Effect of thermal processing on the biochemical constituents of green mussel (*Perna viridis*) in Tin-free-steel cans

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Abstract The effect of thermal processing on the biochemical constituents of green mussel (*Pernaviridis*) in brine was investigated. Depurated mussel samples were thermal processed in tin-free steel cans (TFS) at F_0 8.43. The time temperature data was collected during heat processing using EVAL data recorder and heat penetration characteristics were determined using formula method. The total process time was 27.48 min and the processed cans were found to be commercially sterile. The mineral composition of processed mussel was significantly higher than raw samples. The instrumental texture analysis indicated that product become soft after thermal processing. The thermal process led to a significant decrease in total amino acid and fatty acid content of the samples. Even though the samples showed a decrease in amino acid and fatty acid composition after thermal processing, the mussel meat was nutritionally well balanced with respect to essential amino acids and fatty acids. Hence the product can be considered as a food source with high quality protein and fat to fulfil consumer's requirements. Based on sensory analysis, the processed products were found acceptable during the study period.

Keywords Green mussel · Tin free steel can · F_0 value · Thermal processing

Introduction

The Asian green mussel (*Perna viridis*) is found in the coastal waters of Indo-Pacific region and it can withstand a wide

range of temperatures from 11 to 32 °C and salinity of about 18–33 ppt (<http://www.fao.org/fishery/species/2691/en>). Mussel meat is very popular in several countries and is a source of high quality protein with substantial quantities of glycogen which is responsible for the characteristic sweet flavour in the meat. Like fish meat, mussel meat is also highly perishable in nature (Balachandran and Prabhu 1980). Traditionally tin containers were used for canning seafood products. Tin containers have many disadvantages like high cost, need for special lacquer coating, rusting, imparting metallic taste to the product and also difficulty in opening. One of the recent developments in rigid containers is the use of polymer coated Tin-free Steel (TFS) cans with easy open ends, which solves many disadvantages encountered in conventional tin cans. Suitability of Tin-free Steel cans for canning fish (Mallick et al. 2006) ready to eat squid masala (Gopinath et al. 2007) were studied. Many ready to serve products can be prepared from mussel and products in filling medium like in oil, brine, sauce and curry are popular in several countries. Even though thermal processing improves the keeping quality of many ready to serve products, many workers have reported the negative effects of thermal processing on quality. The present study deals with the effect of thermal processing on the biochemical constituents of green mussel *Perna viridis* in Tin Free Steel (TFS) cans.

Materials and methods

Live green mussel (*Perna viridis*) were procured from the local suppliers at Fort Cochin landing centre, India. They were depurated in running clean sea water for twenty four hours. The mussels were then kept in chlorinated (5 ppm) water for further five minutes. Depurated shell on mussels were blanched (90 °C) in water for two minutes and the meat was shucked out.

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The meat was blanched (90 °C) in 2 % brine for ten minutes, drained and cooled.

Indigenous polymer coated Tin-free Steel cans of 307×109 ($3\frac{7}{16} \times 1\frac{9}{16}$ inch) of 6 oz. capacity manufactured by M/s Amtech packs, Mysore were used for the study. About 130 ± 5 g of shucked meat was packed in cans and filled with hot brine (2 %) leaving some head space (9 mm). The cans were exhausted in steam for ten min. to remove the residual air and seamed immediately in a double seaming machine.

The empty cans were fixed with thermocouple glands (Model no. GkJ 13009 C042, Ellab Co. Rodovre, Denmark) before packing mussels and the thermocouple probe (Model no. SSA 12040 G700 TS, Ellab Co. Rodovre, Denmark) was inserted through it after seaming. Tip of the thermocouple glands were inserted to the centre of the can for recording the core temperature during heat processing. The cans were loaded inside the retort (Model no. 5682, John Fraser and Sons Ltd., Newcastle upon Tyne, U.K) and processed at 121.1 °C till they attained F_0 value of 8. The temperature data was acquired for every minute using Ellab data recorder (Model TM 9608, Ellab Co. Rodovre, Denmark). The lethality (F_0 value) accumulated during the entire processing was calculated from the temperature history by numerical integration (Ball and Olson 1957). After thermal processing, the cans were cooled by pumping water into the retort to reduce the temperature to around 40 °C. The cans were removed from the retort and immersed in potable chilled water and washed thoroughly with detergent solution. They were air drained and kept at ambient temperature for two weeks for conditioning. After conditioning, the samples were taken for various analyses. The lag factor for heating (J_h), slope of the heating curve (fh), time in minutes for sterilization (U) and lag factor for cooling (J_c) were calculated by plotting temperature deficit

