish	76.71 ± 1.54[a]	17.69 ± 1.60[a]	2.34 ± 0.42[a]	3.28 ± 0.22[a]
Fresh mandarin fish	79.68 ± 0.42[b]	17.05 ± 1.07[a]	1.18 ± 0.13[b]	1.11 ± 0.05[b]
Grass carp*	79.33 ± 0.56	18.75 ± 1.04	1.07 ± 0.09	2.28 ± 0.48
Black carp*	79.85 ± 0.79	17.08 ± 1.27	1.50 ± 0.52	1.08 ± 0.21
Bighead*	81.59 ± 1.21	17.00 ± 1.39	1.12 ± 0.15	1.29 ± 0.31
Silver carp*	80.82 ± 1.20	17.42 ± 0.85	1.08 ± 0.10	1.23 ± 0.64
*Pelteobagrus fulvidraco***	82.40	15.37	1.61	0.16
Carp***	73.35	17.48	3.47	1.15

Different lowercase superscripts in similar column indicate significant differences ($p < 0.05$). *Proximate compositions of grass, black, bighead, and silver carp muscles are referred to Yang and Xia (2012). **Proximate composition of *Pelteobagrus fulvidraco* muscle is referred to Huang *et al*. (1999). ***Proximate composition of carp muscle is referred to Zhu *et al*. (2000).

Amino acid composition and hydrolysed protein content

The amino acid profiles from the muscles of fresh and stinky mandarin fish are shown in Table 2. The total amino acid content in stinky mandarin fish on dry weight basis was significantly lower ($p < 0.05$) than that of fresh fish, which might have been due to the initial curing process leading to drip loss in the muscle, accompanied by the loss of free amino acids (Xu *et al*., 2022). Additionally, the protein in mandarin fish meat was decomposed and used by microorganisms during fermentation, resulting in a decrease in the total amount of amino acids. However, due to water seepage (decreased water content) during the pickle salting process, the total amount of amino acids in stinky mandarin fish on wet weight basis increased slightly, but this increase was not statistically significant ($p > 0.05$). In terms of amino acid composition, glutamic acid content was the highest, accounting for 17.82% of the total amino acids in stinky mandarin fish (wet weight basis). It was compositionally followed by aspartic acid, lysine, and leucine.

The closer the protein amino acid pattern in food is to the protein composition pattern of the human body, the easier it is to be absorbed and utilised by the human body, and the higher the physiological value (Wang *et al*., 2021a). Based on the ideal model proposed by FAO/WHO, the ratio of EAA to the total amount of amino acids (TAA) is approximately 0.4, and ratios of EAA to non-essential amino acids (NEAA) > 0.6 indicate high-quality protein sources (Yin *et al*., 2022). As presented in Table 2, the EAA/TAA ratio of meat from stinky mandarin fish was 0.40 and EAA/NEAA ratio was 0.77. The composition ratios were consistent with the FAO/WHO recommended standards.

The ratio of BCAA/AAA content in stinky mandarin fish meat was further measured. BCAA refers to amino acids with branched side chains, including Val, Leu, and Ile. AAA refers to amino acids containing aromatic rings, including Phe and Tyr. The BCAA/AAA ratio of a normal human body is approximately 3.0 - 3.5. When acute liver necrosis (or liver damage) occurs, the BCAA content decreases, and the AAA content increases, leading to BCAA/AAA ratio decreasing to 1.0 - 1.5 (Lu *et al*., 2009). The BCAA/AAA ratio of the stinky mandarin fish was 2.15, which was higher than that of the fresh fish (2.06). Thus, stinky mandarin fish meat has a complete range of amino acids and a suitable amino acid ratio, making it a high-quality protein source.

Nutritional evaluation of amino acids

The nutritional evaluation of the amino acids of stinky mandarin fish meat was performed using the FAO/WHO recommended standard model of amino acids (Lu *et al*., 2009), and the whole egg protein amino acid model proposed by the Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine (Zhu *et al*., 2000). The AAS, CS, and EAAI values of the stinky mandarin fish meat are presented in Table 3.

