



Process Standardization of Ready-to-eat Pasteurized Crab spread from Marine Blue Swimming Crab (*Portunus pelagicus*) Incorporated with Threadfin Bream Surimi

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Abstract

Ready-to-eat pasteurized crab spread was standardized from blue swimming crab (*Portunus pelagicus*) and surimi from threadfin bream (*Nemipterus japonicus*). Spread was prepared incorporating crab meat and surimi in 1:1 ratio, vacuum packed in monolayer retortable polypropylene pouches with screw cap and pasteurized to core temperatures 86°C[T₁] and 89°C[T₂] for a total processing time of 90 min and 50 min, respectively. Both the spreads were subjected to proximate, organoleptic and microbial analysis for the assessment of shelf life under chilled condition (3°C) for six months. The pH values of T₁ and T₂ reduced during the storage period (p<0.001). Total Volatile Base Nitrogen (TVB-N), Trimethylamine Nitrogen (TMA-N), Peroxide Value (PV) and Free Fatty Acid (FFA) values were within the acceptable limits up to 176 days of storage (p<0.001). Both the spreads were analyzed for anaerobic growth and found sterile. The crab spread processed at 86°C (T₁) showed superior sensory characteristics throughout the refrigerated storage (3°C) and found to be stable and acceptable.

Keywords: Pasteurization, crab spread, RTE food, surimi, sensory evaluation

Introduction

Crab meat is considered as one of the most popular marine commodities among the seafood enthusiasts around the world. Crabs are mostly preferred in live

form due to its high perishability which affects its characteristic sweet flavor. Apart from live transportation, the crab meat is marketed in pasteurized form which is a mild heat processing technique giving significant shelf life to the product under chilled conditions without affecting its flavor and textural properties. In India, the crab industry, especially the export market is concentrated on premium crab varieties such as the mud crab (*Scylla serrata*), while marine crab varieties such as blue swimming crab (*Portunus pelagicus*) fetch lower values. But recently due to over exploitation and indiscriminate fishing of juvenile mud crabs there has been a noticeable decline in its populations in the natural habitat throughout Indian coastal waters (Raj et al., 2015) which has increased the demand for other crab varieties.

About 70-80% of blue swimming crabs are currently exported as pasteurized crab meat from India (MPEDA, 2017). Pasteurized crabs meat could be eaten raw right out of the can or mixed in soups or battered and shaped into cakes or served on a salad (Edwards, 2001). Many works has been conducted on standardization and quality evaluation of pasteurized picked crab meat in different packaging (Dima et al., 2016; Tyler et al., 2010; Gates et al., 1993). At the same time only a few works are available regarding development of value added ready-to-eat (RTE) products based on crab meat. The RTE spread products developed from crab meat are mostly based on retort pouch processing (Sreelakshmi et al., 2013) or canning technology (Biji et al., 2013) which are heat sterilization methods significantly affecting the nutritional as well as sensory acceptability of seafood (Bindu et al., 2014) especially crab meat rich in highly volatile flavor compounds (Chen & Zhang, 2006). The only relatable work available

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is by Loaharanu & Lopez (1970) who conducted work on canned, pasteurized crab cake mix, using metal cans for processing. This proves that even though consumer oriented products such as crab spreads and dips are popular, their availability as packaged ready to eat products is still limited.

The Food and Drug Administration (FDA, 2001) defines ready-to-eat food as “animal or plant-derived food that is cooked, frozen, washed, cooked for hot holding, cooled, and processed to be consumed directly or after heating, subjected to many safety criteria *viz.*, zero tolerance level of *Listeria* sp., *Salmonella* sp., *Vibrio* sp. and anaerobic growth”. To prevent post-process surface contamination of convenient products, in-package processing is generally employed. The National Blue Crab Industry Association (NBCIA, 1984) adopted thermal processing guidelines for the blue crab industry. It also established HACCP principles and Critical Control Points (CCPs). As per the guidelines, a minimum pasteurization requirement of 85°C at core temperature for 31 min is to be followed commercially. Therefore, for any crab based product standardization at or above 85°C is commercially feasible. Pasteurization process should be followed by immediate cooling below 10°C and storage in refrigerated conditions (0-2°C). The packaging material used is polypropylene pouches with one end having screw type opening and other end vacuum sealed. Polypropylene (PP) is stronger and lighter than Polyethylene and characterized by good stiffness, heat resistance and suitable for refrigerated storage.

