

# Biochemical change of fermented scallop (*Argopecten Irradians* Lamarck) flour paste in different fermentation periods

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**Abstract** The aim of this paper was to study the biochemical changes in fermented scallop flour paste (SFP) during different fermentation periods. SFP was prepared with scallop muscles, flour koji, salt, rice koji, and neutral protease at 40°C for 12 days. Biochemical changes during fermentation were investigated. By reducing sugar content, formaldehyde nitrogen content, total acid content, and free amino acids (FAAs), the concentration of SFP was increased but water activity was decreased. Most FAAs were increased, and the amounts of glutamic acid, glycine, alanine, and leucine were high. Some amino acid concentrations were higher than their taste threshold, such as the glutamate of umami, the glycine and alanine of sweet and umami, and the arginine and lysine of bitter and sweet. They contributed significantly to the characteristic taste of SFP. Therefore, SFP is a potential seasoning agent with nutritional properties and good taste.

**Keywords** scallop flour paste, fermentation, biochemical changes

## Introduction

Scallop (*Aequipecten irradians*), with a tender meat, delicious taste, and high nutritional value, is one of the three peculiar seafood. It is a popular seafood and an important raw processing material. The rapid development of aquaculture has resulted in a gradual increase in world shellfish yield. Scallop production in China is estimated to be 1172 tons in 2009. Currently, scallop processing mainly comprises primary processing and rough machining, including preserving, freezing and dehydrating treatments (Chung et al., 2002) with a very limited value-adding capacity. The modern microorganism fermentation technologies can be used to ferment the irregular scallop muscle to produce a condiment, which can be developed into a new fermented flavoring to realize the high value added in scallop production, and hence drive regional economic development.

SFP is a mixed product of irregular scallop muscle and wheat flour by solid-state fermentation. It attracts attention from consumers because of its taste and flavor. In some areas,

it is not only a popular condiment as a necessity in households but also the main source of protein in the diet.

Fermentation, as a kind of food processing technology, enhances the nutritional quality and flavor of products (Wonnop et al., 2004; Jiang et al., 2007; Xu et al., 2008). Fermentation is a complex biochemical reaction process and one of its major biochemical events is proteolysis (Je et al., 2005). Proteins are gradually disintegrated by the microbial and autolytic actions of digestive proteases in organisms. During fermentation, degradation products, such as amino acids and reducing sugars, considerably affect the sensory characteristics of products (Je et al., 2005).

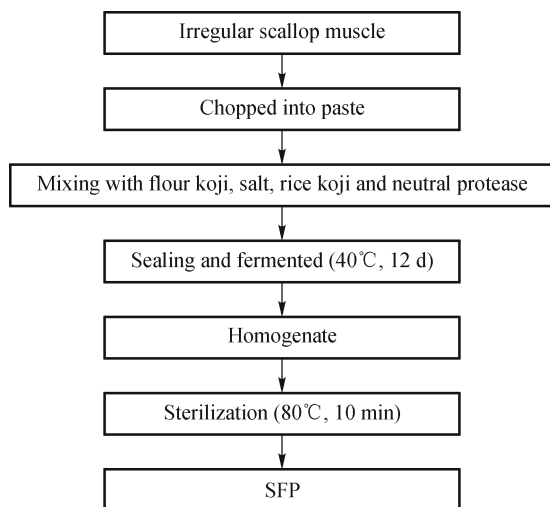
SFP processing, like traditional processes, lacks quality assessment and quality control. It is anticipated that more people would consume it if the production involved good nutritional quality and quality control. The aim of this study was to determine the biochemical changes in SFP during different fermentation periods.

## Material and methods

### Raw materials

The irregular muscle of *Argopecten irradians* Lamarck (water content of about 80%) was obtained from the Bohai Sea in

China. Flour koji and rice koji were obtained from Hebei Huai Mao Co. in China. Neutral protease was purchased from Beijing Solarbio Science & Technology Co., Ltd.. Fig. 1 shows a flow chart of the SFP process.



