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Original Research Articles

The nutritional characteristic of spotted seabass (*Lateolabrax maculatus*) flesh

Siyu Hou[1,2a], Bo Zhang[2,3b], Pengfei Wang[2,3], Chao Zhao[2,3], Lulu Yan[2,3], Yunxiang Lin[3], Feng Lin[4], Xiuting Qiao[1c], Lihua Qiu[2,3,5d]

[1] College of Fisheries, Tianjin Agricultural University, Tianjin 300384, China, [2] Key Laboratory of South China Sea Fishery Resources Exploitation and Utilization, Ministry of Agriculture and Rural Affairs, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, Guangdong 510300, China, [3] Sanya Tropical Fisheries Research Institute, Sanya, Hainan 572019, China, [4] Raoping West Coast Biotechnology Co., LTDRaoping, Guangdong 510300, China, [5] Key Laboratory of Aquatic Genomics, Ministry of Agriculture and Rural Affairs, Chinese Academy of Fishery Science, Beijing 100141, China

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Spotted seabass (*Lateolabrax maculatus*) is a popular food fish in Asian countries. Not only does the fish tasted well, but it is also widely utilized as nutraceuticals. However, the knowledge about the composition of the fish is lack. In this work, the proximate composition of spotted seabass flesh was determined. The content of proximate composition (moisture, proteins, fat, and ash) of spotted seabass flesh was fell within the range of other reported fish species, while it was a high-quality food fish with low oil-very high protein. The ratio of essential amino acids (EAAs) to nonessential amino acids (NEAAs) was 0.74, and the amount of EAAs accounted for approximately 42.34% of the total amino acids. Unsaturated fatty acids composed the majority of the fatty acid components, with linoleic acid was the predominate one. The content of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were abundant in spotted seabass flesh. The proximate composition of the dorsal, ventral, lateral, and tail flesh was similar, except for the ventral flesh exhibited a higher content of fatty acids and lower content of moisture. Our research will be helpful for the general population to know the nutritional traits of spotted seabass.

INTRODUCTION

Nowadays, there is a growing awareness about the nutrition of our eating foods, and fish is gaining momentum because of its distinctive nutritional benefits.[1,2] The main edible part of fish, the flesh, is one of the most important sources of high-quality nutrients for people.[3] It is a unique source of nutrients and easily digestible proteins, has an excellent amino acid and fatty acids composition.[1] The flesh accounts for 15-25% of the total protein weight of fish.[4] Since the above features, fish is commonly consumed all around the world.[1] In addition, fish flesh contains a variety of biologically active compounds. Biologically active proteins and peptides from some fish are of great interest to the nutraceutical, pharmaceutical, and cosmeceutical sectors due to their wide range of bioactivities, including antioxidant, antibacterial, and anti-aging properties (Venkatesan et al., 2017). Antioxidant and antimicrobial peptides derived from fish are used as functional additives in food formulations to improve consumer health and the shelf life of food products (Najafian and Babji, 2012).[5] Many fish lipids, especially polyunsaturated fatty acids (PUFAs), are essential for human health.[6] Therefore, fish is widely used in the fields of human daily diet, biomedicine, health care, etc (Senevirathne and Kim, 2012). Despite the fact that the nutritional value of many fish is well recognized, it is unclear exactly what the nutrients in many species are made up of. Additionally, different fish species have diverse chemical compositions.[6] Thus, designing a scientifically healthy diet and food manufacturing approach is difficult due to a lack of information regarding the nutritious composition of some common food fish.

For the proximate composition of fish, only the genetic regulatory mechanism of adipogenesis has been well-studied at present. Adipogenesis includes two stages: the mes-

a These authors contributed equally to this work

b These authors contributed equally to this work

c Corresponding authors. Xiuting Qiao, qxt6510@126.com

d Corresponding authors. Lihua Qiu, qiugroup_bio@outlook.com

enchymal stem cells (MSCs) fate determination and preadipocytes differentiation. At the initial stage, the commitment of MSCs into adipocyte precursors, which is mainly regulated by transcription factors, such as *Bone Morphogenetic Proteins* (*MBP2*, *MBP4*) and Fibroblast growth factor (*FGF1*, *FGF2*) genes. At the following stage, preadipocytes undergo terminal differentiation into mature fat cells, which is mainly determined by *peroxisome proliferator-activated receptor γ* (*PPARγ*), CCAAT enhancer binding protein α (C/EBPα), *EBF transcription factor 1* (*EBF1*), *Krueppel-like factor 15* (*KLF15*), etc.[7]