In this scenario, the objective of this study was to prepare a ready to eat product from hand-picked

blue swimming crab (*Portunus pelagicus*) meat incorporating surimi from threadfin bream (*Nemipterus japonicus*). The blue swimming crab meat yield is only around 25 to 30% (Pathak et al., 2019) which is considered low on a commercial sustainability basis. In order to mitigate this issue, the study suggested to incorporate surimi, which is a refined fish myofibrillar protein concentrate incorporated with cryoprotectants (Park et al., 2013; Park, 2005). The myofibrillar proteins make surimi an exceptional constituent for developing food products with outstanding gelling properties (Ramirez et al., 1999). Threadfin bream (*Nemipterus japonicus*) a popular fish variety commonly utilized for surimi preparation was identified for the purpose. The surimi acts as a protein rich flavorless filling medium which uniformly distributes the crab meat flavor and also provide acceptable textural properties for the spread.

Materials and Methods

Marine blue swimming crab (*Portunus pelagicus*) was brought from commercial trawlers and transported to the laboratory in iced condition. Threadfin bream (*Nemipterus japonicus*) was also procured in bulk from a nearby fishing harbour for surimi preparation. Other ingredients like mayonnaise, ginger paste, garlic paste, vinegar, and pepper powder were purchased from local supermarket.

The crabs were washed in potable water to remove adhering dirt and boiled at 90°C for 15 min. The meat was picked and packed in polyethylene pouches and subsequently, stored under chilled condition (4°C). Commercial surimi manufacturing process as described by Lee (1984) was followed for

Table 1. Different formulation of spread with varying percentage of crab meat and surimi

Composition	A (100% crab meat)	B (20% crab meat)	C (30% crab meat)	D (40% crab meat)	E (50% crab meat)	F (60% crab meat)
Picked crab meat (gm)	63	12.6	18.9	25.2	31.5	37.8
Surimi (gm)	-	50.4	44.1	37.8	31.5	25.2
Mayonnaise (gm)	6	6	6	6	6	6
Ginger paste (gm)	5	5	5	5	5	5
Garlic paste (gm)	4	4	4	4	4	4
Pepper powder (gm)	1	1	1	1	1	1
Vinegar (ml)	1	1	1	1	1	1

preparation of the required quantity of surimi. Preliminary study was conducted for finalizing the optimum ratio of crab meat and surimi for the preparation of spread (Table 1). The crab spread was prepared using the recipe mentioned under Table 2.

Table 2. Ingredients of crab spread mix

Ingredients	Wt. in grams
Picked Crab Meat	31.5
Surimi	31.5
Mayonnaise	6
Pepper	1
Ginger	5
Garlic	4
Vinegar	1 (ml)

Vacuum-sealing machine (VAC-STAR, 101048, Switzerland), Copper /cupronickel thermocouple probes (Ellab model No: GKJ 13009C042) and data recorder (Ellab –Val Flex M16, 14592, Denmark) and water bath (JEIO TECH, BW-20G, Korea) were used for standardization of process parameters of pasteurized crab spread. Monolayer retortable Poly Propylene Pouches with former end having screw type opening were selected as packaging material. The pouches were supplied by A to Z Packaging Ltd, Ernakulum, Kerala, India. Each pouch was filled with 80 g of formulated crab spread mix. Filling was done through the latter end of pouch, keeping the former end closed by means of screw. Immediately, after filling, latter end was vacuum sealed. For process optimization, total number of filled pouches was divided into two batches (T_1 and T_2). From each batch, five pouches were fitted with thermocouple glands, positioned at geometric centre. For conducting heat penetration studies, thermocouple probes of length of 50 mm and dia. of 1.2 mm were used for measuring the cold spot temperature.