In food protein, limiting amino acids are EAAs found in digested protein that are present in lesser amounts than what is needed for human absorption. Based on the results of AAS, Val had the lowest muscle score among the stinky mandarin fish,

Table 2. Hydrolysed protein amino acids in fresh and stinky mandarin fish muscles (g/100 g).

Amino acids	Dry weight		Wet weight	
	Fresh mandarin fish	Stinky mandarin fish	Fresh mandarin fish	Stinky mandarin fish
Lys	7.24 ± 0.72[a]	6.69 ± 0.43[a]	1.48 ± 0.16[a]	1.54 ± 0.08[a]
Leu	6.73 ± 0.57[a]	6.20 ± 0.40[b]	1.37 ± 0.12[a]	1.42 ± 0.07[a]
Val	2.93 ± 0.21[a]	2.83 ± 0.08[a]	0.60 ± 0.05[a]	0.65 ± 0.01[a]
Ile	2.89 ± 0.24[a]	2.76 ± 0.11[a]	0.59 ± 0.05[a]	0.63 ± 0.02[a]
Thr	3.45 ± 0.25[a]	3.20 ± 0.17[b]	0.70 ± 0.06[a]	0.74 ± 0.03[a]
Phe	3.13 ± 0.31[a]	3.01 ± 0.14[a]	0.64 ± 0.07[a]	0.69 ± 0.02[a]
Met	2.44 ± 0.19[a]	2.25 ± 0.17[b]	0.50 ± 0.04[a]	0.52 ± 0.03[a]
EAA	28.81 ± 1.98[a]	26.93 ± 1.50[b]	5.88 ± 0.54[a]	6.19 ± 0.27[a]
Arg	4.49 ± 0.34[a]	4.05 ± 0.28[b]	0.92 ± 0.08[a]	0.93 ± 0.05[a]
His	1.56 ± 0.27[a]	1.60 ± 0.00[a]	0.32 ± 0.06[a]	0.37 ± 0.00[a]
HEAA	6.05 ± 0.61[a]	5.65 ± 0.28[a]	1.24 ± 0.13[a]	1.30 ± 0.05[a]
Glu	13.00 ± 1.00[a]	11.67 ± 0.89[b]	2.65 ± 0.22[a]	2.68 ± 0.17[a]
Asp	7.86 ± 0.66[a]	7.29 ± 0.46[b]	1.60 ± 0.15[a]	1.67 ± 0.09[a]
Ala	4.91 ± 0.40[a]	4.59 ± 0.28[a]	1.00 ± 0.09[a]	1.05 ± 0.05[a]
Gly	3.18 ± 0.17[a]	3.15 ± 0.09[a]	0.65 ± 0.04[a]	0.72 ± 0.01[b]
Ser	3.25 ± 0.24[a]	2.95 ± 0.17[b]	0.66 ± 0.05[a]	0.68 ± 0.03[a]
Tyr	2.70 ± 0.25[a]	2.47 ± 0.15[b]	0.55 ± 0.05[a]	0.57 ± 0.03[a]
Pro	2.29 ± 0.13[a]	2.21 ± 0.06[a]	0.47 ± 0.03[a]	0.51 ± 0.01[b]
Cys	0.75 ± 0.01[a]	0.79 ± 0.02[a]	0.15 ± 0.00[a]	0.18 ± 0.00[b]
NEAA	37.94 ± 0.72[a]	35.10 ± 1.20[b]	7.75 ± 0.63[a]	8.07 ± 0.39[a]
TAA	72.80 ± 0.20[a]	67.69 ± 0.23[b]	14.87 ± 1.31[a]	15.56 ± 0.70[a]
EAA/TAA	0.40	0.40	0.40	0.40
EAA/NEAA	0.76	0.77	0.76	0.77
BCAA/AAA	2.06	2.15	2.06	2.15

Different lowercase superscripts in similar row indicate significant differences ($p < 0.05$). TAA: total amino acids; EAA: essential amino acids; NEAA: non-essential amino acids; BCAA: branched-chain amino acids, and AAA: aromatic amino acids.

Table 3. Essential amino acid compositions in fresh and stinky mandarin fish muscles (mg/g, on N basis).

