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Research Note

Effect of Steam Shucking on the Biochemical Composition of Black Clam (*Villorita cyprinoides*)

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Abstract

The present study investigates the effect of shucking on the biochemical composition of black clam meat (*Villorita cyprinoides*). The clams were subjected to steaming and the nutritional quality parameters including proximate composition, fatty acid profile and lipid quality indices were estimated. There was an increase in protein content from 5.54% to 12.96%, fat content from 1.33% to 2.56%, and ash content from 0.64% to 1.19% along with a reduction in moisture content after steaming. The alpha amino acid content increased from 210mg% to 224mg% and the non-protein nitrogen content increased from 78mg% to 95mg% by steaming. The lipid oxidation index, thiobarbituric acid reactive substances increased from 0.25 to 0.66mg malonaldehyde/Kg in shucked clam. The palmitic acid and docosahexaenoic acid (DHA) were the major fatty acids present both in fresh and shucked clam meat. However, the saturated fatty acid content (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) had no significant change in their proportion. The PUFA content was 30% in both samples with ~66% constituted by n3 PUFA and ~33% constituted by n6 PUFA. The artherogenic index of fresh meat was 0.72 and shucked meat was 0.79 and thrombogenic index of both shucked and unshucked clam was ~0.29. It can be concluded that steam aided shucking preserves the proximate composition and fatty acids of black clam meat and hence can well be suggested for shucking.

Keywords: Black clam, shucking, steaming, composition, lipid nutritional indices

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Introduction

Clams, mussels and oysters are among the most demanded seafood item due to the Unique flavour and nutritional characteristics. Black clam belongs to the family *Corbiculidae* and contributes to one third of the annual landings of clam in India (Suja & Mohamed, 2010). The shell is removed after shucking the clam and is utilised for making cement, lime and calcium carbide. The meat of black clam is utilised as human and poultry food.

The demand for healthier foods is increasing as consumers are becoming more aware about the nutritional requirements of human body. Dietary lipids are essential nutrients for vital functions as a source of energy for different processes in the body like cell growth. Different processing methods results in changes in nutrient structures (Garcýa-Arias et al., 2003). Cooking is a process that leads to severe reduction in nutritional quality of the product. Changes in temperature can lead to a decrease in the quality of fatty acids, which in turn can cause alterations in protein composition (Ansorena et al., 2010).

There are several reports on the effect of thermal treatments on finfishes whereas the studies on shellfish species is very rare. Even though shucking exists as an essential pre-requisite for consumption of black clam, the effect of shucking on nutritional composition of shucked clam is not studied. Hence in the present study, the fresh clam and shucked clam were compared for proximate composition, fatty acid profile, lipid nutritional quality indices and lipid oxidation index.

The black clam (*Villorita cyprinoides*) was collected from backwaters of Kumbalangi, Cochin, Kerala, India. The clams were bought to the laboratory and were thoroughly washed. Depuration was done for

48 h in chlorinated running water. After depuration, the clams were divided into two batches; Fresh meat along with the water oozed out were collected from the first batch after breaking the shells. The second batch was steamed for 10 minutes using a steamer and the meat was separated from the shell manually for analysis.

Cooking loss was estimated by measuring the weight of fresh clam meat and meat after shucking according to Ovissipour et al. (2013) using the following formula,

Cooking loss (%) = Weight of fresh clam meat — Weight of clam meat after shucking

The proximate composition (moisture, crude protein, crude fat and ash) and NPN of the samples were estimated according to AOAC procedures (AOAC, 2019). Moisture content was analysed by drying the sample in hot air oven at 105±2°C. Total nitrogen was determined using Kjehldahl's method and the crude protein was estimated. Fat content was analysed using soxhlets apparatus and ash content was estimated by heating the sample in a muffle furnace at 550±2°C. The pH of samples was measured using pH meter (Cyberscan 510, Eutech Instruments, Singapore) Spectrophotometric estimation of thiobarbituric acid reactive substances (TBARS) (mg malondialdehyde / Kg sample) was done according to Tarladgis et al. (1960) and the alpha amino nitrogen (AAN) of the sample was determined according to Pope & Stevens (1939). The fatty acid profiling was done using a gas-liquid chromatographer (Varian CP 3800, Palo Alto, CA, USA) after transesterification according to Sreelakshmi et al. (2015). Lipid nutritional indices, hypocholesterolemic / hypercholesterolemic ratio (H/H), index of thrombogenicity (TI) and index of atherogenicity (AI) were calculated according to Ulbricht & Southgate (1991) and Fernandez et al. (2007) using the following formulae:

AI= (C12: 0 + 4 C14: 0 + C16: 0)/ (MUFA + PUFA)

TI= (C14: 0 + C16: 0 + C18: 0)/ (0.5 ΣMUFA + 0.5 Σn3PUFA + 3 Σn6PUFA + n3/n6)

H/H= (C18:1n9 + C18:2n6 + C20:4n + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3) / (C14:0+C16:0)