Spotted seabass (*Lateolabrax maculatus*) is a valuable commercial fish, which is a very popular food fish among Asian consumers, particularly in China, Kore and Japan. The consumption of spotted seabass is keep rising in recent years, and China alone consumed more than 199,000 tons in 2021.[8] In addition to being delectable, the spotted sea bass serves as an essential functional food that is widely utilized in nutraceuticals and dietary therapy.[9] As a traditional Chinese medicine food, spotted seabass can be used to treat a variety of illnesses and inflammation-associated conditions, such as coughs, wounds, miscarriages, cardiovascular disease, ulcerative colitis, and hyperlipidemia.[2,9] A recent study has demonstrated that the extract of spotted seabass can significantly reduce the inflammatory response and increase wound closure rate in mice (Chen et al., 2021). However, the basic chemical compositions of spotted seabass that may be associated with the above characteristics are largely unknown.

In this study, the proximate composition of pond-cultured spotted seabass flesh was systematically determined. Additionally, the flesh composition of spotted seabass and several commonly consumed edible fish species was compared. In theory, this study could inform the general public about the nutritional composition of spotted seabass and offer data for aquaculture nutrition research.

MATERIALS AND METHODS

Experimental fish and sample collection. One year old pond-spotted seabass (n=23, average weight: 650±20 g) were captured from the Zhuhai aquaculture base of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences in August 2021. The rearing environment parameters are as follows, average water temperature: 28 °C, salinity: 8.5‰, dissolved oxygen: 7 mg/L. Before sampling, all fish were anesthetized with 50 mg/L MS-222 (Sigma Aldrich, St. Louis, MO, USA). The flesh was immediately collected and frozen with liquid nitrogen. The samples were subsequently transferred to -80 ° C freezer until use.

Proximate composition detection and evaluation of spotted seabass flesh. To characterize the proximate composition of spotted seabass flesh, the whole flesh was selected for determined. The content of moisture, protein, fat, ash, amino acids and fatty acids were determined according to the method of GB 5009.3-2016, GB 5009.5-2016, GB 5009.6-2016, GB 5009.4-2016, GB 5009.124-2016 and GB 5009.168-2016, respectively. In brief, the moisture content was determined by evaluating the loss of weight after drying the sample in hot air oven at 105℃ overnight until weight stabilized. Protein content was determined by multiplying the nitrogen content obtained by Kjeldahl's method by the conversion factor 6.25. Fat content was determined by extracting fat using Soxhlet method. Ash content was determined in moisture free dry samples in a muffle furnace at 550℃ for 20 h until all organic components of sample were incinerated completely.[10] Data from the different compositional analyses was subjected to the ANOVA method ($P < 0.05$) to investigate differences among different body portions.

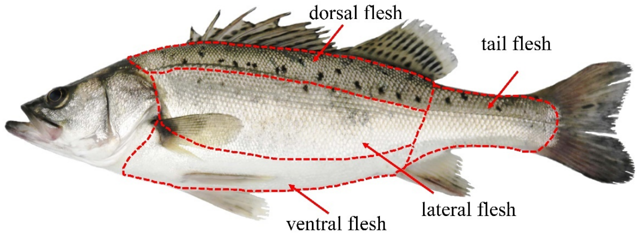

Figure 1. Location of the 4 different spotted seabass flesh portions sampled.

To evaluate the flesh of spotted seabass, the compositional data of several available typical common commercial fishes around the world was considered for comparative analysis. In this study, the selected fish species were Atlantic salmon (*Salmo salar*),[11] Nile tilapia (*Oreochromis niloticus*),[12] European seabass (*Dicentrarchus labrax*),[9] rainbow trout (*Oncorhynchus mykiss*) (Sabetian et al., 2012) and sardine (*Sardina pilchardus*).[13]