Essential amino acid	FAO pattern	Egg protein	AAS		CS		EAAI	
			Fresh	Stinky	Fresh	Stinky	Fresh	Stinky
Ile	2.50	3.31	0.72	0.69	0.55	0.52		
Leu	4.40	5.34	0.96	0.88	0.79	0.73		
Lys	3.40	4.41	1.33	1.23	1.03	0.95		
Thr	2.50	2.92	0.86	0.75	0.74	0.64	65.11	60.89
Val	3.10	4.10	0.59	0.59	0.45	0.45		
Met + Cys	2.20	3.86	0.91	0.86	0.52	0.49		
Phe + Tyr	3.80	5.65	0.96	0.90	0.64	0.61		

AAS: amino acid score; CS: chemistry score; and EAAI: essential amino acid index.

followed by Ile. However, among the CS, Val was the lowest, followed by Met + Cys. Hence, the amino acids that were most limited in stinky mandarin fish muscle were Val, Ile, and Met + Cys. Regardless of AAS or CS, Lys had the highest score, which was relatively close to the whole egg protein standard (CS = 1.00). Lys is usually the first limiting amino acid among people who eat grains as a staple food, and the meat of stinky mandarin fish can compensate for this deficiency (Cheng *et al*., 2021).

Compared with fresh mandarin fish, the AAS and CS values for all of the EAAs in stinky mandarin fish slightly decreased, except for Val, among which Thr decreased the most, followed by lysine and other amino acids such as Leu, Phe + Tyr, Met + Cys, and Iso, which decreased in that order. Taking the EAA of whole egg protein as the standard, the EAAI of fresh and stinky mandarin fish were calculated to be 65.11 and 60.89, respectively, indicating that the amino acid quality of the mandarin fish decreased following traditional fermentation. However, compared with other fish, the EAAI of stinky mandarin fish was close to that of yellow-spotted basket fish (61.07) (Zhuang *et al*., 2008), and was significantly higher than that of the golden pomfret (39.87) (Dai *et al*., 2013b). Therefore, the amino acid nutritional quality of stinky mandarin fish remained relatively high.

Composition and content of free amino acids

FAAs are closely related to the unique umami taste of stinky mandarin fish. The total FAA content observed in fresh mandarin fish was only 33.33 mg/100 g, whereas the total FAA content found in stinky mandarin fish was as high as 468.08 mg/100 g. Thus, the total FAA content in mandarin fish increased significantly by 14 times ($p < 0.05$) following traditional fermentation (Table 4). During the processing of stinky mandarin fish, due to the degradation on its own or exogenous microbial protease, the macromolecular protein of fish meat was continuously degraded into polypeptides and amino acid molecules, resulting in a significant increase in the total FAA amounts observed (Feng *et al*., 2021).

The deliciousness of food is partly attributed to the composition and content of specific delicious amino acids (*i.e.*, Glu, Asp, Gly, and Ala) contained in its proteins. Glu and Asp are characteristic amino acids of umami taste, among which, Glu has the strongest umami taste, whereas Gly and Ala are characteristic amino acids associated with sweet tastes (Lu *et al*., 2009; Wu *et al*., 2019). As shown in Table 5, the content of free umami amino acids in stinky mandarin fish increased significantly ($p < 0.05$), and the contents of Glu, Asp, Gly, and Ala were approximately 12 , 25, 34, and 38 times that of fresh mandarin fish, respectively. The total amount of these amino acids in stinky mandarin fish was 187.81 mg/100 g, which was approximately 20 times that of fresh mandarin fish (8.97 mg/100 g). This accounted for 40.12% of the total FAAs. Therefore, the stinky

Table 4. Free amino acids in fresh and stinky mandarin fish muscles (mg/100 g, wet weight).

