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Determination of Microbiological Contamination Sources of Blue Crabmeat (*Callinectes sapidus* Rathbun, 1896) During Pasteurization Process

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Abstract.- This study has been conducted to determine the major contamination sources during pasteurization processing of blue crabmeat. Five different control points have been examined for the enumeration of total aerobic mesophilic bacteria, coliforms, *Escherichia coli*, *Vibrio parahaemolyticus*, coagulase- positive *Staphylococcus aureus*, *Salmonella* spp., *Listeria* spp., and yeast and molds. From the control points examined, personnel hands and the equipment were found as the primary contamination sources. Environmental air was determined as the secondary contamination sources. Stages of pasteurization process were found to eliminate the overall contamination rate, and therefore, this process has positive effects on the microbiological quality of the final product. Consequently, it has been concluded that the only way to avoid/eliminate these microorganisms is to apply proper cleaning and sanitation procedures.

Keywords: Pasteurization, crabmeat, *Callinectes sapidus*, microbiological contamination.

INTRODUCTION

Blue crab (*Callinectes sapidus* Rathbun, 1896) is one of the most important and commercially valuable crustaceans harvested in southeastern United States, along Atlantic coast and Gulf of Mexico (Ingham *et al*., 1990; Segner, 1992; Chen *et al*., 1996) and originally exists along the eastern coast America between Nova Scotia and Uruguay. Its main distribution area is the North American coast, where it forms an important fishery resource. The existence of *C. sapidus* is also reported on the European coast and Mediterranean (Enzenrob *et al.*, 1997). Since crab is usually consumed by american as well as europeans as well (Adeyeye, 2002), its commercial processing is an important branch of seafood industry (Chung and Cadwallader, 1993).

Crab is commonly marketed either as refrigerated fresh product, frozen or pasteurized chilled meat (Segner, 1992; Chung and Cadwallader, 1993). Crabmeat is processed in the form of (*i*) regular or "special" consisting of white body meat topped with broken lump (*ii*) jumbo and backfin consisting of large pieces of body meat taken from the muscle which controls the back swimming legs (*iii*) super claw and claw consisting of darker meat from the claws (Segner, 1992). In the processing of blue crabs, whole crabs are cooked and cooled, and then the body and claw meat are manually removed from the body of the crab. The picked cooked crab is put manually in plastic cans for pasteurization. The extensive handling of the crab meat during processing increases the risk of contamination by fecal enteric pathogens (Ingham *et al*., 1990) and food pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes*. These cause possible public health problems in fresh handpicked crab meat products (Chen *et al*., 1996).

So far, there have been many studies on the microbiological quality of crabmeat (Ray *et al*., 1976; Ward *et al*., 1977; Wents *et al*., 1985; Gecan *et al*., 1988; Ingham *et al*., 1990; Segner, 1992; Chung and Cadwallader, 1993; Chen *et al*., 1996), but there is none on microbiological quality during the processing stages of pasteurized blue crabmeat meat. The major aim of this study is, therefore, to investigate the possible microbiological contamination sources and their microbial loads during the processing stages of pasteurized blue crabmeat meat. Identifying these points would help us to project several solutions to eliminate or

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minimize the problems caused by these sources. On the other hand, knowledge of the contaminations is very critical in the good hygienic Practices program and effective hygiene as well sanitary procedures in food processing plants. On account of the quality protection and the safety of the process, storage and marketing stages of the food have gained importance in the whole world.

MATERIALS AND METHODS

Sampling procedure and pasteurization process

Live blue crab (*C. sapidus*) was collected from Akyatan Lagoon in the South of Mediterranean Sea and transported alive to the local fish processing plant in Adana, Turkey. The flow diagram of the pasteurized crabmeat processing stages is given in Figure 1.

Crabmeat samples were collected from five pre-determined processing stages: live crab, after steaming, after picking the meat from the shell manually, after closing the can (before pasteurization process), after cold storage.

In addition, knives, cans, counter surface and personnel hands were all involved in the processes. Swab method was used for the examination of the food contact surfaces. The swab was rubbed firmly over approximately 50 cm^2 surface three times with pre-moistened swab in 10 ml with 0.1% sterile peptone water and transported in the same diluent (Erkmen, 2007).