Conventional in-pack pasteurization (Peng et al., 2017) was carried out using a water bath. The process temperature was fixed at 86°C and 89°C for two batches of pouches T_1 and T_2 , respectively. A reference thermocouple was also placed in the water bath to maintain and monitor kettle temperature. Thermocouple probes were in turn connected to a data recorder. The heat penetration data captured by the data recorder were plotted on semi logarithmic graph paper. The time in minutes required to reach product core temperature 85°C was noted and the

channel was selected for standardization of process time and process temperature, respectively. After processing, the pouches were cooled rapidly by unloading the pouches into vessel containing chilled potable water. Then they were wiped dry, labeled, and stored at refrigerated condition (4°C) and were subsequently analyzed for its shelf life quality changes for a period of 6 months. The process parameters like process temperature and process time were optimized using two way ANOVA with the aid of data obtained by sensory evaluation, so as to get the statistical significance of the results without any compromise. The shelf life study of pasteurized surimi based crab spread was done based on biochemical, microbial and organoleptic characteristics of the samples on a fortnightly interval.

Parameters of the proximate analysis (moisture, ash, crude protein, and crude fat) of the developed crab spread were analyzed by following AOAC (2019). The experiments were conducted in Completely Randomized Design (CRD) with two treatments and three replications. Parameters like pH, peroxide value (PV), Free Fatty Acid (FFA), Total Volatile Base Nitrogen (TVB-N), Trimethyl Amine Nitrogen (TMA-N), Total aerobic Plate Count (TPC) were analyzed at 14 days interval up to the 176 days of storage. Shelf life study was done by comparing TVB-N (Conway, 1950), TMA-N (Conway, 1950), PV and FFA (FFA) analysis (AOAC, 2000) under the study. The pH was determined with a pH 700 Digital meter at $25.0 \pm 2^\circ\text{C}$ (Model: Eutech Instruments, Singapore). The pH meter was standardized using a pH buffer of 4.0, 7.0 and 10.2.

For microbiological analysis of raw crab spread mix and processed product, standard procedures mentioned in the bacteriological analytical manual (USFDA, 2001) was followed. Pathogens examined were *Salmonella* sp., *Vibrio cholera*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Escherchia coli* and *Staphylococcus aureus*. Dehydrated bacteriological media and supplements (Himedia Laboratories Pvt. Ltd., Mumbai, India) and Oxoid (Cambridge, UK) were used in the study. Sensory analysis for raw and processed surimi based crab spread mix was carried out by 10 trained panelists using a 9-point hedonic scale as prescribed by Meilgaard et al. (1999). The panelists were asked to score for color, odor, flavor, spreadability and overall acceptability of product sample. A score of above 4.0 was considered as the margin for acceptance.

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS, version 20.0 for windows). Analysis of Variance (one way ANOVA) was performed. The tests for differences in non-parametric sensory scores were done by using Duncan's Multiple Range Comparison Test. Significance of differences was defined at ($p < 0.05$).

Results and Discussion

Yield of manually picked marine crab (*Portunus pelagicus*) meat after cooking was $18 \pm 2\%$. This was identical to the crab yield reported by Pathek et al. (2019). Sensory evaluation of the crab spread mix after pasteurization revealed that crab spread containing crab meat and surimi in the ratio of 1:1 by weight of total meat content, showed better sensory attributes. The average scores for different sensory attributes like color, flavor, spread ability and overall acceptability with its standard deviation for different formulations of the recipe with varying crab meat - surimi ratio coded as A, B, C, D, E and F is shown in Table 3. It was found that adding surimi increased the score for spread ability and color. This may be attributed to the whiteness of surimi as well the gel strength it exhibits when subjected to cooking temperature (Singh et al., 2004). Also, the increasing percentage of crab meat content provided better scores for taste, and flavor. Formulated crab spread mix with crab-surimi ratio 1:1 showed maximum sensory score for spreadability, color, and overall acceptability. There was no marked difference in the average score of flavor with crab-surimi ratio 1:1 and 2:3. hence, 1:1 crab surimi ratio was selected.

The results of thermal process optimization studies for two batches T1 and T2 are represented in Fig. 1 and 2, respectively. The first batch was pasteurized

to attain a core temperature of 86°C and the latter to 89°C . The time in minutes and the corresponding product core temperature, F value, and retort temperature was obtained and is plotted on semi logarithmic graph as depicted in Fig. 1 and 2. The total process time and come up time for the pasteurization process at 86°C (T1) was found to be 92 min and 35 min, respectively. Similarly, total process time and come up time for the pasteurization process at 89°C (T2) was found to be 50 min and 24 min, respectively. Unlike the crab cake mix processing time reported by Loaharanu & Lopez (1970), the current study required only less time owing to the superior heat penetration characteristics (Bindu et al., 2014) of the flexible packaging material used. Table 4 depicts the important heat penetration characteristics of both the processes T1 and T2.