Free amino acid	Fresh mandarin fish	Stinky mandarin fish
Lys	2.56 ± 0.09^a	49.85 ± 1.25^b
Leu	1.34 ± 0.76^a	39.98 ± 2.44^b
Val	2.55 ± 0.42^a	35.18 ± 0.29^b
Ile	0.67 ± 0.38^a	22.53 ± 3.17^b
Thr	0.58 ± 0.41^a	30.09 ± 0.84^b
Phe	2.76 ± 1.52^a	23.30 ± 0.46^b
Met	2.06 ± 1.19^a	16.55 ± 0.94^b
Arg	0.11 ± 0.01^a	0.43 ± 0.01^b
His	0.20 ± 0.12^a	36.26 ± 0.27^b
Glu	5.41 ± 2.33^a	66.78 ± 0.34^b
Asp	0.40 ± 0.17^a	9.97 ± 0.42^b
Ala	1.99 ± 0.21^a	67.13 ± 2.57^b
Gly	1.17 ± 0.74^a	43.93 ± 3.11^b
Ser	0.54 ± 0.01^a	12.93 ± 0.29^b
Tyr	5.31 ± 1.03^a	2.33 ± 0.08^b
Pro	0.11 ± 0.02^a	1.57 ± 0.04^b
Cys	5.57 ± 3.02^a	9.25 ± 0.32^b
Total	33.33 ± 0.92^a	468.08 ± 4.25^b

Different lowercase superscripts in similar row indicate significant differences ($p < 0.05$).

Table 5. Free delicious amino acids in fresh and stinky mandarin fish muscles (mg/100 g, wet weight).

Free delicious amino acid	Fresh mandarin fish	Stinky mandarin fish
Glu	5.41 ± 2.33^a	66.78 ± 0.34^b
Asp	0.40 ± 0.17^a	9.97 ± 0.42^b
Ala	1.99 ± 0.21^a	67.13 ± 2.57^b
Gly	1.17 ± 0.74^a	43.93 ± 3.11^b
Total	8.97 ± 3.45^a	187.81 ± 5.92^b

Different lowercase superscripts in similar row indicate significant differences ($p < 0.05$).

mandarin fish is a type of delicious preserved aquatic food, and the traditional fermentation process is an effective means to enhance the flavour of mandarin fish.

Fatty acid composition and evaluation

As shown in Table 6, a total of 20 fatty acids were detected in fresh and fermented mandarin fish, including six saturated fatty acids (SFA) and 14 unsaturated fatty acids (UFA). Of the UFA, four were monounsaturated fatty acids (MUFA), and ten were polyunsaturated fatty acids (PUFA). The meat of stinky mandarin fish contained rich species of UFA and PUFA. PUFA is a class of active substances with special physiological functions. It can esterify cholesterol, and reduce blood cholesterol and triglyceride levels, which helps prevent hypertension, atherosclerosis, and cardiovascular diseases (Dvoretsky *et al*., 2022).

After fermentation, the SFA content (44.23%) in stinky mandarin fish increased significantly ($p < 0.05$) compared with that in fresh mandarin fish (33.63%). While the UFA content (51.55%) decreased significantly compared with that of fresh mandarin fish (60.51%) ($p < 0.05$), it remained significantly higher than SFA ($p < 0.05$), which was very close to the value in yellow-spotted basket fish (51.43%) (Zhuang *et al*., 2008). This indicated that UFA content in mandarin fish was relatively high. The significant decrease in UFA content (particularly PUFA) might have been due to the complex lipid oxidation reaction of fresh mandarin fish during curing.

Balanced fatty acids in human food affect health and growth significantly. Among the SFA and UFA observed in stinky mandarin fish, the most abundant were palmitic acid (29.59%) and oleic acid (26.14%). These values were significantly higher than those in fresh mandarin fish; the content of palmitic and oleic acids were 21.87 and 24.57%, respectively ($p < 0.05$). The oleic acid content was also higher than in the yellow-spotted basketfish (12.68%) (Zhuang *et al*., 2008).

Additionally, although the contents of UFA such as palmitoleic, α-linolenic, and linoleic acids in stinky mandarin fish meat were significantly lower than those in fresh mandarin fish ($p < 0.05$), they were still relatively high, reaching 8.88, 8.06, and 3.84%, respectively. The essential fatty acids, such as linoleic

Table 6. Fatty acid compositions in fresh and stinky mandarin fish muscles (%).