One way ANOVA was followed by Duncan's test to find the significant difference between the two samples ($p \leq 0.05$) was carried out using SPSS 16.0 (SPSS Inc., USA)

The cooking loss of black clam by shucking was 27%. Marinopoulou & Petridis (2022) reported cooking loss of greater than 20% for steam cooked mussels. The biochemical compositions of both fresh and shucked clams are given in Table 1. The moisture content reduced after shucking because of the loss of exudates during shucking. The protein content increased from 5.56% to 12.93%. Likewise, the crude fat also increased after shucking (1.33% to 2.52%,). This can be the result of loss of water during shucking, which resulted in the increase in concentration of other constituents. This result was similar to Ktari et al. (2015), where the protein content increased by cooking of fish and is in contrast to Bejaoui et al. (2019) where the protein and fat content reduced after cooking clams,

Table 1. Biochemical composition of fresh clam and shucked clam

Parameters	Fresh clam meat	Shucked clam meat
Moisture %	89.97±2.45^a	77.17±1.3^b
Ash %	0.64±0.09^b	1.19±0.10^a
Total Protein %	5.56±0.93^b	12.94±2.94^a
Crude Fat %	1.33±0.57^b	2.52±0.83^a
NPN mg%	210.00±5.38^b	224.00±3.21^a
Alpha amino nitrogen mg%	78.00±1.56^b	95.00±2.29^a
TBA (mg malonaldehyde/Kg)	0.25±0.0^b	0.66±0.01^a
pH	6.64±0.14^b	7.22±0.26^a

Values represent mean±SD

Superscripts with different notations represent signifcant difference among the means (≤0.05)

Ruditapes decussatus. Anjana et al. (2021) reported 13.3% protein, 3.4% fat and 1.13% ash content in black clam from Cochin backwaters. In this study, the alpha amino nitrogen of fresh clam meat was 78mg% which increased to 95mg% after steaming. NPN and TBARS of clam meat have also increased after shucking. Zhang et al. (2013) reported increased NPN and free amino acids in crucian carp after application of temperature. According to Sun et al. (2010), the reason for increase in NPN content is the protein degradation and resultant formation of low molecular weight nitrogen compounds. Gupta & Govindan (1975) reported that the AAN of *Mytilus edulis* varied from 77.5mg% to 161mg%. The biochemical composition of fish differs with species, sex, age, environment, season (Petricorena, 2015) and processing method (Ekanem & Achinewhu, 2000).

There was significant increase in pH of clam meat after steaming. Cooking causes breakage of hydrogen bonds and increase in electrostatic interactions, which results in increased pH (Dhanapal et al., 2012). The content of secondary lipid oxidation products in seafood is generally represented as TBA value. This is mainly constituted by aldehydes and

Table 2. Fatty acid profile of fresh clam and shucked clam

Fatty acid	Name	Fresh meat	Shucked meat
C14:0	Myristic acid	1.90±0.20^{b}	2.72±0.11^{a}
C14:1	Myristoleic acid	0.00±0.00^{b}	0.61±0.01^{a}
C15:0	Pentadecanoic acid	0.74±0.03^{a}	0.77±0.01^{a}
C15:1	Cis-10-Pentadecanoic acid	2.62±0.39^{a}	2.14±0.25^{a}
C16:0	Palmitic acid	25.17±3.56^{a}	25.54±4.94^{a}
C16:1	Palmitoleic acid	3.85±0.59^{a}	4.30±0.73^{a}
C17:0	Heptadecanoic acid	3.65±0.21^{a}	3.90±0.44^{a}
C18:0	Stearic acid	7.67±0.94^{a}	7.58±1.10^{a}
C18:1 cis 9	Oleic acid	6.76±0.87^{a}	6.73±0.83^{a}
C18:2 cis 9,12	Linoleic acid	3.49±0.26^{a}	3.52±0.75^{a}
C20:0	Arachidic acid	0.80±0.01^{a}	0.00±0.00^{b}
C18:3 cis 6,9,12 gamma	Linolenic acid	0.32±0.01^{b}	0.74±0.02^{a}
C20:1 cis 11	cis-11-Eicosenoic acid	2.13±0.11^{a}	1.95±0.38^{a}
C18:3 cis 6,9,12,15 alpha	Linolenic acid	4.07±0.95^{a}	3.71±0.66^{a}
C20:2 cis 11,14	cis-11,14-Eicosadienoic acid	2.30±0.36^{a}	1.96±0.51^{a}
C22:0	Behenic acid	0.40±0.01^{a}	0.00±0.00^{b}
C20:3 cis 8,11,14	Eicosatrienoic acid	0.51±0.01^{a}	0.00±0.00^{b}
C20:4 cis 5,8,11,14	Arachidonic acid	3.71±0.43^{a}	3.39±0.27^{a}
C20:5 cis 5,8,11,14,17	Eicosapentanoic acid	5.07±1.01^{a}	5.33±0.99^{a}
C22:6 cis 4,7,10,13,16,19	Docosahexaenoic acid	10.78±2.45^{a}	11.81±1.75^{a}
Unidentified peaks		14.041±2.03^{a}	13.31±3.33^{a}
SFA		38.44±2.68^{a}	37.79±4.49^{a}
MUFA		15.37±1.60^{a}	15.73±2.68^{a}
PUFA		30.25±4.10^{a}	30.72±2.56^{a}
n3		19.92±1.46^{a}	20.84±2.68^{a}
n6		10.3±0.93^{a}	9.59±1.11^{a}