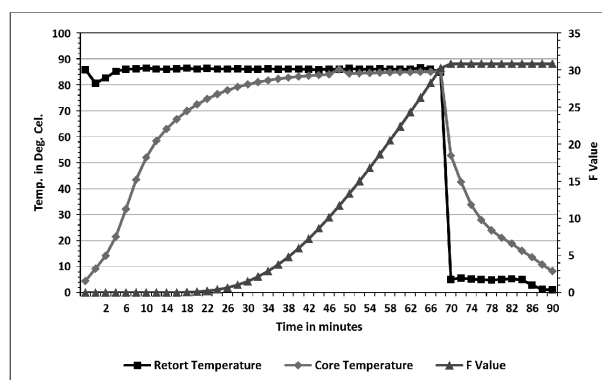


Fig. 1. Heat penetration characteristics of crab spread processed at 86°C (T1)

The average scores for sensory attributes of two treatments viz., T1 and T2 are shown in Table 5. Sensory evaluation scores of attributes such as color, flavor, spread ability and overall acceptability for

Table 3. Sensory evaluation score of sensory attributes obtained for recipe standardization

Sl. No.	Colour	Flavor	Spreadability	Overall acceptability
A	3.5 ± 0.52^a	6.6 ± 0.51^a	3.5 ± 0.56^a	3.8 ± 0.53^a
B	4.4 ± 0.51^b	6.9 ± 0.51^b	4.4 ± 0.50^b	4.3 ± 0.53^b
C	7.5 ± 0.52^c	8.4 ± 0.51^d	7.0 ± 0.50^c	6.88 ± 0.52^c
D	8.4 ± 0.51^d	8.2 ± 0.52^c	8.8 ± 0.44^e	8.33 ± 0.50^d
E	8.6 ± 0.50^d	8.2 ± 0.50^c	8.5 ± 0.50^d	8.66 ± 0.50^e
F	8.5 ± 0.50^d	8.2 ± 0.50^c	8.45 ± 0.51^d	8.32 ± 0.50^d

Values are mean $\pm$ standard deviations of three replicates. Superscripts in the same column represents significant difference (< 0.001)

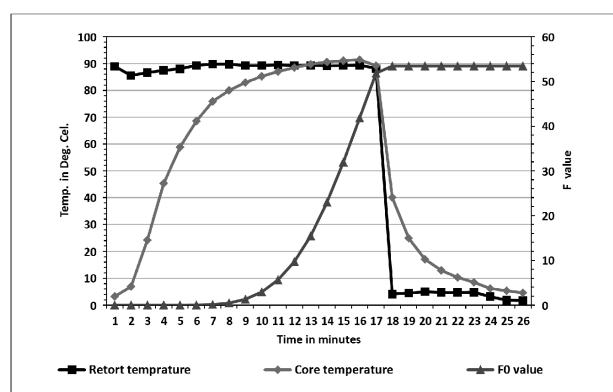


Fig. 2. Heat penetration characteristics of crab spread processed at 89°C (T2)

two batches of product (T1 and T2) were analyzed statistically using the Chi-square test. Statistically, there existed no significant difference in color, flavor and overall acceptability between two treatments ($p < 0.05$). But, a significant difference was observed for spread ability between two treatments (T1 and T2) which for T1 was observed to be 8.66 ± 0.60 , whereas for T2, it was only 4.13 ± 0.62 . The results showed that a temperature of 86°C and total process time of 92 min were found optimum for the pasteurization of surimi based crab spread for preserving the sensory attributes like color, flavor, spread ability and overall acceptability.

Table 4. Summary of Thermal validation study conducted

Heat penetration characteristics	T1(86°C)	T2 (89°C)
Come Up Time(CUT)	35 min	24 min
Heating Time(fh)	35 min	10 min
Cooling Time	22 min	16 min
Total Process Time	92 min	50 min

Table 5. Average sensory evaluation scores of sensory attributes of treatments T1 and T2

Sensory attribute	T1	T2
Color	8.56 ± 0.60	8.51 ± 0.58
Flavor	8.71 ± 0.52^a	8.82 ± 0.53^b
Spread ability	8.66 ± 0.60^a	4.13 ± 0.62^b
Overall acceptability	8.8 ± 0.60^a	8.75 ± 0.60^b