Comparative analysis of spotted seabass flesh composition and adipogenesis in different body portions. To analyze the compositional variance of the flesh from various body portions, the fleshes from dorsal, ventral, lateral and tail of spotted seabass were collected (**Figure 1**). The proximate composition analysis was the same as above. Key genes implicated in the signaling pathway of adipogenesis were detected by quantitative real-time PCR (qRT-PCR) using specific primers (**Supplementary table 1**), which were designed using Primer 5.0 software (Plymouth, UK). In brief, the total RNA of the above four portions were isolated with the high Pure RNA isolation kit (Tiangen, China). Then the RNAs were used to synthesize the first strand cDNA using the ReverTra Ace® qPCR RT Kit (ToYoBo, Japanese). Finally, the cDNAs were used as the template for qRT-PCR with SYBR Premix Ex-Taq (Takara, Japan).*β-actin* gene was used as the internal reference gene. All analyses were performed in triplicate. For data analysis, the 2-ΔΔCt method (Livak and Schmittgen, 2001) was used to calculate the relative mRNA expression of each gene. All data in this study were expressed as the mean ± standard error.

RESULTS

The proximate composition of spotted seabass flesh. In the present study, five fundamental whole flesh compositions of spotted seabass were systematically determined. Like other fishes, the proximate composition of spotted seabass

Table 1. Proximate composition of different food fish species.

Species	Moisture[a]	Protein[a]	fat[a]	Ash[a]	Ref
Spotted seabass	74.81±2.30	21.09±1.35	2.61±0.06	1.48±0.04	The present study
Atlantic salmon	75.17±0.99	20.32±0.47	2.90±0.37	0.91±0.02	Kristinsson and Rasco[11]
Tilapia	81.36±1.86	14.93±1.73	1.08±0.43	0.52±0.02	Islam et al.[12]
European seabass	72.00±0.40	21.00±0.08	4.00±0.50	1.30±0.02	Munekata et al.[9]
Rainbow trout	71.70±1.90	19.65±1.20	4.46±0.20	1.33±0.10	Sabetian et al., 2012
Sardine	74.04±0.02	18.30±0.20	3.71±0.05	2.36±0.02	Bagthasingh et al.[13]

[a]. Data are expressed as mean ± SE (%).

flesh was made up of moisture. It accounted for about 74.81% of the weight of the entire flesh, followed by proteins (about 21.09%), fat (about 2.61%), and ash (about 1.48%) (Figure 2A, Table 1).

As the second abundant component in spotted seabass flesh, the exact amino acid composition of protein was analyzed. The results showed that the most abundant amino acid composition in spotted seabass flesh protein was glutamic acid (Glu), which accounted for roughly 16.10% of the total amino acids. The essential amino acids (EAAs) for human beings accounted for roughly 42.34% of the total amino acids, of which the content of lysine (Lys) was the highest, followed by leucine (Leu), arginine (Arg), valine (Val), threonine (Thr), isoleucine (Ile), phenylalanine (Phe), methionine (Met) and histidine (His) in decreasing amounts. The ratio of essential amino acids to nonessential amino acids (EAAs/NEAAs) was 0.74. Important flavor amino acids (FAAs, aspartic (Asp), Glu, glycine (Gly), alanine (Ala), Phe, and tyrosine (Try)) accounted for roughly 41.55% of the overall content, with Glu being the most abundant (**Supplementary table 2**).

Like other fish, fat was the third abundant component of spotted seabass flesh. The fatty acid profile revealed that 14 fatty acid components were detectable in spotted seabass flesh. The result showed that unsaturated fatty acids were the predominant components of fatty acids. Linoleic acid (LA, C18:2 n-6c) was the most abundant, followed by oleic acid (OA, C18:1 n-9). Eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) were also found to be in high amount, with ratios of 2.91% and 7.23%, respectively. For the case of saturated fatty acids (SFAs), the palmitic acid (C16:0) was the dominant component (**Supplementary table 3**).

Compositional comparing of common food fish species. To better understand the nutritional character of spotted seabass flesh, a comparison analysis was conducted based on the data of several available commonly consumed fish species. The results showed that the ratio of moisture, protein, fat and ash in flesh was similar across species, there were significant differences among species ($p<0.05$). The moisture, the predominant components of flesh, accounted for 71.70% to 81.36% of the total weight among the compared species. The moisture content of spotted seabass (~74.81%) fell within the prescribed limit. The ratio of protein to moisture was 0.28 in spotted seabass, which was just slightly lower than that of European seabass (0.29) among the six evaluated species. Among the compared six species, the ratio of fat to water was relatively low in spotted seabass and tilapia (Figure 2B, Table 1).

