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Variation of Amino Acids in the Muscle of Crab *Portunus sanguinolentus* During Storage

[1]E. Rethna Priya, [2]K.L. Jeyalakshmi Kala, [1]S. Ravichandran and [3]M. Chandran
[1]Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, 608502, Tamil Nadu, India
[2]Department of Engineering Chemistry, Rajas International Institute of Technology for Women, Nagercoil, 629 001, Tamil Nadu, India
[3]Department of Chemistry, Vivekananda College, Agasteeswaram, Kanyakumari, 629701, Tamil Nadu, India

Corresponding Author: S. Ravichandran, Center of Advanced Study in Marine Biology (Annamalai University), Parangipettai, 608502, Tamil Nadu, India

ABSTRACT

Amino acids are at the basis of all life processes, as they are absolutely essential for every metabolic process. In the present study variation of amino acids in the muscle of the crab *Portunus sanguinolentus* during frozen storage was analyzed. In the freshly caught crabs the levels of Glycine and Arginine were the highest, followed by glutamine (Gln) Ala and proline (Pro) together they accounted for 83% of the total amount of FAA. Taurine (Tau) and Glu were also in fairly large amounts. High levels of Gly, Arg, Pro, Tau and Ala are generally present in the muscle of crabs. The *P. sanguinolentus* muscle was also rich in Gln but low in Tau. The total amount of FAA altered slight throughout storage, but increased by 24-38% prior to the preliminary decomposition stage. The overall quantity of Arg, Gly, Ala and Glu before storage averaged 1272 mg/100 g. the total amount of these four taste-active amino acids increased by12-48% within 4 days of storage at 4°C, but hardly reduced after storage. Significant differences, except glutamic acid and tyrosine, were observed among the amino acid values changed during storage at 4°C. Major FAA such as Gly, Ala, Pro, Gln, Arg and Tau also increased, whereas, their levels usually exhibited small changes or even a decrease except that the Arg and Tau contents increased to some extent. The Glu content fluctuated during the early storage periods but its level reduced after 1 day of storage.

Key words: Crab meat, amino acids, FAA

INTRODUCTION

An adequate supply of dietary protein is required for survival, growth, development, reproduction and maintaining health throughout life. Food and tissue proteins contain 20 different amino acids of nutritional importance. They are nevertheless equally, as important as the indispensable amino acids for the nutrition of cells and for normal cell and organ function (Harper and Yoshimura, 1992, Tapiero *et al*., 2002). The extractive components in fish and shellfish are known to contribute to the taste properties and taste specificity of sea foods. Regarding the snow crab meat, alanine (Ala), arginine (Arg), glutamic acid (Glu), glycine (Gly), Glycine Betaine (GB), adenosine monophosphate (AMP), cytosine monophosphate and guanosine monophosphate are the taste-active components (Hayashi *et al*., 1981).

Similarly, crab meat deteriorates rapidly at refrigerated temperatures. Its high moisture content and loose connectivity between the collagen fibers, provide a rich environment for microbial growth (Suyama and Konosu, 1987). Many publications are available on the amino acid composition (Naczk *et al*., 2004; Chen and Zhang, 2007) of different crab species in various parts of the world. However, nothing is known of amino acid composition of *P. sanguinolentus* crab meat during preservation. Therefore, this study has been carried out to determine the variation of amino acid composition of the edible crab *P. sanguinolentus* muscle during storage.

MATERIALS AND METHODS

Samples: All crabs of *P. sanguinolentus* were caught from the Colachel coastal environment, Southwest coast of India. After catching they were transferred to the laboratory alive. Subsequently, the claw meat and breast meat muscle were dissected blend to homogenize and preserved at 4°C with proper labelling.

Amino acid composition analysis: For amino acid analysis, the samples were hydrolyzed with 6 mol L^{-1} HCL and determined by amino acids analyzer (Biochrom Ltd., Cambridge, UK) with a C18 column (25 cm×4.6 mm), according to the method of Chinese Standard GB/T 5009.124-2003. For measuring tryptophan content, each sample was hydrolyzed with 5 mol L^{-1} NaOH before analysis. The amino acid content was calculated by comparison with retention time and the peak areas of standard amino acids.