Values are mean $\pm$ standard deviations of three replicates. Superscripts in the same column represents significant difference (< 0.001)

Table 6. Proximate composition of pasteurized crab spread (T1&T2)

Sl. No.	Constituents	Percentage	
		T1	T2
1.	Moisture	72.33 ± 0.57^a	71.42 ± 0.52^b
2.	Protein	17.03 ± 0.51^a	17.5 ± 0.62^b
3.	Fat	5.90 ± 0.42^a	6.1 ± 0.39^b
4.	Ash	1.50 ± 0.34^a	1.8 ± 0.36^b

Values are mean $\pm$ standard deviations of three replicates. Superscripts in the same column represents significant difference (< 0.001)

Results of proximate composition studies of pasteurized surimi based crab spread was determined (Table 6). The product exhibited moderate moisture and reasonable protein content for both the products processed at 86°C (T1) and 89°C (T2). Compared to pasteurized crab cake mix as reported by Loaharanu & Lopez (1970) in previous studies, this product reported better moisture and protein values and lower fat and ash content which may be due to its higher percentage of crab meat and surimi content.

A decreasing trend in pH was observed during the refrigerated storage life of 176 days (Fig. 3) unlike standard pasteurized crab meat which showed an increasing trend during storage (Yerlikaya & Gokoglu, 2004). This could be attributed to the presence of acetic acid as an ingredient in the recipe. Statistically no significant difference was observed between the treatments ($p < 0.05$). A gradual increasing trend in TVB-N, as indicated in many studies (Yerlikaya & Gokoglu, 2004; Loaharanu & Lopez, 1970) with values ranging from 25.26 ± 0.05 to 28.1 ± 0.05 mg 100 g⁻¹ was observed for T1. T2 batch showed comparatively high values with an average of 27.703 ± 0.039 than that of T1 (26.687 ± 0.039) (Fig. 4). Ludorf & Meyer (1973) recommended levels of 30-35 mg TVB-N 100 g⁻¹ as the upper limit of adequate freshness. However, statistically no significant difference was observed ($p < 0.05$). An increasing trend in TMA-N content was observed for both batches throughout the refrigerated shelf life period of 176 days (Fig. 5). T2 batch showed higher values of TMA-N content (7.5 - 9.23 mg 100 g⁻¹) compared to T1 (8.13 - 10.56). Reports suggest a value of 10 mg TMA-N 100 g⁻¹ as acceptable quality (Varlik et al., 1993). In the present study after 112 days of storage, the values increased beyond 10 mg 100 g⁻¹. Loaharanu & Lopez (1970) has observed that the

TMA-N content in pasteurized crab cakemix could not be considered as a sensitive index of spoilage of the product. However, statistically no significant difference was observed ($p>0.05$) between two batches during the storage period.

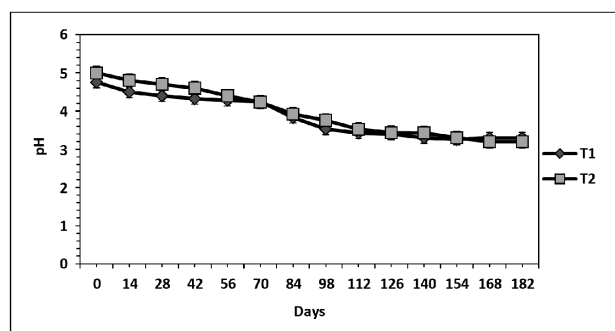


Fig. 3. Variation in pH of two batches of pasteurized crab spread under refrigerated storage condition

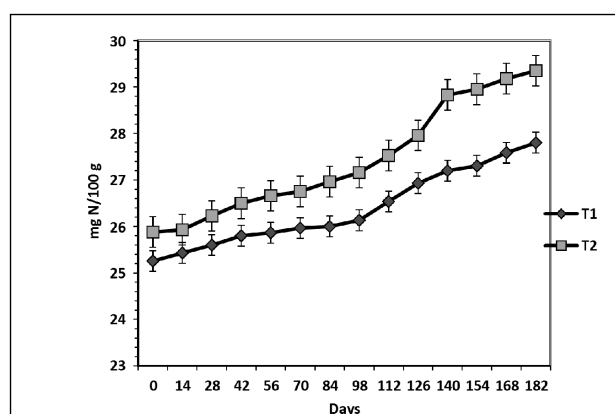


Fig. 4. Variation in TVBN of two batches of pasteurised crab spread under refrigerated storage condition