The amino acid profiles of the six fish showed a remarkably similar pattern in the amount of amino acids content. Except for tilapia, the content of EAA was higher than 40% in all fish species. Among the six evaluated fish species, the FAAs content of spotted seabass was only lower than that of European seabass (51.74%). Yet, compared to European seabass, spotted seabass had a more balanced distribution of FAAs composition (Figure 2C, **Supplementary Table 2**).

The fatty acid composition varies among the compared fish species. The predominant fatty acid compositions were analyzed in this study. It could be ranked as tilapia, spotted seabass, rainbow trout, sardine, Atlantic salmon, and European seabass in the decreasing order of unsaturated fatty acid content. Among the examined fish species, palmitic acid (C16:0) was the most prevalent saturated fatty acid (SFA). Only tilapia (23.92%) had a lower ratio of palmitic acid than spotted seabass (28.06%). For the group of monounsaturated fatty acids (MUFAs), the oleic acid (OA, C18:1 n-9) was the predominant component in most of the compared fish species. Among the compared 6 fish species, both of the MUFAs presented a middle-level in spotted seabass. In the case of PUFAs, the linoleic acid (LA, C18:2 n-6c), DHA and EPA were the three most abundant components in the evaluated fish species, although their content varied between species. In spotted seabass, the major polyunsaturated fatty acid was LA (27.17%), which was the highest among the evaluated six fish species. The amounts of DHA and EPA were also relatively high in spotted seabass (7.23% and 2.91%, respectively) (Figure 2D, **Supplementary Table 3**).

Among the four proximate compositions analyzed, the amount of ash was the least in the evaluated six fish species. It varied between 0.52 and 2.36. Sardine presented the highest content, and tilapia was the lowest. It accounts for 1.48% of the wet weight in spotted seabass flesh, which placed it in the middle-level of the 6 fish species being compared (Figure 2B, Table 1).

THE COMPOSITION DIFFERENCE OF FLESH FROM DIFFERENT PORTIONS

The proximate composition of the fleshes from the dorsal, lateral, ventral, and tail of spotted seabass were detected to analyze their compositional difference. The result showed

