REGULAR CHANGES OF FREE AMINO ACID AND TAURINE DURING OYSTER FRESHNESS PRESERVATION

by

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ABSTRACT

Changes of various free amino acids in *Ostrea rivularis*, *Ostrea plicatula* and *Ostrea gigas* were followed at different storage temperatures. The experimental results show some common features. The content of various free amino acids decreased slightly during early storage and then increased irregularly. During storage, changes in the content of various free amino acids are closely associated with the R-group polar of the amino acids. Increase of the non-polar R-group amino acids is more than polar R-group amino acids with or without electric charge. The higher the storage temperature the faster the increase. Taurine content decreased a little during 15 days storage in *Ostrea rivularis*, and *Ostrea plicatula*, but increased a little in *Ostrea gigas*.

INTRODUCTION

Oyster is an aquatic animal of high (Tan Guili *et al.*, 1993) nutritive value. The nutritive components in the cells not only regulate osmotic pressure, but also give the oyster its special taste. According to the reports (Shen Yuyun, 1986) changes in contents of free amino acids and taurine are closely associated with the original contents, autolysis and microbial level (Michiyo Murata, 1986). In the early eighties Japanese researchers did a lot in this field (M, Sakaguchi, 1984) with cold stored fish, such as *Pneumatophorus japonicus, Katsuwonus pelamis, Thunnus* tonggol. They studied the relationship between changes in contents of FAA and indices of freshness. With further study and elucidation of the mechanisms, processing and preservation techniques were improved. Studies in this field started later in China and the information was limited.

Oysters are abundant in China. *Ostrea plicatula, Ostrea gigas* and *Ostrea rivulans* are important species. At present oysters are eaten directly without processing. The study was conducted to learn the biochemistry of free amino acids and taurine in oyster during cold storage, in order to search for new processing techniques.

MATERIALS AND METHODS

Materials: *Ostrea gigas* was obtained from Luoyuan farm; *Ostrea plicatula* and *Ostrea rivulans* were obtained from Longhai farm.

Sample preparation: shelled oyster meat was put in bags and sealed, then stored in three groups at 0~2°C, 3~5°C and 6~8°C.

Determination method

A 30g sample was homogenized with 1.5g sulfosalicylic acid and stored in a refrigerator for one hour. After centrifuging the supernatant was frozen for determination. Before analysis, the liquid was thawed, then determined with Waters' PICO-TAG method. Separation column: PICO TAO c18 3.9x150mm; mobile phase as liquid A:19.0g NaAc +0.5ml triethylamine, dissolved in 940ml distilled water, adjust pH to 6.4, with 60ml CH_3 CN; liquid B: 60% CH_3CN, flow speed: 1.5ml/min, UV detector, wave length: 254nm.

RESULTS

Changes of total amount of free amino acids

Changes of total free amino acids in *Ostrea gigas*, *Ostrea rivulans* and *Ostrea plicatula* stored at different temperature are shown in Fig.1. On the first day, the total of free amount of free amino acids in these oysters were significantly different: 225~252mg/100g in *Ostrea plicatula*, 254~285mg/100g in *Ostrea rivulans* and 472~508mg/100g in *Ostrea gigas*. The contents of amino acids in all oysters increased to different levels during storage. At 0~2°C, the total amount of free amino acids increased slowly. After 15 days the total amount of free amino acids in *Ostrea plicatula*, *Ostrea rivulans* and reached 296mg/100g, 289mg/100g and 498mg/100g respectively. At 3~5°C, total free amino acids increased more quickly, after 15 days, in *Ostrea plicatula*, *Ostrea rivulans* and *Ostrea gigas*, they reached 390mg/100g, 426mg/100g and 662mg/100g respectively. At 6~8°C, total amount of free amino acids increased most quickly. After 15 days, they respectively reached to 605mg/100g, 687mg/100g and 718mg/100g. Increase and decrease of total free amino acids in these oysters occurred during storage, but in general the main trend was an increase.

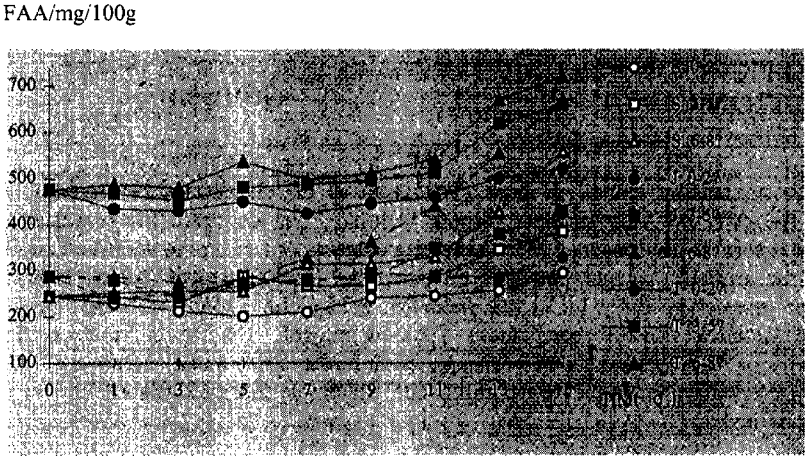

Fig. 1. Changes of total amount of free amino acids in oyster stored in several temperature ranges.

