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Amino acid changes in fermented oyster (*Crassostrea gigas*) sauce with different fermentation periods

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Abstract

Fermented oyster sauce (FOS) was prepared with 25% NaCl (w/w) at 20 °C with different fermentation periods. Biochemical changes during the fermentation were investigated. Results revealed that, during fermentation, protein contents were increased whereas carbohydrate contents were decreased. Amino acid composition showed that the contents of aspartic acid, lysine, glutamic acid, glycine and alanine were higher than other amino acids. Free amino acid contents of FOS fluctuated during the fermentation period but most of the free amino acids increased. Particularly, the contents of taurine, glutamic acid, lysine, glycine and alanine were high, which may contribute to the taste and flavour of FOS.

Keywords: Oyster sauce; Amino acids; Fermentation; Chemical composition

1. Introduction

Fermentation is one of the oldest techniques in food preservation as it not only extends the shelf-life but enhances the flavour and nutritional quality of the product (Visessanguan, Benjakul, Riebroy, & Thepkasikul, 2004). Proteolysis is one of the major biochemical events during fermentation. Fish sauce, a fermented product, is a highly appreciated food product in southeast Asia, where the annual production is about 250,000 tons (Stefansson & Steingrimsdottir, 1990). It is basically produced from a mixture of fish and salt (3:1) that has been allowed to ferment for a period of 6–12 months. During fermentation its degradation products, amino acids and peptides, have a considerable effect on the sensory characteristics of fish sauce. In southeast Asia, fish sauce is not only popular as a condiment but, in some areas and certain social classes in the region, it is the main source of protein in the diet and has become a necessity in the household. Fish sauces contain about 20 g/l of nitrogen, of which 80% is in the form of amino acids; thus they may be considered an important sources of protein. Fish sauces have a strong taste; therefore food chemists have been interested in characterizing their chemical composition for a very long time (Kurokawa, 1986; Mizutani, Kimizuka, Ruddle, & Ishige, 1992; Tsuji, Kaneko, Kim, Otaguro, & Kaneda, 1994).

Fish sauce is a product that can be made cheaply from various fish raw materials, which are not normally used for food. This product is still not well known in the western world, but at present there is a growing interest in investigating the suitability of different raw materials for such production (Gildberg, 2001).

In Korea, the production of oyster was estimated to be 182,229 tons in 2002, and only a few fish and shellfish sauces have survived in the local areas in Korea. However, fish and shellfish sauces have been recently rediscovered because of higher consumer interest in their

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taste and flavour. The objective of this study was to investigate the changes of amino acids during different fermentation periods.

2. Materials and methods

2.1. Preparation of fermented oyster sauce (FOS)

Fresh oyster (*Crassostrea gigas*) was purchased from a local shellfish market, and oyster sauce was prepared by mixing oyster (10 kg) with 25% (w/w) salt. The fermentation was carried out at about 25 °C and allowed to continue for 6 months. The FOS obtained after different fermentation periods were filtered on a sieve (40-mesh), desalted by an electrodialyzer, and lyophilized on a freeze-drier for analysis of proximate composition and amino acids.

2.2. Analysis of proximate composition

Moisture, ash, protein, and lipid contents of FOS were determined using the AOAC (1990) methods with some modifications, and carbohydrate content was determined by the phenol–sulphuric acid method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956) in three replicates.

2.3. Amino acid analysis

The FOS were hydrolyzed, and analyzed with an amino acid analyzer (Biochrom 20, Biochrom Ltd., Cambridge, UK). Briefly, 50 mg of FOS were hydrolyzed with 6.0 N HCl in a sealed-vaccum ampoule at 110 °C for 24 h for amino acid composition analysis. The HCl was removed from the hydrolyzed sample on a rotary evaporator, brought to a known volume (10 ml) with 0.2 M sodium citrate buffer (pH 2.2). Amino acids were determined on a Biochrom 20 amino acid analyzer (Pharmacia Biotech., Cambridge, UK) using ninhydrin as colour reactant and on a single ion-exchange resin column (4.0×150 mm).

For determination of free amino acids, 3.0 g of FOS were homogenized at 12,000 rpm twice for 2 min with 20 ml of ice-cold 6.0% (v/v) perchloric acid in an ice bath using an ACE homogenizer (Nissei AM-7, Nihonseikei Kaisha Ltd., Tokyo, Japan). The homogenized sample was then incubated for 30 min in ice before centrifuging at 2000it g for 15 min. The residue was re-extracted with 20 ml ice-cold perchloric acid and centrifuged, as described above. The supernatants from the first and second extraction were combined and filtered through a Whatman No. 41 filter paper. The pH of the filtrate was adjusted to 7.0 using a 33% (w/v) KOH solution, and centrifuged at 2000*g* 10 min to remove the precipitate of potassium perchlorate. The supernatant was acidified to pH 2.2 with a 10 M HCl solution and then diluted to 50 ml with distilled water. Two millilitres of the extract were transferred into a clean tube and 1.0 ml of lithium citrate buffer (pH 2.2) was added to it. Samples were then analyzed on the same amino acid analyzer.