Values represent mean±SD

Superscripts with different alphabets in a row indicate significant difference in mean values (≤0.05)

contribute to off flavours in meat. Steaming the clam for shucking had a significant effect on the TBA value ($p<0.05$). The value increased from 0.25mg malonaldehyde/kg to 0.66mg malonaldehyde/kg. Lesser TBA values are more acceptable as unfavourable flavours and odours are usually associated with TBA value of 1–2mg MDA/kg (Sreelakshmi, 2022). The TBA value of clam meat after steam shucking is lesser and hence there is no risk associated with lipid oxidation.

Different cooking methods have different effects on fatty acid content of fish (Hosseini et al., 2014). The fatty acid profiles of fresh and shucked clam meat are given in Table 2. Nineteen fatty acids were present in clam meat with a minimum amount of 0.1%. The fatty acids were present as saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids. Palmitic acid and docosahexaenoic acid are predominant in both fresh and shucked clam meat.

The unsaturated fatty acid content was almost similar in both samples. The 8, 11, 14-eicosatrienoic acid, which inhibits the aggregation of human platelets (Falardeau et al., 1976) was present in fresh clam, which was not seen in shucked clam. Oleic acid and cis-11-eicosenoic acid were the monounsaturated fractions present in both the samples. This constituted 15.37% and 15.73%, respectively in fresh and shucked meat. The health benefits of aquatic food including reducing cognition decline, antithrombotic activity, lowering the occurrence of cancer and hypertension reduction, anti-inflammatory activity, photoreception, anti-arrhythmic activity, foetal neurodevelopment and vasodilatory activity are because of the n-3 PUFA, Eicosapentaenoic acid and Docosahexaenoic acid (Gladyshev et al., 2006). The total PUFA contributed to almost 30% of the total lipids in both samples with around 66% was constituted by n-3 PUFA and around 33% is constituted by n-6 PUFA. The EPA values increased from 5.07% to 5.33% and DHA values increased from 10.78% to 11.81% by shucking.

The P/S, H/H, AI, TI and n3/n6 values can be used as better evaluation of nutritional quality (Sreelakshmi et al., 2019). Fats with low P/S values will induce hypercholesterolaemia (Santos-Silva et al., 2002) and the value greater than 0.45 is preferred in foods (Hmso, 1994). The P/S ratios of samples are given in Table 3. For fresh meat and shucked meat, the P/S ratio was ~0.80. The atherogenic index (AI) is the ratio between pro-atherogenic and anti-atherogenic fatty acids. In other words, it is the ratio of fatty acids that promote the buildup of plaque in arteries to those that help to prevent plaque formation. The TI is the ratio of pro-thrombogenetic fatty acids to anti-thrombogenetic fatty acids. The H/H values of the samples were found to be almost similar (~1.26). According to Wu et al. (2012) higher the H/H values, the fat is more beneficial nutritionally. Marine finfishes were reported to have H/H values ranging from 0.87 to 2.46 (Fernandes et al., 2014). Higher TI and AI values increase the risk of chronic heart disease (Ulbricht & Southgate, 1991). The AI of fresh clam meat was 0.72 and shucked clam meat was 0.78 and TI was ~0.29. *Leognathus bindus* are reported to have AI and TI values of 1.4 and 0.8 while for *Upeneus sulphureus*, the values were 1.3 and 0.8 (Ghaeni et al., 2013). In this study, n-3 to n-6 ratio of fresh meat was 1.93 and shucked meat was 2.17. A recommended dietary value of n3/n6 is 0.1 - 0.2 and higher ratios are more beneficial to health of humans (FAO/WHO, 1993). The n3/n6 index in oyster meat was 4.66 (Asha et al., 2014) and in puffer fish was reported to be 3.27 (Sreelakshmi et al., 2016).

Table 3. Lipid nutritional quality indices of fresh clam and shucked clam

Index	Fresh meat	Shucked meat
H/H	1.26	1.25
AI	0.72	0.79
TI	0.29	0.29
N3/N6	1.93	2.17
P/S	0.79	0.81

Black clam forms a major inland fishery in India, especially in southern parts. Shucking is an important process for extraction of clam meat from shell. This study evaluated the nutritional quality of clam before and after steam shucking. There was an increase in the major nutritional constituents of meat except moisture. The MUFA and PUFA did not show significant change. Majority of the fatty acids were preserved during treatment. The lipid quality indices have also shown that the nutritional quality of clam meat was not affected by steam shucking.

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