S- *Ostrea plicatula* J- *Ostrea rivulans* T- *Ostrea gigas*

Changes of various free amino acids

Free amino acids in oyster stored at different temperatures changed at different rates, more quickly at higher storage temperature. There were some differences in changes of various free amino acids. According to the presence of side chains in polar amino acids, free amino acids are classified as non-polar amino acids, or polar amino acids with electric charge (Shen Tong *et al.*, 1980). During storage, non-polar amino acids increased more quickly than polar amino acids. Changes in the non-polar amino acids of Ostrea rivulans stored at 6~8°C for 15 days were: Met increased from 1.4mg/100g to 20.2mg/100g, Phe increased from 1.1mg/100 to 29.1mg/100g. Pro increased from 9.7mg/100g to 14.7mg/100g. Iso increased from 1.8mg/100g to 31.2mg/100g. Leu increased from 2.4mg/100g to 51.3mg/100g, Val increased from 5.8mg/100g to 37.5mg/100g. Ala increased from 49.2mg/100g to 89.6mg/100g. Polar amino acids without electric charge increased more slowly. Ser increased from 36.0mg/100g to 53.1mg/100g, Thr increased from 13.2mg/100g to 52.1mg/100g, Tyr increased from 3.9mg/100g to 25.8mg/100g, Cys decreased a little; Polar amino acids carrying electric charge changed slowest: Glu increased from 61mg/100g to 98mg/100g, His increased from 2.2mg/100g to 10.3mg/100g, Arg increased from 26.2mg/100g to 41.3mg/100g, Asp increased a little, and Lys was almost unchanged after 15 days storage. Changes of *Ostrea plicatula* and *Ostrea gigas* were the same. Essential amino acids such as Met, Phe, Leu, Ile and Val are non-polar amino acids, their contents increase rapidly during cold storage.

Changes of taurine

Content of taurine is high in oyster. In *Ostrea gigas*, *Ostrea rivulans* and *Ostrea plicatula* content was 765mg/100g, 250mg/100g and 356mg/100g respectively. During storage, taurine in *Ostrea gigas* increased slightly. After 15 days storage at temperature ranges of 0~2°C, 3~5°C and 6~8°C, they increased to 760mg/100g, 850mg/100g and 854mg/100g respectively, while the content of taurine in *Ostrea rivulans* and *Ostrea plicatula* both decreased slightly, to 270mg/100g, 225mg/100g, 240mg/100g and 376mg/100g, 362mg/100g, 367mg/100g respectively at the three temperature ranges.

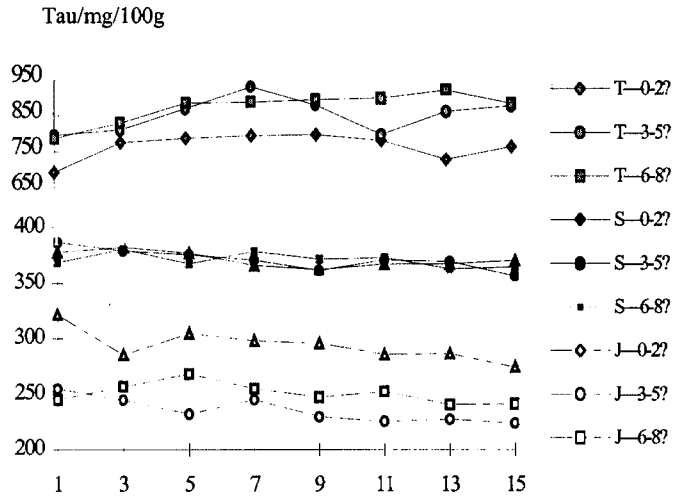

Fig. 2. Changes of taurine in oyster stored in several temperature ranges.

S- *Ostrea plicatula*□ J- *Ostrea rivulans*□ T- *Ostrea gigas*

DISCUSSION

Total amount of FAA in oyster decreased during early storage and then increased in later storage. Shown in Fig.1, total amount of FAA in oyster stored at different temperature changed at different rates. For example, at 0~2°C, total amount of FAA in *Ostrea rivulans* decreased, after 5 days of storage it began to increase, but at 3~5°C and 6~8°C, it began to increase irregularly after 3 days. Changes in *Ostrea plicatula* and *Ostrea gigas* were the same as *Ostrea rivulans*. We consider that these changes are associated with post-mortem physiological changes in the oyster. Fresh oyster meat is neutral or weakly alkaline. A series of biochemical reactions inside the oyster did not stop during storage. Firstly, glycogen was decomposed to lactate, then phytic acid was decomposed to phosphoric acids. The accumulation of lactate and phosphoric acid made the oyster acidity. When the pH of oyster meat reached 5.6~6.0, fibrillin lost solubility due to its lower moisture retention.. At the same time, some free amino acids were used iup as a nitrogen source for bacterial propagation and some free amino acids were decomposed by autolysis (Liu Yongchen, 1996; Wu Guanghong *et al.*, 1990). So FAA in oyster decreased during primary storage but the decrease varied with different storage conditions and different species of oyster. With time of storage on one hand, some proteins were decomposed to peptides, peptones and amino acids due to autolysis in the oyster meat (Wangzhang, 1990), while on the other some were decomposed due to bacterial activity. This led to a combination of amino acid decomposition and synthesis. Total amount of FAA increased when the speed of amino acid synthesis was faster than the speed of amino acids decomposition. We postulate that this is the primary factor in determining changes in total amount of FAA in oyster during storage.