3. Results and discussion

Fish and shellfish sauces are generally prepared with high salt content in east-southern Asian countries such as Indonesia, Thailand, China, Japan, and Korea. However, high salt content in fish and shellfish sauces has limited nutrient value because it cannot be consumed in large quantities (Aryanta, Fleet, & Buckle, 1991). Therefore, it is important to remove salt from fish and shellfish sauces. In this study, we employed the electrodialyzer to remove salt from the FOS and to enhance seasoning and nutrition. The moisture, crude protein, crude lipid, ash and carbohydrate contents of FOS with different fermentation periods are shown Table 1. Moisture content was changed during the fermentation period from 27.82 to 16.01%. Crude protein contents during fermentation were increased by up to 65.6% at 6 months. These results were caused by action of bacteria or mould, and they produce small proteins and peptides. Carbohydrate contents, however, were decreased and little change could be observed in crude lipid or ash contents.

The percentage composition of amino acids of FOS with different fermentation periods is summarized in Table 2. FOS contained high amounts of glutamic acid, glycine, alanine, lysine and aspartic acid. The compari-

Table 1
Proximate compositions of FOS

Components	Contents (%)		
	2 Month	4 Month	6 Month
Moisture	27.82	18.37	16.01
Crude protein	36.6	61.3	65.6
Crude lipid	1.36	1.13	1.12
Crude ash	1.61	1.61	1.49
Crude carbohydrate	32.6	17.6	15.8

Table 2
Percentage composition of amino acids in FOS

Amino acids	FOS		
	2 Month	4 Month	6 Month
Aspartic acid	9.74	11.4	11.4
Threonine[a]	1.75	1.53	1.25
Serine	0.70	0.58	0.22
Glutamic acid	17.8	17.6	17.7
Proline	7.86	6.86	6.61
Glycine	11.1	10.0	9.77
Alanine	9.06	8.29	8.44
Cystine	1.18	0.88	0.64
Valine[a]	5.23	5.69	5.74
Methionine[a]	2.40	2.41	2.33
Isoleucine[a]	3.39	3.91	4.23
Leucine[a]	5.88	6.81	7.61
Tyrosine	1.70	2.07	2.21
Phenylalanine[a]	3.98	4.50	4.50
Histidine[a]	2.35	2.45	2.69
Lysine[a]	9.78	9.41	8.97
Arginine	6.14	5.57	5.68
Anserine	1.23	–	–
Carnosine	1.55	1.84	1.59
Arginine	4.15	4.06	4.08

[a] Essential amino acid

son of amino acid composition of FOS with different fermentation periods showed an increase in aspartic acid, leucine and isoleucine, but decreases in glycine, proline and lysine. When the contents of the essential amino acids in the FOS were compared, lysine was found to be most abundant at 2 months' fermentation. At 6 months' fermentation, the contents of leucine, isoleucine and phenylalanine were increased, whereas the content of lysine was decreased.

The contents of individual free amino acids of FOS with different fermentations are shown in Table 3. The major free amino acids found throughout the whole fermentation period were taurine, glutamic aicd, glycine, leucine, alanine and lysine. Although the contents of some individual free amino acids in FOS fluctuated during the fermentation period, most free amino acids were increased as expected. These compounds are released by microbial action, (mainly by the microbial enzymes), through the biochemical reactions that take place during fermentation. Especially, taurine was the highest in composition of FOS during fermentation and it is well known to have several beneficial physiological actions, including antioxidation, detoxification, osmoregulation, cell membrane stabilization, and neuromodulation (Huxtable, 1992; Kuriyama, 1980; Pasantes, Wright, & Gaull, 1985; Thurston, Hauhart, & Dirgo, 1980; Wright, Tallan, & Lin, 1986). FOS also had high contents of glutamic acid, glycine, lysine and alanine, which are regarded as important contributors to the flavour and taste of FOS.

In conclusion, we analyzed the changes of chemical composition and amino acids of the FOS with different fermentation periods after desalting by an electodialyzer. Free amino acids, such as glutamic acid, glycine, lysine and alanine, were abundant and are recognized as being important in the tastes of fish and shellfish sauces Therefore, these results suggest that the FOS is also a potential seasoning agent with good taste and nutritional properties.

Table 3
Percentage composition of free amino acids in FOS

Amino acids	FOS		
	2 month	4 month	6 month
Phosphoserine	–	–	–
Taurine	15.1	13.9	14.3
Phosphoethanolamine	–	–	–
Aspartic acid	2.74	3.05	3.19
Hydoxyproline	–	–	–
Threonine	3.79	4.26	4.45
Serine	3.77	4.07	4.06
Asparaginine	1.80	1.63	1.70
Glutamic acid	8.45	8.98	8.90
Sarcosine	–	–	–
α-Aminoapidic acid	–	–	–
Proline	5.61	5.74	6.14
Glycine	5.31	5.76	5.33
Alanine	7.11	7.26	7.00
Citrulline	0.67	0.65	0.64
α-Aminobuytric acid	0.63	0.59	0.54
Valine	4.29	5.09	5.12
Cystine	–	–	–
Methionine	2.58	2.89	2.64
Cystathionine	–	–	–
Isoleucine	3.58	4.36	4.43
Leucine	5.88	6.55	6.55
Tyrosine	3.43	3.66	2.87
β-Alanine	1.39	1.20	1.11
Phenylalanine	3.36	3.72	3.89
β-aminoisobutyric acid	0.77	–	–
γ-Aminobutyric acid	0.84	–	–
5-Hydroxylysine	1.68	1.44	1.85
Ornithine	0.97	0.85	0.92
Lysine	5.52	5.05	5.31
1-Methylhistidine	–	–	–
Histidine	1.67	1.54	1.58
3-Methylhistidine	1.83	1.92	1.87

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