Fatty acid	Fresh mandarin fish	Stinky mandarin fish
C14:0	2.93 ± 0.02^a	3.13 ± 0.20^a
C15:0	1.30 ± 0.03^a	1.10 ± 0.14^a
C16:1n-6	10.56 ± 0.17^a	8.88 ± 1.42^b
C16:0	21.87 ± 0.49^a	29.59 ± 1.18^b
C17:1n-8	0.70 ± 0.03^a	0.75 ± 0.23^a
C17:0	1.63 ± 0.09^a	2.64 ± 0.18^b
C18:3n-7	0.45 ± 0.04^a	0.15 ± 0.10^b
C18:4n-3	1.72 ± 0.23^a	0.61 ± 0.00^b
C18:2n-6	5.76 ± 0.93^a	3.84 ± 2.35^a
C18:1n-9	24.57 ± 1.27^a	26.14 ± 1.60^b
C18:3n-3	11.07 ± 0.97^a	8.06 ± 0.03^b
C18:0	5.07 ± 0.37^a	6.50 ± 0.59^a
C20:4n-3	0.12 ± 0.02^a	0.31 ± 0.33^a
C20:4n-6	1.07 ± 0.16^a	0.48 ± 0.28^b
C20:5n-3	1.07 ± 0.05^a	0.28 ± 0.04^b
C20:3n-3	0.55 ± 0.04^a	0.26 ± 0.07^b
C21:5n-3	0.94 ± 0.05^a	0.25 ± 0.00^b
C21:1n-9	1.34 ± 0.01^a	1.89 ± 0.00^b
C22:6n-3	0.42 ± 0.03^a	0.17 ± 0.00^a
C23:0	0.15 ± 0.02^a	0.12 ± 0.07^a
ΣSFA	33.63 ± 0.87^a	44.23 ± 1.39^b
ΣUFA	60.51 ± 3.56^a	51.55 ± 1.06^b
ΣMUFA	37.18 ± 1.41^a	37.65 ± 0.60^a
ΣPUFA	23.34 ± 2.26^a	13.90 ± 1.51^b
Σn-3 PUFA	15.78 ± 1.27^a	9.35 ± 0.43^b
Σn-6 PUFA	17.39 ± 1.06^a	12.98 ± 0.98^b
Σn-6/ Σn-3	1.10	1.38
ΣPUFA/ ΣSFA	0.69	0.31

Different lowercase superscripts in similar row indicate significant differences ($p < 0.05$). ΣSFA: total amount of saturated fatty acids; ΣUFA: total amount of unsaturated fatty acids; ΣMUFA: total amount of monounsaturated fatty acids; ΣPUFA: total amount of polyunsaturated fatty acids; Σn-3 PUFA: total amount of n-3 series polyunsaturated fatty acids; Σn-6 PUFA: total amount of n-6 series polyunsaturated fatty acids; AA: arachidonic acid; EPA: eicosapentaenoic acid; and DHA: docosahexaenoic acid.

and linolenic acids, of stinky mandarin fish were higher than those of other common freshwater fish (*e.g.*, grass carp) (Xiao *et al*., 2004). In stinky mandarin fish meat, other important UFAs, such as arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were also detected in small quantities, but their contents were significantly lower than those observed in fresh mandarin fish (p < 0.05), and the total amount was approximately 0.91%.

The ratio of n-6 series PUFA to n-3 series PUFA (n-6 PUFA/n-3 PUFA) is an important index for measuring the nutritional value of fatty acids in seafood. The nutritional value increases with a decrease in the ratio (Dai *et al*., 2013b). The recommended maximum safe level of n-6 PUFA/n-3 PUFA in human food is 4.0. If food exceeding this value is ingested over a long period, it may lead to cardiovascular disease, affecting human health. The minimum safe limit of PUFA/SFA in the food is 0.45, and the higher the ratio, the greater the nutritional value of food (Cai *et al*., 2016). The n-6 PUFA/n-3 PUFA of stinky mandarin fish meat was 1.38, which was higher than that of fresh mandarin fish (1.10). The PUFA/SFA value was 0.31, which was lower than that of fresh mandarin fish (0.69) (Table 6), indicating that the fatty acid quality of meat in fermented mandarin fish showed a sharply decreasing trend. However, its n-6 PUFA/n-3 PUFA ratio was far lower than the recommended maximum limit; thus, the lipid quality of stinky mandarin fish was high for cultivation and consumption.