Calculations and statistical analysis: Amino Acid Score (AAS) was calculated according to FAO/WHO reference amino acid standard for adults and the Chemical Score (CS) was calculated with respect to the amino acid composition of egg protein. The AAS and CS were calculated using the following formula (Biel *et al*., 2009; Gong *et al*., 2013).

Analyses were carried out in triplicate and the results were presented as mean and standard deviation (Means±SD). The data were analyzed by one-way analysis of variance (ANOVA) through SAS 9.0 statistical software (SAS Institute Inc., Cary, NC, USA). Significant ($p<0.05$) differences among the samples were ranked by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Crab meat has a higher composition of free amino acids compared with finfish and the mechanism of decomposition is somewhat different. In the present study changes in the levels of Free Amino Acids (FAA) were shown in Table 1. Before storage, levels of Glycine and Arginine were the highest, followed by glutamine (Gln), alanine (Ala) and proline (Pro). The five amino acids together accounted for 83% of the total amount of FAA. Taurine (Tau) and Glu were also in fairly large amounts. High levels of Gly, Arg, Pro, Tau and Ala are generally present in the muscle of crabs. In comparison with horsehair and snow crabs, the *P. sanguinolentus* muscles were also rich in Gln but low in Tau. The total amount of FAA changed little during storage, but increased 24-38% prior to the initial decomposition stage. Major FAA, such as; Gly, Ala, Pro, Gln, Arg and Tau also increased, whereas, their levels usually, exhibited small changes, except that the Arg and Tau contents increased somewhat. The Glu content fluctuated during the early storage, but its level reduced after 1 day of storage. The total amount of Arg, Gly, Ala and Glu before storage averaged 1272 mg/100 g. Like the total FAA, the total amount of these four taste-active amino acids increased by12-48% within 4 days of storage at 4°C, but hardly reduced after 1 day of storage.

Table 1: Variation of amino acids (mg/100 g) in the muscle during storage at 10°C

	Days at (4°C)						
Amino acids	0	1	2	4	6	8	10
Aspartic acid	19±2	7±1	7±0	5±2	6±3	5±0	4±3
Serine	6±1	6±1	8±3	13±1	21±2	23±1	25±0
Histidine*	11±2	12±2	14±1	15±0	19±3	25±2	29±1
Tyrosine	19±3	16±2	24±4	32±6	40±3	54±7	52±6
Valine*	22±6	20 ±3	26±6	31±14	39±21	55±4	47±22
Phosphoserine	1±1	1±0	2±1	2±0	3±1	3±1	4±1
Threonine*	11±3	9±1	12±2	15±1	20±1	23±2	26±3
Taurine	82±20	87±8	132±13	144±18	153±25	167±18	193±23
Alanine	187±17	172±18	180±16	173±17	243±24	325±87	376±28
Proline	173±25	187±12	196±19	205±16	212±21	253±21	267±32
Glycine	523±76	607±78	556±41	484±42	442±64	648±67	566±72
Glutamic acid	58±17	31±12	17±3	27±1	31±1	34±4	38±6
Glutamine	208±25	187±25	210±46	218±21	237±22	256±18	252±76
Arginine	554±37	608±49	577±14	543±85	537±42	460±54	430±49
Sarcosine	1±0	3±1	3±0	4±1	2±1	5±2	4±1
Cystine	0±0	1±1	1±1	3±2	4±4	6±5	8±3
Methionine*	13±1	10±1	14±3	18±4	20±2	38±2	44±6
Isoleucine*	10±2	11±3	15±1	20±5	28±4	37±3	43±5
Leucine*	18±2	16±1	20±2	33±7	72±5	80±11	91±8
Phenylalanine*	18±1	17±3	22±5	30±3	36±5	42±5	48±6
Tryptophan	5±2	8±0	12±1	18±2	22±2	24±3	27±0
Ornithine	1±0	1±0	2±1	6±4	18±3	30±2	56±15
Lysine*	37±3	32±5	46±13	53±16	68±14	85±11	102±12

