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Fatty Acid Contents of Lyophilized and Frozen *Portunus sanguinolentus* Crab Meat

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ABSTRACT

Crab meat is a popular delicacy sold as a high market price. Crab meat contains small amounts of Omega-3 fatty acid, polyunsaturated fats, known to be essential for healthy functioning of the heart. In the present study, freezing and freeze drying, a fatty acid content of lyophilized and frozen meat of crab *Portunus sanguinolentus* was analyzed. The physical destruction during freeze drying was minimal. When preserved in sealed bags at room temperature for 2 months. No destruction of pigment, such as; discoloration or browning was observed in the meat or viscera. The major contents of fatty acid after rehydration were similar to that before rehydration the most prevalent fatty acid was oleic acid. Lyophilization and rehydration of meat usually, it causes little shrinkage or toughening of the sample and due to the reduction of the surrounding pressure and their sublimation from solid phase to liquid phase, there will not be huge effect on chemical composition and their nutritive value. The body flesh of *P. sanguinolentus* contains a considerable amount of both Eicosa Pentaenoic Acid (EPA) and Docosa Hexaenoic Acid (DHA). About 24 fatty acids have been found to occur in the lyophilized and frozen crab body tissue. Mono Unsaturated Fatty Acids (MUFAs) were the most abundant of the total.

Key words: Crab meat, *Portunus sanguinolentus*, lyophilization, rehydration, fatty acid

INTRODUCTION

Generally seafood is regarded as beneficial to human health, mainly due to its content of the long-chained fatty acids EPA (Eicosa Pentsenic Acid) and DHA (Docosa Hexaenoic Acid) as well as the content of zinc, selenium and iodide. They are incorporated in many parts of the body including cell membranes (Lazzarin *et al*., 2009) and play a role in anti-inflammatory processes and in the viscosity of cell membranes (Conquer *et al*., 2000; Smith *et al*., 2011). The EPA and DHA are also the precursors of several metabolites that are potent lipid mediators, considered by many investigators to be beneficial in the prevention or treatment of several diseases (Serhan *et al*., 2008). Freeze drying is reported to be the best method of dehydration. It freezes samples and then reduces the surrounding pressure to allow frozen water in the samples to sublime directly from the solid phase to the gas phase. The ice inside the samples and the low temperature required during freeze drying protects the products from deterioration and microbiological reactions, which results

in the final products having excellent physical and biological qualities (Lihong *et al*., 2012). Hence, it assume that freeze drying may be a promising method for high-value crab processing, so, the present study evaluated the physical changes, rehydration ability and fatty acid composition of freeze dried *Portunus sanguinolentus* crabs.

MATERIALS AND METHODS

Freezing and freeze drying: Live crabs were collected from the fish landing centre of Colachel, South west coast of India and transported live to the laboratory. The crabs were then frozen and stored at deep freeze (-80°C) before analysis the level of fatty acid. The frozen right or left half of one crab was taken out at random and lyophilized under high-vacuum conditions in a Free Zone 6 Litter Freeze Dry System (Labconco Corp., Kansas City, MO, USA) to produce freeze dried samples. The freeze dried samples were kept in sealed bags at room temperature until biochemical analysis. The remaining frozen halves were used as controls.

Rehydration of freeze-dried crabs: Freeze-dried crabs that had been preserved at room temperature for two months at 70°C, until they reached a constant weight, to estimate their water content. The freeze-dried samples were immersed in distilled water at room temperature and rehydrated for 30-60 min, respectively. Then, they were weighed after removing the superficial water with tissue paper. The rehydration ratio was calculated, as the ratio of the mass of the rehydrated sample to that of the dried sample.

Analysis of fatty acid composition: Fatty acid profiles of the crab samples were determined according to AOAC (1995) method and their composition was determined by Gas Chromatography (GC). Extraction was performed with a (2:1) Chloroform/methanol mixture in a Soxhlet device (Folch *et al*., 1957). Fatty acid composition was expressed, as the percentage of each fatty acid relative to the total fatty acids.

Statistical analysis: The data obtained from experiments were subject to appropriate statistical analysis by using star personal XT computer. Analysis of ANOVA (F-test). Degree of correlation, regression lines between different parameters were drawn by calculating with the help of least square method (Walpole, 1982). The differences between freeze-dried crabs and the control crabs were analyzed using Chi-Squared Analysis. Pearson Correlations (r) were performed between fresh group and frozen group for biochemical parametric test. Statistical significance for all data was accepted at the 95% confidence level ($p \leq 0.05$), statistically analyses were performed using the Statistical Package for Social Sciences (SPSS).