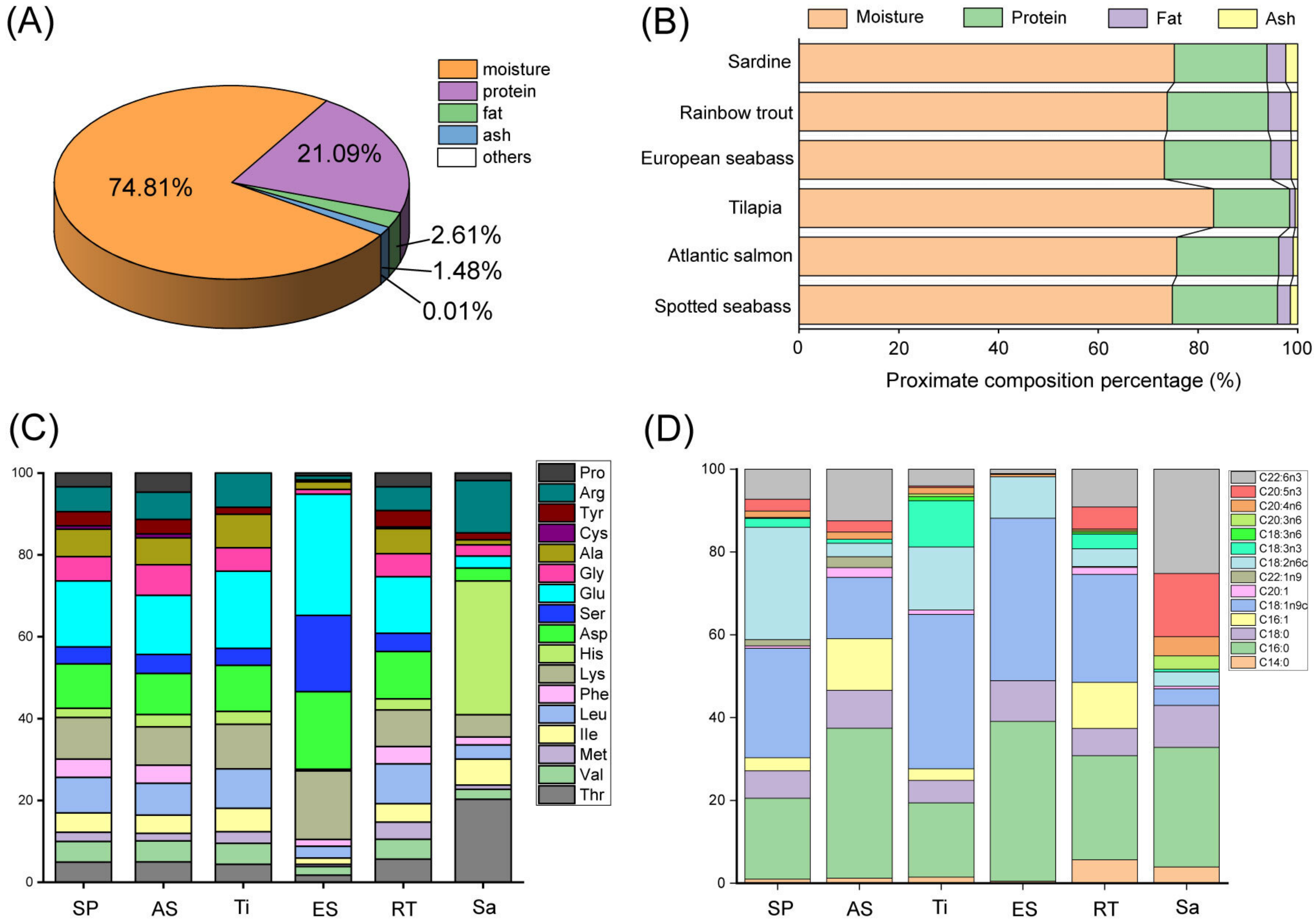

Figure 2. Comparative analysis of the proximate composition in 6 common food fish species.

(A) The whole-flesh proximate composition of spotted seabass. (B) The proximate composition of 6 fish species. (C) The mainly amino acid composition of 6 fish species. (D) The fatty acids composition of 6 fish species. SP, Spotted seabass; AS, Atlantic salmon; Ti, Tilapia; ES, European seabass; RT, Rainbow trout; Sa, Sardine.

Table 2. Proximate composition of spotted seabass flesh from different body portions (wet weight).

Proximate composition	Dorsal[a]	Lateral[a]	Ventral[a]	Tail[a]
Moisture (g/100g)	75.83±0.05	74.37±0.60	70.33±0.61	75.03±0.21
Protein (g/100g)	21.26±0.16	20.44±0.15	20.92±0.36	20.92±0.46
Fat (g/100g)	0.93±0.06	1.58±0.43	5.96±0.54	1.86±0.13
Ash (g/100g)	1.20±0.01	1.67±0.09	1.55±0.07	1.44±0.04

[a]. Data are expressed as means ± SE (%).

that, with the exception of the fat and moisture content in the ventral region, the four detected regions did not differ in other proximate composition (moisture, proteins, fat, and ash). The ventral flesh had a lower content of moisture and a higher content of fat when compared to the other three regions (*p<0.05*). The detailed flesh compositional profiles of the four regions revealed that there were no significant variations in amino acids, while variances in fatty acids (*p<0.05*). The variances of fatty acid were primarily arisen by variations in the contents of several components (**Figure 3A, D, E**, **Table 2**, **Supplementary Table 4, 5**). In terms of saturated fatty acids, the content of myristic acid (C16:0) was significantly lower in dorsal flesh than the other three portions (*p<0.05*), instead, the content of stearic acid (C18:0) was much higher in dorsal flesh (*P<0.05*). The primary variances of the unsaturated fatty acids were DHA (22:6 n-3), which was highest in the dorsal region and lowest in the ventral region.

The genetic regulation under the fat content difference. To clarify the genetic regulation that account for the difference of fat content in different flesh portions, the expression of several key adipogenesis genes were detected. The result showed that genes involved in the transformation of MSCs to preadipocytes, the *MBP2*, *MBP4*, *FGF1* and *FGF2*, were highly expressed in ventral tissue. So did the genes, *PPARγ*, *C/EBPα*, *EBF1*, *KLF15*, driving the differentiation of preadipocytes into mature fat cells (**Figure 3B, C**, **Supplementary Figure 1**). The results indicated that adipogenesis was more active in the ventral flesh.