Data is Mean±SD (n = 3), *Essential amino acid

Significant differences, were observed the amino acid values changed during storage at 4°C. In contrast, the ornithine and citrulline contents before storage were small and their levels began to increase when the initial decomposition progressed. The total amount of FAA changed little during storage at 4°C, but increased to the initial decomposition stage. Major FAA such as Gly, Ala, Pro, Gln, Arg and Tau also increased whereas their levels usually exhibited small changes or even a decrease except that the Arg and Tau contents increased somewhat. The Glu content fluctuated during the early storage periods but its level reduced after 1 day of storage.

Crustaceans are beneficial and highly appreciated due to their favorable taste and nutritional quality. The chemical composition and nutritional value of crustaceans have been investigated intensively (Skonberg and Perkins, 2002; Celik *et al*., 2004; Naczk *et al*., 2004; Kucukgulmez *et al*., 2006; Chen and Zhang, 2007; Barrento *et al*., 2009a, b, 2010; Marques *et al*., 2010; Tsape *et al*., 2010; Wu *et al*., 2010; Maulvault *et al*., 2012; Risso and Carelli, 2012). The taste and nutritional quality of crab meat is to a large extent related to the presence of amino acids and fatty acids (Chen and Zhang, 2007). And a balanced amino acid and fatty acid composition is essential for promoting good health as well (Wu *et al*., 2010). The meat protein from *P. sanguinolentus* crabs are relative well-balanced in their essential amino acid compositions. In this present study highly recommend *P. sanguinolentus* crab can be regards as a high quality protein source for human consumption. Taurine plays a vital role in physiological functions during development and aging. Since, human have limitation in biosynthesizing of taurine, the dietary supplementation seems to be necessary. No significant difference in any single amino acid content was found among the samples in the present study. Since humans have limitation in biosynthesizing of taurine, the dietary supplementation seems to be necessary.

Krzeczkowski and Stone (1974) found lower phenylalanine quantities in snow crab compared to this study. The results of the present investigation the essential amino acid values are favorably comparable with the published reports in milk, beef and egg. The World Health Organization

recommended leucine and isoleucine requirements for adults of 14 and 10 mg amino acid/kg body weight/day. In the present investigation found good amount of both leucine and isoleucine were observed from the meat of *P. sanguinolentus* which is much essential for human well-being. Iwasaki and Harada (1985) reported that essential amino acid/nonessential amino acid ratio of many fish species is 0.70 on average. The present amino acid values of crab tissues compared well with the USDA Nutrient Database for Standard Reference (Ahuja *et al*., 2012) U.S. Department of Agriculture, 2004 pattern. However, in the present study, threonine was found to be high whereas glutamic acid and aspartic acid had lower ratios according to this study. The total essential amino acid and non-essential amino acid contents of present study were similar to those of spider crab (Marques *et al*., 2010), but were a little higher than those of blue swimmer crab (Wu *et al*., 2010) and were a higher as almost 2 times than those of wild-caught Chinese mitten crab.

According to Hayashi *et al.* (1981) who have conducted sensory analysis of taste-active components in the extracts of boiled snow crab meat, Arg, Gly, Ala and Glu are the amino acids that contribute to the characteristic taste of the meat. A marked increase in Orn during storage of horsehair crab (Tokunaga *et al*., 1983), snow crab (Miyagawa *et al*., 1990; Matsumoto and Yamanaka, 1992) and Japanese spiny lobster (Yamanaka and Shimada, 1996) was also reported. In conclusion, the limit of acceptable quality of the *P. sanguinolentus* crab as being fresh was about 4 days. The reason may be that the energetic status of the crabs decreased suddenly during the first 2 days of storage. A rapid degradation of nucleotides and a small change in the total amounts of FAA and taste-active amino acids prior to the initial decomposition.

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