Mineral composition

Minerals are essential substances for sustaining life and normal metabolism. As shown in Table 7, stinky mandarin fish meat was rich in minerals. Among the major elements, Na content (786.11 mg/100 g) was the highest, which was primarily due to the addition of sodium chloride during the curing process, followed by K (178.85 mg/100 g), P (126.46 mg/100 g), Ca (52.48 mg/100 g), and Mg (23.21 mg/100 g). Among the trace elements, Zn (1.21 mg/100 g) was the highest, followed by Fe (0.96 mg/100 g), and Cu (0.05 mg/100 g).

Table 7. Mineral contents in fresh and stinky mandarin fish, and other fish muscles (mg/100 g, wet weight).

Mineral	Fresh mandarin fish	Stinky mandarin fish	Silver carp**	Yellowfin tuna***
Ca	27.75	52.48	177.00	3.83
P	143.01	126.46	116.00	275
K	203.28	178.85	539.20	492.58
Mg	27.40	23.21	16.80	29.08
Na	63.31	786.11	68.70	86.60
*Zn	1.08	1.21	0.69	0.33
*Fe	2.38	0.96	0.79	0.97
*Cu	0.05	0.05	0.07	0.16

*Essential trace element for human. **Mineral content of silver carp muscle is referred to Jin and Li (1998). ***Mineral content of yellowfin tuna muscle is referred to Luo *et al*. (2008).

Ca is primarily involved in the growth and development of bones, teeth, and nerves in the body, and closely associated with the cardiovascular system, nerve conduction, and muscle contraction (Jin and Li, 1998). The Ca content of stinky mandarin fish meat was 52.48 mg/100 g, which was not as high as that of silver carp (177.00 mg/100 g) (Luo *et al*., 2008), but was much higher than that of yellowfin tuna (3.83 mg/100g) (Jin and Li, 1998). Fe, Zn, Cu, and other trace elements are important in the formation of various active enzyme centres, and act as activators of certain enzymes, playing a vital role in the synthesis of proteins, nucleic acids, carbohydrates, and lipids, and in various immune processes (Gómez-Limia *et al*., 2021). The observed Zn content of stinky mandarin fish was 1.21 mg/100 g, higher than that of yellowfin tuna (0.33 mg/100 g) (Luo *et al*., 2008) and silver carp (0.69 mg/100 g) (Jin and Li, 1998). Given that nearly all of the Zn and Ca currently ingested by humans are derived from food, stinky mandarin fish could be regarded as a potential source of Zn and Ca in the human diet.

Discussion

Salting is a traditional fish preservation method. During salting, the salt solute diffuses into the tissue of the fish, causing water to flow outward from the fish. When it finally reaches a balance, it increases the osmotic pressure of the fish, and reduces water activity, thereby partly inhibiting the spoilage activities of certain microorganisms and enzymes (Czerner and Yeannes, 2013). Salt (sodium chloride) is crucial in the fermentation of stinky mandarin fish by penetrating the fish body, and increasing saltiness. After the mandarin fish is cured, osmotic pressure causes the moisture content of the finished product to drop to 76.91%, and the protein, crude fat, and ash contents to increase to 17.69, 2.34, and 3.28%, respectively (Figure 2). Cai *et al*. (2016) reported that the water content of Chinese lees fish made from the large yellow croaker (a traditional fermented fish product from southern China) was 51.50%, the crude protein content was 19.00%, the crude fat content was 13.01%, and the ash content was 2.67%. The chemical composition of six types of *Suanyu* prepared using traditional fish fermentation processes from the Xiangxi Autonomous Prefecture, Hunan, using grass carp and carp as raw materials, were analysed. The moisture content of the *Suanyu* was 52.9 - 58.1%, protein content 17.2 - 22.9%, fat content 0.88 - 1.48%, and ash content 4.18 - 8.18% (Zeng, 2013). The moisture content of stinky mandarin fish was significantly higher than that of Chinese lees fish and *Suanyu*, and the notable difference in the water content might be the primary reason for the distinctive creamy texture in stinky mandarin fish compared with those in the other two traditional fermented freshwater fish dishes (Yang *et al*., 2017). Thus, stinky mandarin fish was found to have a smooth and tender taste along with healthy dietary attributes of low fat and high protein.