DISCUSSION

Fish is commonly regarded as one of the healthiest and highest-quality sources of animal protein and play a significant role in our daily diet.[1] Spotted seabass is a valuable commercial fish, wildly consumed in Asian countries. Although spotted seabass is a well-known tasty and functional food fish,[9] little is known about the composition of the fish's main edible portion, the flesh. In this study, a comprehensive analysis of the composition of spotted seabass flesh was conducted.

The chemical composition of fish flesh is widely used as a key indicator to evaluate the nutritional value and quality of fish.[14] According to previous studies, the main components of fish flesh are moisture, proteins, fats, minerals, and carbohydrates.[1] In general, the mineral and carbohydrate content of fish is usually too low (less than 2% of the total body weight, with some notable inaccuracies), the nutritional value of fish was commonly evaluated using the approximate composition of moisture, protein, and fat.[15] Moisture is essential to all living organisms, including fish, since it serves as a medium for the cells' biological processes, such as osmotic pressure maintenance, material dissolution, nutrition delivery, and biological metabolisms.[1] The bulk of fish species reported moisture content that typically ranged from 60% to 81%.[16] By investigating the proximate composition of nine marine fish, Kland discovered that the moisture content of those fish ranged from 87.20 to 79.00% (Kland et al., 2005). The moisture content of six indigenous small fish species of Bangladesh ranged from 65.88% to 78.62%.[17] According to the study that is currently available, the muscle with the highest moisture content was found to be that of the Bombay ducks (*Harpodon nehereus*), whose muscle had a moisture level up to 89.1%.[18] In this study, we found that the moisture content of spotted seabass was about 74.81%, which was comparable to the content of most fish species and suggested that the moisture content of spotted seabass was at a normal level among fish.

Protein is an essential component for the structural and biological function of all living organisms. Fish flesh is known to be an excellent source of high-quality protein for human beings.[1] The protein content of fish flesh accounts for 15-25% of the total weight.[4] In this study, the protein content of the spotted seabass was about 21.09%, with the primary amino acids being Glu, Asp, Lys and Leu. The similar result also observed in other fish. The protein content of rainbow trout muscle was 19.65%, and the major amino acids were comparable to those in spotted seabass (Sabetian et al., 2012). Despite differences in content and ranking of several amino acids, European seabass, a close relative of spotted seabass, showed similar flesh protein content (about 20%) and the main amino acid composition.[9] Considering the low content of fat, spotted seabass can be categorized as a "low oil-very high protein" food fish based on the fish classification system developed by Stansby and Olcott.[19]

The amino acid content of the protein, particularly the quantity of EAAs and FAAs, is an important indicator of food quality in the field of food science.[20] It is known that the EAAs are vital in maintaining human health[3] and promoting wound healing.[9] Fish protein contains all the EAAs in a balanced ratio and is more than 90% digestible for humans (Pigott & Tucker, 2017). According to previous studies, the Lys, Leu, and Arg are three EAAs, which unique abundant in fish species (Fuentes et al., 2010). The content of EAAs in European seabass accounted for about 51.74%