RESULTS

Lyophilization and rehydration: Freeze drying is reported to be the best method of dehydration. Live fresh *P. sanguinolentus* crabs were freeze dried. The moisture content, rehydration ratio and fatty acid composition of freeze-dried crabs were analyzed. The meat and viscera were breakable and retained their physical constitution. There was no significant reduction in volume or change in shape, such as; contraction, wrinkling or deformation. This indicated that physical destruction during freeze drying was minimal. When preserved in sealed bags at room temperature for 2 months. No destruction of pigment, such as; discoloration or browning was observed in the meat or viscera. When immersed in distilled water at room temperature for 30 min, the rehydration ratio reached 2.11, which was very close to that for 60 min (Table 1), indicating good rehydration ability of freeze-dried crabs.

Table 1: Average rehydration ratio of freeze-dried after rehydration for different time (n = 6)

Rehydration time (min)	Rehydration ratio
30	2.11±0.15
60	2.17±0.20

Valves are give in Mean±SD

Table 2: Fatty acid contents of lyophilized and frozen crab

Fatty acids	Lyophilized	Frozen
C14:0	0.83±0.07	0.62±0.02
C15:0	0.56±0.09	0.27±0.04
C16:0	15.64±0.02	19.54±0.03
C17:0	0.87±0.24	0.67±0.75
C18:0	10.45±1.32	12.19±0.15
ΣSFA	28.35±1.74	33.29±0.99
C14: In7	2.40±1.88	3.46±6.05
C16: In7	8.66±1.01	7.82±0.07
C16: In5	0.62±0.46	2.91±0.14
C17:1	0.44±1.15	0.48±0.15
C18: In9	19.61±0.14	17.19±0.21
C18: In7	4.12±0.06	3.54±0.03
C20: In9	1.73±1.88	1.91±0.37
C20: In7	0.88±0.52	0.83±0.10
ΣMUFA	38.46±7.10	38.14±6.91
C18: 2n6	11.24±0.24	9.44±0.67
C18: 3n4	1.95±1.29	1.25±0.53
C18: 3n3	0.21±0.04	0.19±0.27
C18: 4n3	0.15±0.05	0.26±0.14
C20: 2n6	0.82±0.24	1.73±0.15
C20: 3n6	0.63±0.19	0.75±0.13
C20: 4n6	2.83±0.24	4.16±0.54
C20: 5n3	4.80±0.15	5.54±0.17
C22: 2n6	0.13±0.02	0.07±0.09
C22: 5n3	0.43±0.13	0.65±0.48
C22: 6n3	3.10±1.50	3.43±0.17
ΣPUFA	24.41±0.56	32.66±0.28
Σn3PUFA	7.84±1.64	8.93±0.13
Σn6PUFA	15.30±0.20	21.66±1.52
n-3/n-6	0.58±0.20	0.65±1.49
DHA/EPA	0.78±0.65	0.70±0.05
ΣHUFA (>20.3n)	10.38±1.62	13.08±0.08

SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acids, PUFA: Poly-unsaturated fatty acids, DHA: Docosahexaenoic acid, EPA: Eicosa pentaenoic acid, HUFA: Highly unsaturated fatty acid

Considering that lyophilized crabs must be rehydrated fully before cooking, so, further analyzed the fatty acid profiles of the lyophilized crabs after full rehydration. As shown in Table 1, the major contents of fatty acid after rehydration were similar to that before rehydration (Table 2): The most prevalent fatty acid was oleic acid (C18:1, 18-25%), followed by palmitic acid (C16:0, 12-21%), linoleic acid (C18:2 n-6, 5-18%) and palmitoleic acid (C16:1, 6-12%). The EPA and DHA were the major n-3 PUFAs and accounted for 2-6% of the total fatty acids.

Lyophilization and rehydration of meat usually it causes little shrinkage or toughening of the sample and due to the reduction of the surrounding pressure and their sublimation from solid phase to liquid phase there will not be huge effect on chemical composition and their nutritive value.

Fatty acid contents of lyophilized and frozen crab: The fatty acid contents of lyophilized and frozen crab are shown in Table 2. The present investigation has revealed that the body flesh of *P. sanguinolentus* contains a considerable amount of both Eicosa Pentaenoic Acid (EPA) and

Docosa Hexaenoic Acid (DHA). About 24 fatty acids have been found to occur in the lyophilized and frozen crab body tissue. Mono Unsaturated Fatty Acids (MUFAs) were the most abundant and accounted for 45-47% of the total fatty acids, followed by Poly-Unsaturated Fatty Acids (PUFAs), which accounted for 27-37% of the total. Oleic acid (C18:1) was the pre-dominant fatty acid (28-30%), followed by palmitic acid (C16:0, 14-17%), palmitoleic acid (C16:1, 10%) and linoleic acid (C18:2 n-6, 6-12%). The major n-3 PUFAs were Eicosa Pentaenoic Acid (EPA, 20:5 n-3) and Docosa Hexaenoic acid (DHA, 22:6 n-3); both of which were more abundant in crabs.