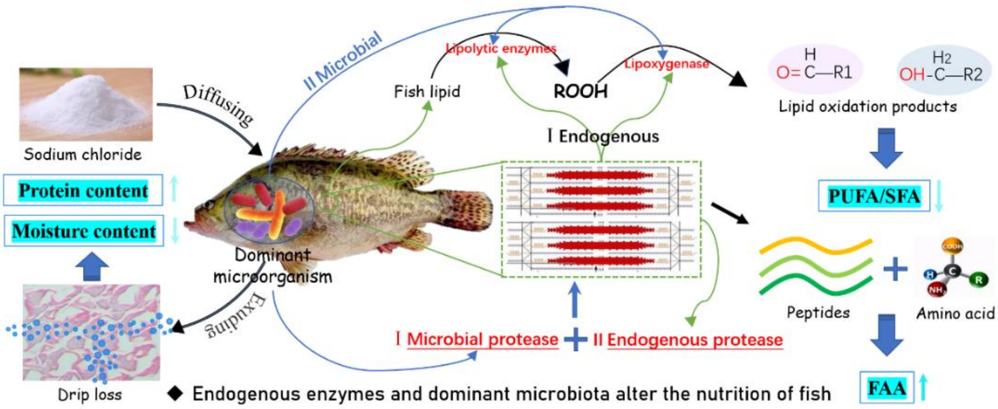

Figure 2. Proposed formation mechanism of nutritional quality of traditional fermented freshwater fish.

Although salting can inhibit the growth of some microorganisms (Wang *et al*., 2021b), under suitable environmental conditions, microorganisms associated with freshwater fish can use fish meat as a "culture medium" for rapid reproduction, and therefore become dominant strains. This leads to the initiation of natural fermentation processes within fish (Dai *et al*., 2013a). Additionally, endogenous proteases, such as cathepsin, can decompose abundant proteins in fish after slaughter (Xu *et al*., 2021). Wang (2017) studied the protein degradation of *Suanyu* made from carp during fermentation, and reported that during the initial stage of fermentation, microorganisms secreted protease, which had a weak ability to hydrolyse sarcoplasmic and myofibrillar proteins, but could still decompose small molecular proteins in fish to obtain amino acids for metabolism and reproduction. Following the start of the fermentation, the endogenous proteases of the fish were activated by the acidified environment, and a large number of macromolecular proteins, such as sarcoplasmic and myofibrillar proteins, were decomposed to generate peptides and amino acids. In the subsequent process, some amino acids were deaminated, decarboxylated, or further metabolised to produce small molecules such as aldehydes and ketones (Wang *et al*., 2017). Therefore, microbial metabolism during fermentation, in conjunction with endogenous enzyme activities, could alter the amino acid content and composition of freshwater fish (Yang *et al*., 2020). The present work found that traditional fermentation did not change the

EAA/TAA ratio in mandarin fish, but the nutritional evaluation results revealed that following short-term fermentation, the EAAI level in the mandarin fish decreased from 65.11 to 60.89, indicating that the quality of amino acids decreased slightly. This was consistent with the trend observed in Chinese lees fish made from large yellow croaker (Cai *et al*., 2016). This indicated that amino acid quality could be improved or supplemented by the metabolic regulation of the fermentation process, or by pairing appropriate side dishes with meals.