**Figure 1** Flowchart of SFP process.

### SFP sample preparation

The irregular scallop muscle was chopped into a paste using an organization homogenate device. SFP was prepared by fermenting the mixture of muscle paste, flour koji (50%, koji/paste, w/w), salt (23.21%, salt/paste, w/w), rice koji (5.36%, koji/paste, w/w), and neutral protease (1300 U per gram protein). The mixture was placed in a suitable fermentation container, sealed with frischhaltefolie and isolated from aerobic conditions. The mixture was fermented at about 40°C for 12 d. Finally, the SFP sample was processed by a colloid mill and sterilized at 80°C for 10 min. During fermentation, SFP was analyzed for reducing sugar content, total acid content, formaldehyde nitrogen content, and water activity on the 0, 2nd, 4th, 6th, 8th, 10th and 12th day respectively. FAAs were analyzed on the 0th, 4th, 8th and 12th day.

### Collection of paste

At the designated time, ground SFP was used for analysis.

### Biochemical analysis

*Determining reducing sugar, formaldehyde nitrogen, total acid, and water activity*

Reducing sugar was analyzed by the dinitrosalicylic acid method using a dinitrosalicylic acid reagent, glucose standard, and a Shimadzu UV-540 nm spectrophotometer (Miller, 1959). Formaldehyde nitrogen was determined by titration (Beddows et al., 1976). A 20 mL diluted sample was mixed with 60 mL distilled water and titrated with 0.05 mol/L NaOH to pH 8.2 before a 10 mL formalin solution (37%) was

added. The volume consumed was recorded to determine the total acid of SFP. SFP was finally titrated to pH 9.2 with 0.05 mol/L NaOH. Water activity was determined by a France GBX Laboratory Master-aw using a 2.5 g sample.

### FAA analysis

The samples were used to determine the contents of 18 kinds of FAAs using the precolumn derivatization-high performance liquid chromatography method (Zhao et al., 2007). Briefly, the diluting proportion of SFP and ultra-pure water was 1:3. The 100 L centrifuged sample was placed in a 1.5 mL Eppendorf centrifuge tube with 200 µL of buffer (pH 9.0) and 100 µL derivative reagent to avoid light reaction at 90°C for 1.5 h. Then 50 µL of 10% acetic acid and 550 µL ultra-pure water were added in the Eppendorf centrifuge tube. The mixture was filtered by a 0.45 µm filter and used to determine FAA content using an Agilent 1200 high performance liquid chromatograph. The concentration of 18 kinds of FAAs in SFP samples was calculated by calibrating with standard amino acids.

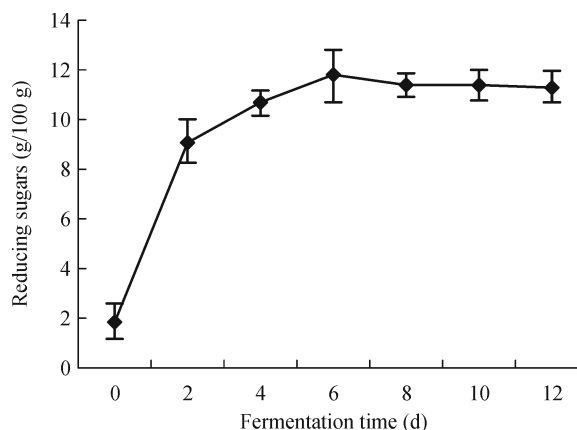
### Statistical analysis

Analysis of variance (ANOVA) was used to search for significant differences between the mean values presented as means ± standard deviation. Parallels ( $N = 3$ ) were used for all analyses.

## Results and discussion

### Reducing sugar content, formaldehyde nitrogen content, total acid content, and water activity

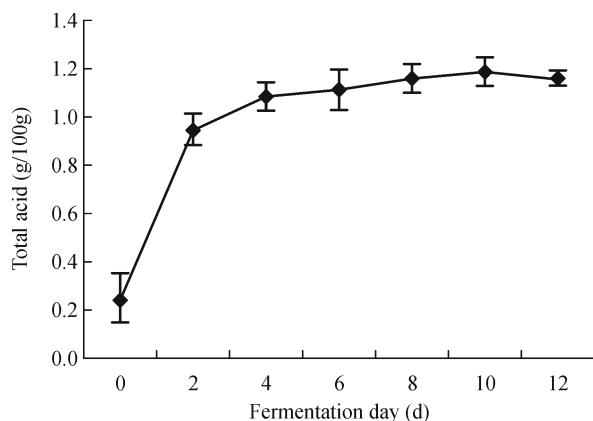
A slight hint of sweetness was found in SFP. The reducing sugar contents at different fermentation periods are shown in Fig. 2. The reducing sugar content of SFP increased fast during the first 2 days. Then from the 2nd day to the 6th day, the reducing sugar content began a slow increase. However,