(RT-Tc) against time on semi log paper. The process time (B) was calculated by mathematical method (Stumbo 1973) by adding 58 % of the come up time (CUT) to B . Cook value (C_g), a measure of heat treatment with respect to nutrient degradation and textural changes that occur during processing, was also determined by measuring the extent of cooking and nutritional loss during processing in a manner similar to D value.

After thermal processing, the cans were examined for external defects and cut open. Brine was drained out completely and the meat was used for further analysis.

Thermal processed mussel was tested for commercial sterility (IS 2168 1971). The proximate composition of the samples was determined by AOAC (2000) method. pH was measured according to APHA (1998) using a digital pH meter (Cyberscan 510, Eutech instruments, Singapore). Total volatile base nitrogen (TVB-N) and Trimethylamine (TMA) were estimated by the micro diffusion method (Conway 1950). Amino acid concentration was determined by Ishida et al. (1981) method and Tryptophan content was estimated by the method of Sastry and Tummuru (1985). Fatty acid composition was estimated by AOCS (1989) method. The minerals like sodium, potassium, calcium were analyzed according to APHA (1998) using flame photometer. (BWB Technologies, United Kingdom). Texture profile analysis was measured with a Universal Testing Machine (Lloyd instruments LRX plus, UK) with a cylindrical probe of 50 mm diameter. The load cell used was of 50 N capacity, 12 mm/min speed with a trigger force of 50 g. Hardness, cohesiveness, springiness, springiness index, gumminess, adhesive force, adhesiveness, stiffness, chewiness, and fracture force were calculated as defined in the texture analyzer user manual. Sensory analysis of canned mussel was carried out by ten trained panelists using

Fig. 1 The heat penetration characteristics of mussel meat processed at 121.1 °C

Table 1 Thermal process parameters of mussel meat in brine medium in TFS cans

Parameter	Value
Come up time (min)	11.02
Heat rate index F_h (min)	16
Heating lag factor (J_h)	0.567
Cooling lag factor (J_c)	1.07
F_0 Value (min)	8.43
Temperature deficit (g)	1.92
Ball process time, B (min)	21.09
Total process time, T (min)	27.48
Operator process time (min)	16.47

a 9 point hedonic scale as prescribed by Meilgaard et al. (1999). The sensory attributes evaluated were appearance, colour, odour, flavour, firmness and taste. The overall impression of the product on the assessor was recorded as overall acceptability. A score of above 4 was considered as the margin of acceptance.

All analyses were carried out in triplicate. The results were expressed as mean values $\pm$ standard deviation. Data were analysed by using one way ANOVA and the least significant difference was calculated at the probability level $p < 0.05$.

Results and Discussion

The F_0 recommended for fish products ranges from 5 to 20 (Fott and Lewis 1994). The thermal processing of mussel was done at F_0 of 8. The heat penetration characteristics of samples processed at 121 °C are given Fig. 1. The various process parameters obtained by plotting the time temperature data on a semi-logarithmic paper are given in Table 1. The come up time to reach 121 °C was 11.02 min. The total process time was 27.48 min, which included 58 % of the come up time. The

Table 2 Changes in proximate composition and other quality parameters of raw and processed mussel meat