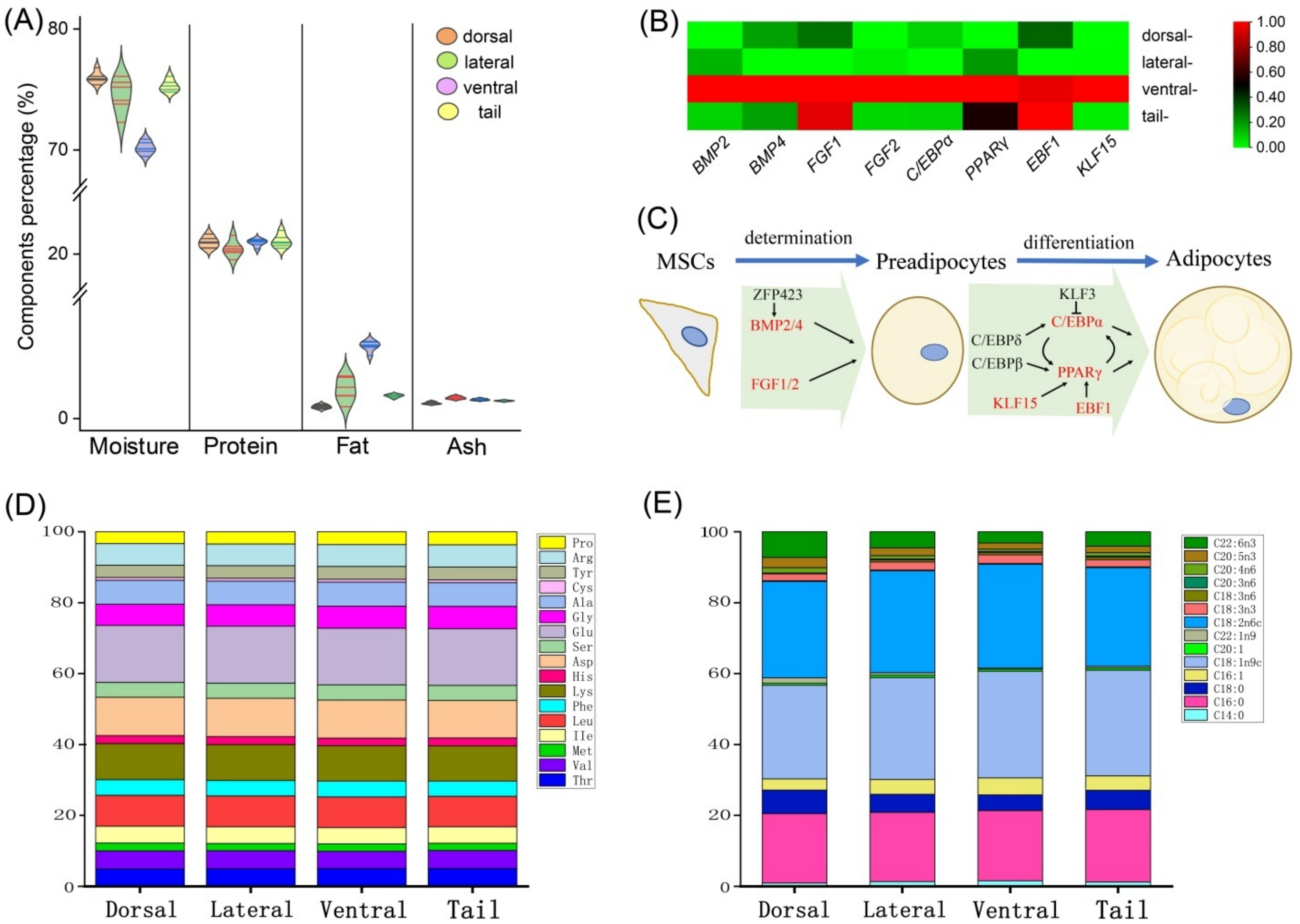

Figure 3. Comparative analysis of flesh components in 4 different portions.

(A) The proximate compositional analysis of flesh from four body regions (n=6). (B) Analysis of relative expression of adipogenesis related genes in 4 different flesh portions (n=3). (C) Schematic of adipogenesis in spotted seabass. Genes highlighted in red indicated the significantly up-regulated genes in ventral flesh. MSC, mesenchymal stem cell. (D) The main amino acid composition of the 4 different portions. (E) The fatty acids composition of the 4 different portions.

of the total amino acids, the EAAs/NEAAs was1.07, and Arg and Leu being the two most abundant amino acid compositions.[9] In rainbow trout, the EAAs/NEAAs was 0.68, Leu and Lys were the two most abundant amino acids, which account for 40.27% of total amino acids (Sabetian et al., 2012). In this study, the most abundant EAAs were Lys and Leu, accounted for 42.34% of the total amino acids, and the EAAs/NEAAs was 0.74. Based on the defining of FAO/WHO's for the quality of protein (1991), spotted seabass protein is a high-quality protein, which would be related to its capacity to serve as a functional food. FAA is in charge of the flavor of food.[20] Previous research demonstrated that the flavor of fish is determined by the characteristic FAAs, such as Asp, Glu, Gly, and Ala (Fuentes et al., 2010).[20] In this study, the content of FAAs was comparatively high and well-balanced, which would account for its flavorful appearance.