**Figure 2** The change in reducing sugar content during fermentation.

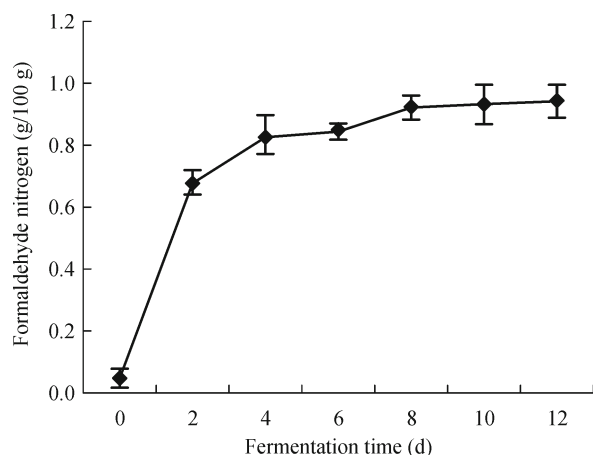
after a little decrease from the 6th to the 8th day, it again increased slightly. The change in reducing sugar content was probably due to the saccharification of diastatic enzymes from flour koji and rice koji during fermentation (Shih et al., 2003), resulting in a  $(11.34 \pm 0.61)$  g/100 g fermented end-product which tasted sweet.

The change in total acid content shown in Fig. 3 revealed a sharp increase from  $(0.246 \pm 0.104)$  g/100 g to  $(0.945 \pm 0.064)$  g/100 g during the first 2 days, and thereafter a slow increase. The peak  $(1.181 \pm 0.056)$  g/100 g appeared on the 10th day, followed by a slight decrease to  $(1.164 \pm 0.027)$  g/100 g on the 12th day of fermentation. The acids may come from raw materials and microbial metabolism (Liu et al., 2004).



**Figure 3** The change in total acid content during fermentation.

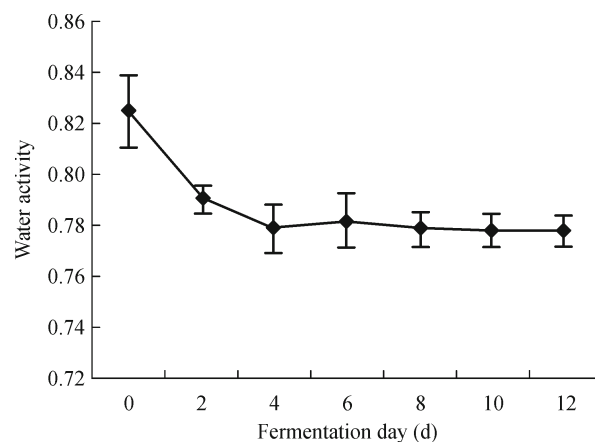
Formaldehyde nitrogen is an important index used to classify the quality of fermented condiments in China (Xu et al., 2008), playing an important role as an indicator of the degree of protein hydrolysis (Byun et al., 2000; Zhao et al., 2009). As shown in Fig. 4, the formaldehyde nitrogen content increased gradually during the whole fermentation, with a quick increase during the first 2 days of fermentation followed by a slow increase, indicating that protein was hydrolyzed



**Figure 4** The change of formaldehyde nitrogen content during fermentation.

gradually by the endogenous enzyme and microbial proteases, and the nutritional value was higher. The formaldehyde nitrogen content was  $(0.95 \pm 0.06)$  g/100 g on the 12th day of fermentation.

In developed countries, water activity is widely used in the food industry as an indicator of food corruption properties and shelf life (Zhao et al., 2008). The change in water activity in Fig. 5 reveals that the water activity decreased sharply from  $(0.825 \pm 0.015)$  to  $(0.790 \pm 0.006)$  during the first 4 days, increased slightly from the 4th day to the 6th day, and then began to decrease slowly, which was attributed to NaCl and biodegradation, with a  $0.777 \pm 0.006$  water activity at the end of fermentation.