Parameter	Raw mussel	Processed mussel
Moisture	79.75 $\pm$ 0.24	68.27 $\pm$ 0.19
Protein	12.83 $\pm$ 0.13	15.80 $\pm$ 0.14
Fat	2.24 $\pm$ 0.17	3.94 $\pm$ 0.31
Ash	3.72 $\pm$ 0.45	5.83 $\pm$ 0.29
pH	6.59 $\pm$ 0.16	5.74 $\pm$ 0.17
TVN	18.70 $\pm$ 0.14	28.48 $\pm$ 0.16
TMA	5.50 $\pm$ 0.65	10.39 $\pm$ 0.17
Na (g % in dry weight)	1.78 $\pm$ 0.03	2.28 $\pm$ 0.43
K (g % in dry weight)	0.147 $\pm$ 0.21	0.903 $\pm$ 0.25
Ca (g % in dry weight)	0.051 $\pm$ 0.09	0.2029 $\pm$ 0.15

All values are the means $\pm$ standard deviations of three replicates

heating lag factor was 0.567 and the cooling lag factor was 1.07. Similar results were reported for prawn kuruma packed in aluminium cans (Mohan et al. 2008), squid masala in TFS cans (Sreenath et al. 2007), and ready to serve yellow fin Tuna and vegetables in TFS cans (Ravishankar et al. 2010).

Canned samples were tested for commercial sterility. Microbial growth was not observed in any of the samples incubated in Thioglycollate broth. This indicated that the process given was sufficient to commercially sterilize the product. Changes in proximate composition of raw and processed mussel are presented in the Table 2. During thermal processing, loss of moisture content with an increase in protein, fat and ash was observed ($p < 0.05$). A similar result was also reported by Gallardo et al. (1990); Ruiz-Roso et al. (1998); Mai et al. (1978); Puwastien et al. (1998); Gall et al. (1983); Gokoglu et al. (2003) in thermal processed fish products. Water loss in mussel meat can be explained in terms of heat treatment and protein degradation of muscle, this leading to a decreasing water holding capacity of the myofibrillar protein fraction (Castrillón et al. 1996; García-Arias et al. 1994). From the present study it was observed that most of the cook loss was water. According to Bell et al. (2001), Thermal denaturation of muscle proteins is the primary mechanism leading to the moisture loss. Water content located within the narrow channels between the thick and thin filaments of myofibrils (Offer et al. 1984; Bertola et al. 1994) releases due to the heat denaturation and subsequent shrinkage of myofibrils (Ofstad et al. 1993). The increased protein, fat and ash content in the canned samples may be due to the water loss from the muscle during sterilization (Castrillón et al. 1996; García-Arias et al. 1994).

Table 3 Change in Amino acid profiles (g/16 g N) of raw and processed mussel meat

Amino acids	Raw mussel	processed mussel
Aspartic acid	11.74 $\pm$ 0.12	9.27 $\pm$ 0.21
Treonine	5.04 $\pm$ 0.13	4.73 $\pm$ 0.15
Serine	5.79 $\pm$ 0.2	5.27 $\pm$ 0.12
Glutamic acid	13.97 $\pm$ 0.36	12.77 $\pm$ 0.10
Proline	5.16 $\pm$ 0.41	3.94 $\pm$ 0.32
Glycine	8.58 $\pm$ 0.11	6.72 $\pm$ 0.12
Alanine	4.07 $\pm$ 0.64	4.07 $\pm$ 0.05
Valine	4.69 $\pm$ 0.11	4.32 $\pm$ 0.17
Methionine	2.17 $\pm$ 0.15	1.49 $\pm$ 0.11
Isoleucine	5.27 $\pm$ 0.08	4.35 $\pm$ 0.09
Leucine	7.69 $\pm$ 0.12	6.64 $\pm$ 0.15
Tyrosine	13.18 $\pm$ 0.16	5.86 $\pm$ 0.11
Phenylalanine	4.65 $\pm$ 0.13	3.73 $\pm$ 0.12
Histidine	5.53 $\pm$ 0.07	5.01 $\pm$ 0.24
Tryptophan	2.36 $\pm$ 0.16	2.36 $\pm$ 0.54
Σ EAA	32.36393	28.338