As freshwater fish is fermented, its proteins and basic nutrients are degraded to produce polypeptides, small peptides, amino acids, and ammonia, and these small molecules contain flavour-causing substances (flavour-causing amino acids and peptides), flavour precursor substances, or flavour-enhancing agents (Hao *et al*., 2023), imparting fermented fish products a delicious taste (Feng *et al*., 2021). Chen *et al*. (2023) reported that amino acids with an umami flavour could be responsible for the distinctive taste of the stinky mandarin fish. Yang *et al*. (2022a) studied the umami peptides of stinky mandarin fish based on the umami receptor T1R1/T1R3 *via* molecular docking, and 400 umami peptides with molecular weights < 1,500 Da were identified, in which the ratio of glutamic and aspartic acid was > 30%. These peptides were proteolytically formed from 77 precursors, including myosin, troponin, and actin. Peptidomics- and metagenomics-based analyses revealed that proteases from *Vagococcus*, *Peptostreptococcus*, *Acinetobacter*, *Psychrobacter*, and *Enterococcus* were crucial in the formation of umami peptides in stinky mandarin fish, and it was believed that the key umami peptides were primarily derived from troponin and myosin, while the lactic acid bacteria contributed the most to the hydrolysis production of umami peptides (Yang *et al*., 2022b). The findings of the present work revealed that the FAA content in mandarin fish increased substantially after fermentation, and delicious amino acids increased from 8.97 to 187.81 mg/100 g. The traditional fermenting process produced more FAAs and umami peptides, which imparted a spicy flavour to stinky mandarin fish.

The decomposition and oxidation of fish lipids during fermentation is one of the primary methods for creating flavour substances in traditional fermented freshwater fish, and lipid content is another important index useful in evaluating the nutritional quality of fermented fish. During the early stage of fermentation, the initial degradation of freshwater fish lipids is primarily induced by endogenous lipases (lipolytic enzymes and lipoxygenases). Fish lipids are decomposed by the lipolytic enzymes (neutral lipase, acid lipase, and phospholipase), and release FAAs, which are subsequently mostly oxidised through lipoxygenases. During fermentation, the dominant microorganisms also secrete exogenous enzymes that can decompose and oxidise fat, thus being involved in lipid degradation in fish. In addition, environment-mediated auto-oxidation during fermentation may be another important cause of lipid changes in freshwater fish (Feng *et al*., 2021). The present work found that in stinky mandarin fish, the ratio of n-6/n-3 fatty acids increased, and the PUFA/SFA ratio decreased, indicating that the fatty acid quality of mandarin fish decreased after fermentation. Xie (2018) found that mixed strain fermentation (*i.e*., consisting of three fermented strains: *Staphylococcus xylosus* 135, *Saccharomyces cerevisiae* 31, and *Lactobacillus plantarum* 120) could effectively improve the fatty acid quality of *Suanyu* compared with that of single strain fermentation. Xu *et al*. (2022) showed that the combined use of papain synergistic fermentation could enhance the release of EPA and DHA in stinky mandarin fish. Thus, further investigation is required to control fat oxidation and breakdown, and improve the quality of fatty acids in fermented mandarin fish products.

Conclusion

Compared with raw material, the stinky mandarin fish muscle moisture content decreased significantly (p <0.05) after salting, with corresponding increases in crude protein, lipids, and ash contents of 17.69, 2.34, and 3.28%, respectively. The stinky mandarin fish fermented using the traditional Huangshan process maintained higher water content compared with that of other fermented fish. High protein and low fat resulting from high-quality amino acids (EAA accounting for 40.00% of TAA) and fatty acids (UFA accounting for 51.55%) were observed. Moreover, traditional fermentation increased total FAA content in mandarin fish muscle by nearly 14 times. However, despite these findings, further research is needed into how to use modern processing technology to improve the nutritional quality of fermented mandarin fish.

Acknowledgement

The present work was financially supported by the China Agriculture Research System (grant no.: CARS-48), Anhui Provincial Modern Agro-industry Technology Research System (grant no.: AHCYJSTX-08), Key Natural Science Research Projects of Colleges and Universities in Anhui Province (grant no.: 2023AH051540), and Projects of Quality Engineering at Huainan Normal University (grant no.: 2022hsjyxm26). and the Cooperative Projects of Hefei University of Technology-Huangshan Wanxin Huisan Food Supply Chain Co. Ltd. (W2022JSKF0866).

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