There were some differences in changes of the various free amino acids during storage. These differences are associated with composition and transformation of amino acids in the oyster protein (Zhou

Runqi *et al.*, 1990), and are also associated with the polarity of amino acids. With ongoing time of storage increase of non-polar R-group amino acids is more marked than polar R-group amino acids with or without electric charge. The reason is that polar R-group amino acids with electric charge such as Lys, Arg and Asp are hydrophilic and easily dissolved in water due to the electric charge. Polar R-group amino acids without electric charge such as Cys and Gly can form hydrogen bonds with water and easily dissolve due to having dissociated polar groups in their side-chains. Among them, Ser, Thr and Tyr are polar due to their hydroxy groups (Shen Tong *et al.*, 1980). FAA are dissolved and thus spread throughout the oyster. FAA come into intimate contact with autolytic enzymes and microorganisms. They are easily autolysed and are used up as a nitrogen source for propagation by microorganisms. Non-polar R-group amino acids such as Leu, Val and Ile dissolve with difficulty due to their hydrophobic groups. Amino acids hydrolyzed from proteins attach themselves to proteins so they move more slowly and have less contact with autolytic enzymes and microorganisms. Non-polar R-group amino acids increase rapidly when the speed of hydrolysis to amino acids from proteins is faster than the speed of amino acid decomposition. This phenomenon is distinct during higher temperature storage.

The glutamate in three species of oyster is high. During storage, content of glutamate is 100mg/100g in *Ostrea gigas*, 60-70mg/100g in *Ostrea rivulans* and 50-60mg/100g in *Ostrea plicatula*. This probably relates to the biochemical process of free amino acids, which is synthesized around 2-ketoglutarate and glutamate (Zhou Runqi *et al.*, 1990). The majority of amino acids, such as Gly, SER, Thr, Cys, Val, Pro, Arg, lys, Leu and Ile are probably combined with 2-ketoglutarate to change to Glu and relevant ketonic acids. Arg is synthesized from Glu's N-2acyl intermediate product by microorganisms. This intermediate product is also a precursor of Pro for bacteria. On the contrary, various amino acids can be synthesized from Glu and the relevant ketonic acids by transamination. Some transaminations will only occur in the presence of proteolytic enzymes from bacteria (Cheng Guocheng, 1982).

Content of Asp is relatively stable in oyster, and is about 40mg/100g in *Ostrea gigas*, about 10mg/100g in *Ostrea plicatula* and *Ostrea rivulans*. Synthesized from Asp, Thr can also be changed to Ile. Except for Val, Leu, aryl amino acids and His, the majority of amino acids can be synthesized from Glu, Asp and Gly (Zhang Qianheng *et al.*, 1995).

Like amino acids, taurine will be used up as a nitrogen source for bacterial growth after the oyster's death. Taurine is a non-protein amino acid. It is considered that taurine is changed from a sulphur-bearing amino acid (Zhang Qianheng *et al.*, 1995). During storage, content of taurine in *Ostrea rivulans* and *Ostrea plicatula* decreased slightly, while in *Ostrea gigas* it increased slightly. According to Li Deming (1995), contents of sulphur-bearing amino acids such as Cys and Met are high in oyster protein. They are 510.4mg/100g and 641.6mg/100g respectively. Shown in Fig.2, Met increased slightly, and Cys changed little during storage. For example, at 0-2°C, 3-5°C and 6-8°C, Met in Ostrea plicatula increased to 6mg/100g, 6.2mg/100g and 22mg/100g from 3mg/100g respectively, while Cys in *Ostrea rivulans* and *Ostrea plicatula* was only 6mg/100g, Cys in *Ostrea gigas* was only 9mg/100g. This illustrates that taurine is probably derived from cysteine and leucine which are in turn derived from protein by hydrolysis. Cys in *Ostrea plicatula*, *Ostrea gigas* and *Ostrea rivulans* can be changed from Met and Ser. The sulphur in Cys is from Met by transsulfuration, and the carbon frame in Cys is from Ser. It was reported by Zhang Qianheng that taurine can be formed from Cys through two ways. One way is that Cys can change to sulfonic acid alanine by oxidation, then change to taurine by de-carboxylation. Another pathway is for Cys to change a sulphuric acid group by oxidation and decomposition. Cys loses mercapto- and amino- directly to change to pyruvic acid, ammonia and H2S, then H2S is oxidised to sulphuric acid. The mercapto- in Cys can be oxidised to sulfonic-, then change to sulphuric acid.

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