All values are the means $\pm$ standard deviations of three replicates

Table 4 Changes in Total Fatty acid (%) composition of raw and processed mussel meat

Fatty acids	Raw mussel	Processed mussel
Myristic Acid Methyl ester (C14:0)	4.13 ± 0.02	3.02 ± 0.31
Myristoleic Acid methyl esters (C 14:1)	0.78 ± 0.14	0.53 ± 0.17
Pentadecanoic Acid (C15: 0)	1.51 ± 0.13	1.52 ± 0.11
Palmitic Acid methyl Ester (C16:0)	22.2 ± 0.06	19.64 ± 0.05
Palmitoleic Acid Methyl Ester (C16:1)	6.44 ± 0.03	6.31 ± 0.04
Heptadecanoic Acid Methyl Ester (17:1)	1.30 ± 0.14	1.31 ± 0.11
Stearic Acid Methyl Ester (C18:0)	4.65 ± 0.15	4.62 ± 0.17
Elaidic Acid Methyl Ester (C18:1)	2.13 ± 0.05	1.84 ± 0.11
Linoleic Acid Methyl ester (C18:2)	3.96 ± 0.01	3.99 ± 0.19
Linolenic Acid Methyl Ester (C18:3)	1.34 ± 0.06	1.23 ± 0.09
Arachidic Acid Methyl Ester (C20: 4)	1.40 ± 0.14	1.29 ± 0.08
Cis-11-Eicosanoic Acid Methyl Ester (C21: 0)	2.28 ± 0.06	2.73 ± 0.1
Heneicosanoic Acid Methyl Ester (C21:0)	3.78 ± 0.12	3.17 ± 0.21
Cis-5,8,11,14,17- Eicosapentaenoic Acid Methyl Ester (20:5 n-3)	16.56 ± 0.02	14.53 ± 0.09
Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester (22:6 n-3)	33.14 ± 0.04	29.58 ± 0.13
Σ SFA	38.55	34.7
Σ MUFA	10.65	9.99
Σ PUFA	56.4	50.62

All values are the means ± standard deviations of three replicates

In the present study the pH of the raw mussel meat sample was around 6.59 suggesting the mussel used was of good quality (Riaz and Quadri 1985). Changes in the pH of raw and processed mussel meat are presented in the Table 2. It was observed that pH of the samples decreased on heat processing.

The TVB-N quantifies a wide range of basic volatile compounds including ammonia, methylamine, Dimethylamine (DMA), Trimethylamine (TMA), etc. Trimethylamine oxide (TMAO) is presented in large number of fish and shell fish and it is the main source of TMA and DMA. Changes in the TVB-N content are presented in the Table 2. TVBN content of

thermal processed samples were significantly ($p < 0.05$) higher than raw samples. This result agrees to previous research on canned tuna fish (Gallardo et al. 1990; Gill et al. 1987), canned farmed coho salmon (Rodríguez et al. 2009) and Monterey sardine (Uriarte-Montoya et al. 2010). The increase in TVB-N can be due to the thermal breakdown of TMAO during the cooking and sterilization steps (Gallardo et al. 1990; Gill et al. 1987).

TMA, a volatile amine, represents the majority of TVBN and is generally produced by bacterial enzyme action, reducing the TMAO to TMA. Changes in the TMA content during

Fig. 2 Changes in texture profile of fresh and processed mussel meat. All values are the means ± standard deviations of three replicates

Table 5 Mean sensory scores of thermal processed mussel meat

Sensory attributes	Scores
Can appearance internal	8.5 ± 0.95
Can appearance external	8.3 ± 0.98
Color	7.5 ± 1.4
Flavour	7.7 ± 0.99
Odour	7.5 ± 1.3
Taste	7.5 ± 1.2
Firmness	8 ± 0.56
Overall acceptability	7.5 ± 1.2

All values are the means ± standard deviations of ten replicates

thermal process is shown in the Table 2. The average TMA value of raw mussel meat was 5.50 mg TMA/100 g, which indicates that the mussel used in the study was in fresh condition (Connel 1995). A significant ($p < 0.05$) increase of TMA content in canned samples were observed during the present study. TMA presence in the canned samples can be explained by means of two different pathways: (i) As a result of Trimethylamine oxide (TMAO) bacterial catalysis breakdown during the chilled storage, and (ii) TMA can be produced from TMAO by thermal breakdown during the cooking and sterilization steps. The increase in TMA during canning process led to the conclusion that the thermal processes have exerted a higher effect on the TMA formation. This result agrees to previous research on fish canning by Gallardo et al. (1990) and Rodríguez et al. (2009).