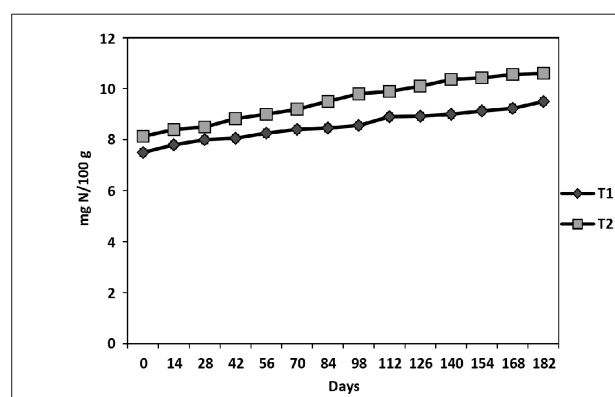


Fig. 5. Variation in TMAN content of two batches of pasteurized crab spread under refrigerated storage condition

According to Biji et al. (2013), the lipid oxidation products can highly influence the overall quality and flavor of thermal processed products. In this study an increasing trend in peroxide value was observed for both batches throughout the refrigerated shelf life period of 176 days. Initially both batches indicated similar values and gradual increase in trend was noticed. However from the Fig. 6, it was clear that PV of T2 batch showed a comparatively steeper increase after 70 days of storage life. O'Connell, 1975, has reported that if the peroxide value is kept below 10–20 meq kg⁻¹ fat, rancid flavor could be avoided. In this study, the PV was well below that limit throughout the storage period. No significant difference was observed ($p<0.05$) between two batches during the shelf life study. Thermal treatment could result in breakdown of high-molecular weight lipids such as triglycerides and phospholipids which could contribute towards new FFA formation (Aubourg et al., 1997). The lower FFA content indicates higher quality and lower oxidation which could be attributed to the lower processing temperature for a shorter period of time (Ahmed et al., 2017). The acceptable limit of FFA in edible crude fish oil was suggested as 2–5% (Young, 1985). The FFA values of both the samples were within acceptable limit during the storage period. In the study, initially both batches showed lower but similar values. But later the T2 batch indicated a significant increase in FFA compared to T1 (Fig. 7). It was also observed that T1 (86°C) and T2 (89°C) were significantly different ($p<0.05$) during the storage study period.

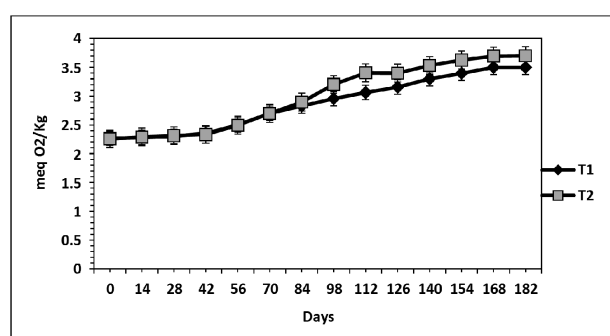


Fig. 6. Variation in PV of two batches of pasteurized crab spread under refrigerated storage condition

Appropriate pasteurization of crab meat could eliminate non-proteolytic *Clostridium botulinum* Type E and *Listeria monocytogenes* (USFDA, 2001) even though pasteurization does not result in a commercially sterile product (Cockey & Chai, 1991; Segner,

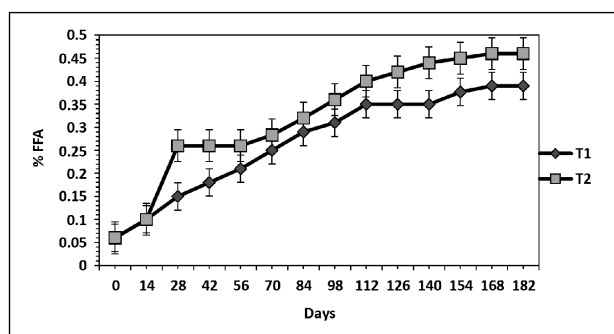


Fig. 7. Variation in FFA of two batches of pasteurized crab spread under refrigerated storage condition