Fish fatty acids, particularly PUFA, play important roles in human health.[6,21] Omega-3 fatty acids (n-3 FAs), a subgroup of PUFAs, have been linked to human healthy throughout the lifetime. Because the endogenous production of several n-3 FAs are extremely limited in humans, it is necessary to intake sufficient amounts of n-3 FAs from exogenous food.[22] Therefore, the n-3 FAs are often used as one of the indicators to evaluate the nutritional value of fish.[22] Among the variety of n-3 FAs, DHA and EPA have attracted much attention since they are essential for human health. A deficiency of EPA and DHA cause a variety of disorders, such as inflammation, peripheral artery disease, etc.[23]

In this study, the comparison of numerous popular food fish species revealed that the EPA and DHA concentration in spotted seabass flesh was in the middle range, suggesting that EPA and DHA were not the fish's most distinguishing nutritional characteristics. Spotted seabass can be used as a nutritious diet with a normal need for EPA and DHA. In this study, the result showed that spotted sea bass may be utilized as a food fish with normal demands for DHA and EPA because the content of DHA and EPA in spotted seabass was at a moderate level among commonly consumed fish species. Interesting, another important UPFA, the LA was abundant in spotted seabass flesh (about 27.17%). LA is an essential fatty acid that must be obtained from diet as it cannot be synthesized in the human body.[24] The ω-6 series fatty acids in human body are derived from cis-LA.[25] Conjugated LA has been proven to affect immunological function and to be protective against cancer, obesity, diabetes, and atherosclerosis.[26] According to the data that is currently available, the LA content of fish species ranges from 2.20% to 15.27%.[27] In longsnout catfish (*Leiocassis longirostris*) muscle, the content of LA was 6.52%.[27] In rainbow trout, the content of LA was 3.93% (Sabetian et al., 2012). Thus, the content of LA in spotted seabass was much higher than that of the most common food fish species. Given that the spotted seabass can be used as a functional food in traditional Chinese medicine, our findings may shed some light on the fish's potential therapeutic value.

Understanding the nutritional properties of the meat from various body parts is essential for food science and food processing. The compositional variance between various fish regions has been shown in previously studies. Barbosa[28] found that the center muscle had less fat than the edge samples in flat fish (*Lepidorhombus whiffiagonis*). Likewise, Testi[29] reported that the dorsal and ventral fillet regions of three fish species, European sea bass (*D. labrax*), gilthead sea bream (*Sparus aurata*), and rainbow trout (*O. mykiss*), differed in terms of moisture and lipid content. As well as, they demonstrated the considerable differences of fatty acid components between dorsal and ventral muscle.

In this study, we compared the flesh from four different portions of seabass and found that the primary distinction was the content of fat content: the ventral flesh had a significantly higher amount of fat content than that of the other three portions. Studies have confirmed that the content of fat in fish tissue depends on the number of adipocytes.[30] The adipocytes are derived from MSCs and undergo a two-stage cell transformation that is regulated by a series of adipogenesis genes. Our study showed that the key genes involved in positive regulation of adipogenesis were highly expressed in ventral flesh of spotted seabass. The result indicated a more active adipogenesis in ventral flesh, which would explain the genetic mechanism of higher fat content in ventral flesh of spotted seabass. Our findings were consistent with the previous research.

In conclusion, the spotted seabass was a high-quality food fish with low oil-very high protein. The fatty acid of spotted seabass was dominantly composed of UPFA, of which rich in linoleic acid, DHA and EPA. Except for the higher content of fat in ventral flesh, the other proximate composition of fleshes showed no significant difference among different portions.

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AUTHORS' CONTRIBUTION PER CREDIT

Methodology: Siyu Hou (Equal), Pengfei Wang (Equal). Formal Analysis: Siyu Hou (Equal), Bo Zhang (Equal). Investigation: Siyu Hou (Equal), Bo Zhang (Equal). Writing – original draft: Siyu Hou (Equal), Bo Zhang (Equal), Lulu Yan (Equal). Resources: Siyu Hou (Equal), Yunxiang Lin (Equal), Feng Lin (Equal). Conceptualization: Bo Zhang (Equal), Xiuting Qiao (Equal), Lihua Qiu (Equal). Writing – review & editing: Bo Zhang (Equal), Chao Zhao (Equal). Funding acquisition: Bo Zhang (Equal), Pengfei Wang (Equal), Chao Zhao (Equal), Lihua Qiu (Equal). Supervision: Xiuting Qiao (Equal), Lihua Qiu (Equal).

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SUPPLEMENTARY MATERIALS

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