Changes in the amino acid composition are given in the Table 3. The major amino acids found in raw mussel were tyrosine, glutamic acid, and aspartic acid. A significant decrease ($p < 0.05$) in amino acid composition was observed in thermal processed samples, which may be due to the effect of thermal processing on the amino acids. The changes in amino acids could also be due to the heat sensitive nature of amino acids (Mohan et al. 2008). Even though the samples showed a decrease in amino acid composition after thermal processing, the mussel meat was well balanced with respect to essential amino acids. Hence it can be considered as a food source with high quality proteins to fulfil consumer's requirements.

The fatty acid profile of both raw and thermal processed mussel meat are presented in the Table 4. The majority of marine species are rich sources of long chain PUFA, particularly 22:6 n-3 and 20:5 n-3 (Murphy et al. 2003). In the present study the dominant n-3 PUFA was 22:6 n-3 both in raw and processed mussel meat. The dominant fatty acids in the raw samples were Docosahexaenoic Acid (29.58 %), Palmitic Acid (22.20 %) and Eicosapentaenoic Acid (14.53 %). Palmitoleic acid (16:1) was present up to 6.31 %, while stearic acid (18:0) was up to 4.62 in raw mussel meat. In the present study, a significant decrease ($p < 0.05$) in the fatty acid composition was observed after thermal processing. That may be due to the effect of thermal processing on fatty acids. Even

though the samples showed a decrease in fatty acid composition after thermal processing, the mussel meat was well balanced with respect to essential fatty acids.

Changes in the minerals composition are presented in the Table 2. There was an increase in the mineral composition in thermal processed samples. This may be due to the inclusion of minerals from the brine used for the thermal processing as the liquid medium. The increase of minerals after thermal processing is in accordance with the total ash content observed in processed samples.

Texture profile of raw and processed mussel meat are given in the Fig. 2. There was a significant decrease in hardness in samples after thermal processing. The change in the hardness is mainly due to heat processing which causes conversion of collagen to gelatin and dissociation of muscle protein (Bindu et al. 2007). Cohesiveness, the ratio of the positive force during the second compression to that during first, decreased from 0.240 in raw mussel meats to 0.167 in thermal processed samples. The gumminess which is the product of hardness 1 and cohesiveness also showed a decreasing trend from raw mussel to thermal processed ones. Springiness is the elastic recovery property which reduced from 1.657 to 0.875 mm. From the total texture profile results, it was confirmed that the mussel meat becomes softer after thermal processing. This may be due to the gelatinization of muscle collagen and destruction of muscle cells during cooking (Kugino and Kugino 1994; Gopinath et al. 2007).

According to Mendez and Gallardo Abuin (2006), the effect of thermal processing on sensory quality in marine products is difficult to predict because of intra and inter specific variability of fish species and factors such as appearance, odour, colour, flavour, and texture. The results of the sensory evaluation analysis are given in the Table 5. It shows that the samples were well acceptable by the sensory panel members after thermal processing.

Conclusion

The Green mussel processed in tin free steel cans with F_0 8 was found to be suitable for thermal processing of mussel meat in brine. The product was commercially sterile and sensorily acceptable. The thermal treatment has led to the formation of volatile amines. Reduction of moisture content with an increase of protein, fat and ash content was observed to the samples after thermal processing. Thermal process has led to a reduction of amino acid and fatty acid content in mussel samples. However the thermal processed mussel meat was well balanced with essential amino acids and fatty acids to meet the needs of public.

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