**Figure 5** The change of water activity during fermentation.

### Change of FAAs in different fermentation periods

The overwhelming majority of food contains amino acids either in the free form or in the form of partially hydrolyzed protein or intact protein. In recent years, changes in FADs have become more commonly analyzed in food and nutritional products (López-Cervantes et al., 2006). Generally, fish and scallop have high nutrition, owing to their essential amino acid and protein contents (María et al., 2007). FAAs can contribute a lot to the flavor of products (Zhang et al., 2004). The percentage compositions of the FAAs during the different fermentation periods are summarized in Table 1, with the SFP found to be rich in glutamic acid, glycine, alanine and leucine. During fermentation, it was found that levels of aspartic acid, glutamic acid, histidine, serine, arginine, threonine, proline, alanine, valine, isoleucine, leucine, tryptophan, phenylalanine, lysine, and tyrosine were increased, whereas glycine and taurine levels were decreased. The methionine content increased from mixing day to the 8th day of fermentation, and then decreased slightly. After calculating, it was found that the contents of essential amino acids, semi-essential amino acids and the 18 kinds of FAAs were increased during different fermentation periods. The essential amino acid and semi-essential amino acid content accounted for 29.6% of the total FAA content.

**Table 1** The percentage compositions of FAAs in SFP during different fermentation periods

FAAs	Percentage compositions during different fermentation periods (g/kg)			
	On the mixing day	On the 4th day	On the 8th day	On the 12th day
Aspartic acid	0.08±0.05	0.69±0.11	0.97±0.02	1.00±0.01
Glutamic acid	0.50±0.12	3.72±0.62	4.30±0.28	4.31±0.19
Histidine	0.04±0.01	0.29±0.06	0.31±0.13	0.32±0.03
Serine	0.19±0.04	1.17±0.11	1.26±0.21	1.27±0.19
Arginine	0.25±0.04	0.30±0.06	0.31±0.01	0.32±0.02
Glycine	4.79±1.10	4.75±0.33	4.73±0.62	4.71±0.14
Threonine	–	0.62±0.03	0.83±0.04	0.84±0.06
Taurine	1.47±0.46	1.30±0.12	1.17±0.21	1.15±0.11
Proline	0.36±0.02	1.45±0.12	1.49±0.10	1.50±0.03
Alanine	0.40±0.03	4.89±0.23	5.56±0.16	5.63±0.07
Valine	0.11±0.01	0.95±0.01	0.98±0.02	1.06±0.01
Methionine	0.02±0.01	0.37±0.02	0.41±0.01	0.39±0.01
Isoleucine	0.09±0.01	1.55±0.12	1.72±0.07	1.72±0.12
Leucine	0.17±0.01	1.92±0.17	2.07±0.08	2.11±0.10
Tryptophan	–	0.48±0.10	0.49±0.08	0.50±0.04
Phenylalanine	0.12±0.02	1.13±0.23	1.17±0.10	1.17±0.12
Lysine	0.15±0.03	0.94±0.06	0.94±0.03	0.95±0.08
Tyrosine	0.79±0.03	1.44±0.27	1.66±0.08	1.71±0.08
Essential amino acid	0.68±0.03	8.00±0.10	8.69±0.20	8.81±0.30
Semi-essential amino acid	0.29±0.04	0.59±0.05	0.62±0.13	0.64±0.03
Total <sup>a</sup>	9.55±1.38	27.99±0.64	30.45±1.10	30.73±0.71

<sup>a</sup> means total of 18 kinds of FAAs.