1992). Swift cooling followed by refrigeration is necessary to preserve product quality and safety (Rippen et al., 1989). The Maryland Crabmeat Quality Assurance Program (MCQAP) emphasize that the maximum permissible bacterial count for fresh crab meat is 1.0×10^5 CFU g^{-1} whereas the maximum allowable bacterial count for pasteurized crab meat, according to the MCQAP is 2.5×10^4 CFU g^{-1} (Rippen & Sieling, 2008). In this study, a gradual increase in Total Plate Count (TPC) was observed for both batches during the storage but was within the permissible range (Fig. 8). Log values of TPC were taken for statistical analysis between two treatments. A significant difference ($p < 0.05$) was noted in TPC of two batches with storage period. No pathogens of public health significance like *Escherichia coli*, *Staphylococcus aureus*, and *Vibrio cholera*, *V. parahaemolyticus*, *Salmonella sp.* and *Listeria monocytogenes* were detected throughout the storage period of 176 days under chilled condition.

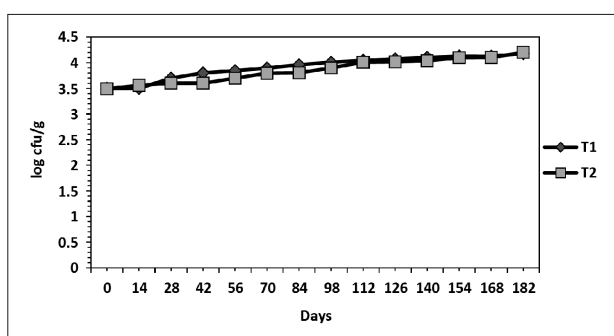


Fig. 8. Variation of Log TPC values of two batches of pasteurized crab spread under refrigerated storage condition

Sensory evaluation revealed that T1 remained acceptable throughout the entire storage life with average scores varying from 8 to 9 (Fig. 9a). T2

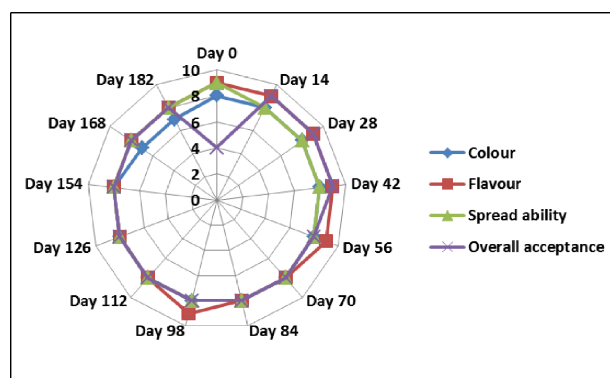


Fig. 9a. Sensory properties of T1 (86°C) process under chilled storage conditions

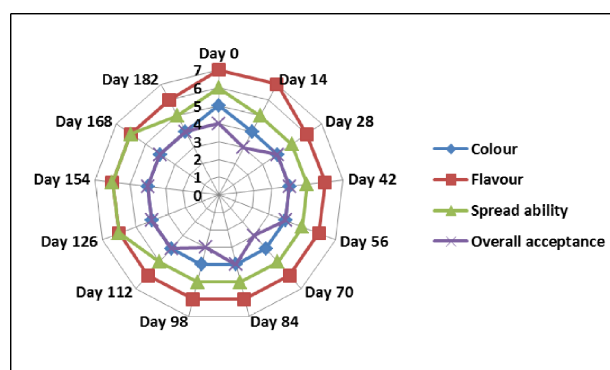


Fig. 9b. Sensory properties of T2 (89°C) process under chilled storage conditions

process on the other hand reported a lower acceptance score especially in color and overall acceptability throughout the storage period (Fig. 9b).

The study was an original attempt for developing an RTE product from crab meat incorporating surimi using mild heat processing technique such as pasteurization in a flexible container. The study demonstrated that pasteurization accompanied by refrigerated storage makes surimi based crab spread a consumer safe product with minimum loss of sensory attributes. The spread having crab meat and surimi in the ratio 1:1, pasteurized at 86°C for 92 minutes reported maximum sensory acceptability throughout the refrigerated storage period of 176 days with acceptable biochemical and microbiological quality parameters. This could lead to development of more RTE products utilizing low value crab species incorporating surimi resulting in higher value realization and value addition options for the industry.

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