DISCUSSION

The present investigation has revealed that the body flesh of *P. sanguinolentus* crabs contains a considerable amount of both Eicosa Pentaenoic Acid (EPA) and Docosa Hexaenoic Acid (DHA). The occurrence of considerably higher levels of EPA and DHA in the body flesh is common in detritivorous benthic animals of Sundarbans estuarine complex (Misra *et al*., 1983). About 24 fatty acids have been found to occur in the body flesh, of *P. sanguinolentus*. The contents of EPA and DHA in lyophilized *P. sanguinolentus* crabs were comparable to that reported in marine crabs (Celik *et al*., 2004; Cherif *et al*., 2008; Wu *et al*., 2010). In addition, some monomethyl and multiple methyl branched fatty acids were also detected in our study, among which monomethyl hexadecanoic acid [C16:0(15ME), C16:0(14ME)] and 3, 7, 11, 15-tetra- methyl-hexadecanoic acid [C16:0(3, 7, 11,15ME)] were predominant. These branched fatty acids could be converted to branched carbohydrates *in vivo*.

The ratio of n-3 to n-6 PUFAs was 0.33-0.86, which was higher than that recommended by the FAO/WHO (Lihong *et al*., 2012). The FAO/WHO recommend that the ratio of n-3 PUFA to n-6 PUFA in the diet should be at least 0.1-0.2 and a higher ratio (>0.2) is more beneficial to human health (FAO/WHO, 1999). A higher ratio of n-3 PUFA to n-6 PUFA in the food indicates a much higher nutritional value (Chen *et al*., 2007; Kuley *et al*., 2008). In this study the ratio of n-3 to n-6 PUFAs was indicating that both the frozen and lyophilized crabs' *P. sanguinolentus* have high nutritional value.

Lipids play an important role in marine nutrition for provision of both Energy and Fatty Acids (EFA) (Sargent *et al*., 1989). The effects of different types of lipids on growth and tissue fatty acid composition have been investigation for a number of cultures species (Lochmann and Gatlin III, 1993; Fair *et al*., 1993; Nematipour *et al*., 1992). In the past decades, studies on crustacean fatty acids have focused on the nutritional requirements of brooders during ovarian development (Teshima *et al*., 1983) or juvenile growth (Xu *et al*., 1994; Wen *et al*., 2003). Large quantities of fatty acids were found to be necessary for the development of ovaries (Teshima *et al*., 1983). The DHA and EPA can lower blood pressure and have antithrombotic and antitumor effects, as well as prevent heart disease and improve physiological functions. Increased DHA intake can significantly reduce breast cancer risk in women (Yu and Yan, 2014). However, no detailed information is available for fatty acid composition and variation in lyophilized and frozen of *P. sanguinolentus* crabs.

The Chi-Squared Analysis shown that there are not much difference into the freeze-dried and control meat crab. And several statistical hypothesis and parametric test in comparison to previous results (Lihong *et al*., 2012), it was suggested that the total fatty acid measured composition is not shown more differences significantly. Based upon the pervious literature and statistical data has been shown that the changes in the fatty acid concentrations were found to be useful as an index of freshness and retention of nutritional composition significantly.

The changes in Saturated Fatty Acids (SFA's), Mono Unsaturated Fatty Acids (MUFA's), Poly-Unsaturated Fatty Acids (PUFA's), EPA+DHA/C16. N-3 PUFA/n-6 PUFA (n-3/n-6) and Poly-Unsaturated Fatty Acids (PUFA/SFA) and also long free fatty acids were investigated in frozen and lyophilized stored sample of marine crab meat. The percentile composition overall result stated that the variation is little and significant. In the freeze-dried condition the retention of moisture content in the meat or any freeze material is very important criteria for long term storage and retention of nutritive chemical composition. In this study, after the lyophilization, the average moistures content was measured 6.17% and this shows effective and promising techniques for long term storage and transportation. The statistics of PUFAs and MPUFAs reported that freeze-dried or frozen, rehydrated or dehydrated ratios were higher than that those recommended by the FAO/WHO (1999), indicating that the *P. sanguinolentus* crab meat shown to be higher content of nutritional value even after long term of storage.

The regression analysis between frozen and control demonstrates, there is no effect on Long-Chain Free Fatty Acid (LC-FFA) concentration, per oxidizability index and lipid peroxidation-derived product content. The inverse association between lower temperature and LC-FFA persisted after correction for size, texture and fatty acid composition. These results indicate that the retention of fatty acid composition is an optimized feature associated with freezing of meat at lower temperature emerging the potential and suitable techniques for long term storage. The results indicate that the frozen on-lyophilized have no destruction to the nutritive value of species *P. sanguinolentus* crabs with regards to the fatty acid compositions.

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