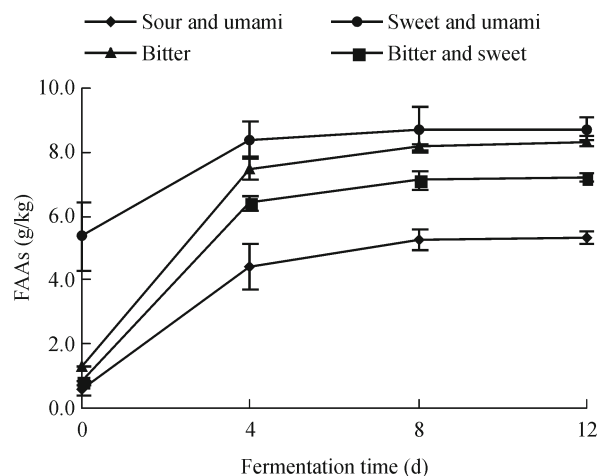
The amount of FAAs at different fermentation periods were statistically analyzed and results indicated that there existed significant differences ( $P < 0.05$ ) in the contents of proline, threonine, aspartic acid, essential amino acids and the 18 kinds of FAAs from mixing day to the 8th day. The amounts of glutamic acid, histidine, serine, alanine, valine, methionine, isoleucine, leucine, tryptophan, phenylalanine, lysine, tyrosine, and semi-essential amino acids were significantly different ( $P < 0.05$ ) from mixing day to the 4th day. However, in other treatments no significant differences were found. From here we see that the reaction rate of protein enzymatic hydrolysis was fast during the first 4 days of fermentation.

Amino acids contribute significantly to the taste of SFP (Jiang et al., 2007). Because every amino acid has different characteristic tastes, sodium glutamate is full of mainly 71.4% umami, 13.5% saltiness, 3.4% sourness, and 9.8% sweetness. However, tryptophan is marked by 87.6% bitterness and 1.2% umami (Liu, 1989; Jinap et al., 2010). Generally, mild hydrolysis of protein produces  $\alpha$ -amino acids which belong to L-amino acids (Shen and Wang, 1990). The tastes of L-amino acids depend on the structure of the side chain. Glutamic acid and aspartic acid taste sour and umami, threonine, serine, alanine, glycine and methionine taste sweet and umami, valine, leucine, isoleucine, phenylalanine, tyrosine, and tryptophan have a bitter taste while histidine, lysine, arginine, and proline have a bitter and sweet taste (Yang et al., 2009).

As for the tastes of FAAs in SFP during different

fermentation periods, Fig. 6 reveals that four sets of tastes of free amino acids increased during fermentation, of which the sweet and umami taste is that of the basic amino acids. At the end of fermentation, it was found that the percentage of sweet and umami was 28.4%, bitter amino acid was 27.1%, sweet and slightly bitter amino acid was 23.5%, and sour and umami amino acid was 17.3%.

When amino acid content is up to the taste threshold, it will stimulate the taste buds of humans. The relationship between



**Figure 6** Change of the flavor free amino acids content with different fermentation time.

**Table 2** Relationship between FAAs concentration and taste threshold of SFP

FAAs	Content (g/kg)	Taste threshold (g/kg)	Multiples relationship
Aspartic acid	1.00	1.00	1.00
Glutamic acid	4.31	0.30	14.37
Histidine	0.32	0.20	1.60
Serine	1.27	1.50	0.85
Arginine	0.32	0.10	3.20
Glycine	4.71	1.10	4.28
Threonine	0.84	2.60	0.32
Proline	5.63	3.00	1.88
Alanine	1.50	0.60	2.50
Valine	1.06	1.50	0.71
Methionine	0.39	0.30	1.30
Isoleucine	1.72	0.90	1.91
Leucine	2.11	3.80	0.56
Phenylalanine	1.17	1.50	0.78
Lysine	0.96	0.50	1.92

concentration and taste threshold of FAAs at the end of fermentation in Table 2 reveals that some FAAs were much higher than the taste threshold, such as the umami sodium glutamate, sweet and umami glycine and alanine, and bitter and sweet arginine and lysine, which were contributed more to the characteristic taste of SFP.

## Conclusions

This study showed that the fermentation of SFP was a complex biochemical reaction process. The amounts of reducing sugar, formaldehyde nitrogen, total titratable acid and most of FAA concentrations were increased during different fermentation periods, while water activity was decreased. Thus, the fermenting technology can significantly improve the nutritive value and safety quality of SFP. At the end of fermentation, glutamic acid, glycine, alanine, and leucine were plentiful. The amount of essential amino acid and semi-essential amino acid accounted for 29.6% of the total FAA content. Some FAAs were recognized as important taste constituents of SFP, including umami sodium glutamate, sweet-and-umami glycine and alanine, and bitter-and-sweet arginine and lysine. In conclusion, these results suggest that SFP is a potential seasoning agent with good nutritional and taste properties.

## Acknowledgements

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