Air samples were obtained from the cooling room and handling unit of the processing plant. When sampling the environmental air, plate count agar (PCA-Merck) plates without lids were kept open in a place for 15min. where there was normal air circulation during processing (Erkmen, 2007; Temelli *et al*., 2006).

Twenty five grams of crabmeat was sampled for the determination of *Listeria* spp. *Salmonella* spp. and *Vibrio* spp. Ten grams of snail meat was sampled for determination of other microorganisms indicated in microbiological analysis section. Samples from the personnel hands were taken as follows: workers were made to wear sterile latex gloves and 20 ml 0.1%. Sterile peptone water was carefully pipetted into the gloves. Hands in gloves were massaged completely and the gloves were carefully taken off and transferred to the laboratory. All samples were diluted up to 10^{-6} with 0.1% sterile peptone water and were plated using

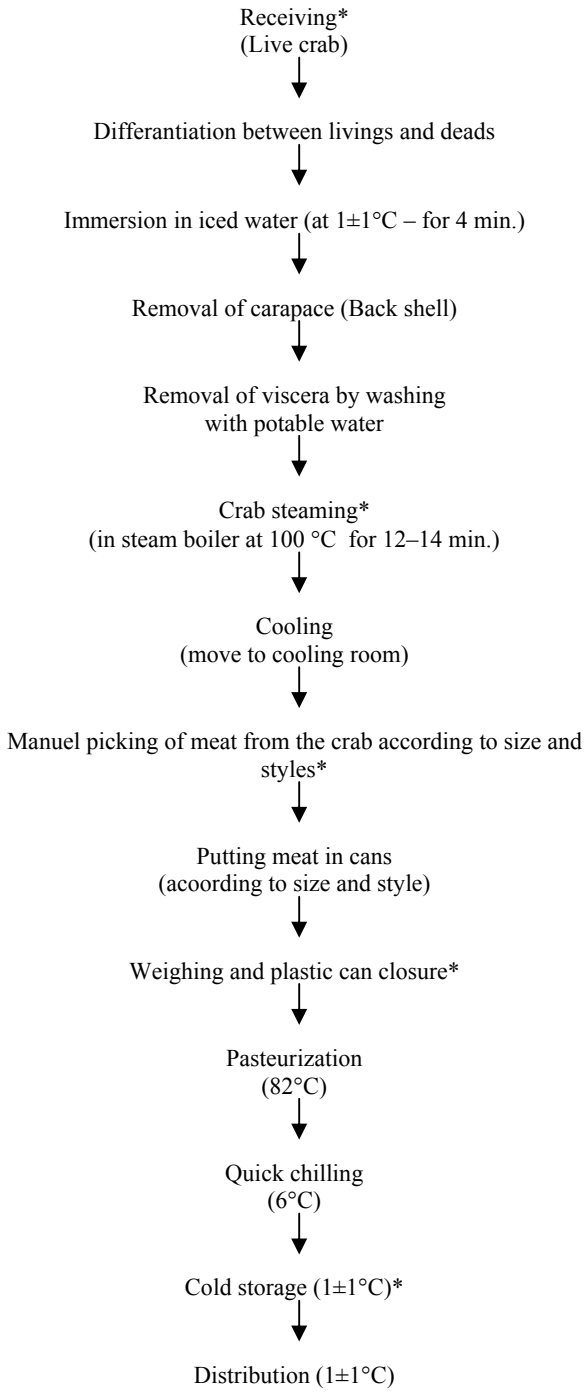

Fig. 1. Flow diagram of crabmeat pasteurizing process.
*:sampling points

appropriate methods for the bacteria indicated in microbiological analysis section.

Microbiological analysis

The aerobic plate count was obtained on plate count agar medium incubated for 2 days at 30°C. Potato dextrose agar was used as the medium for total mould–yeast counts. Plates were incubated 30°C for 5 days (Merck, 2005).

The most probable number (3-tube) method was used to determine the level of the tolal coliforms, and *E.coli* in crab samples. Coliform and *E.coli* were determined using fluorocult lauryl sulfate broth incubated for 48 h at 37°C. Gas positive tubes were recorded as coliform and controlled through UV portable torch of 366 nm long wavelength in a gloomy ambiance. Tubes with fluorescence were marked *E. coli* and final confirmation was made by applying the indole test with the addition 1 ml of Kovacs Indole Reagent (Merck, 1998).

For the estimation of *S. aureus*, dilutions of crabmeat homogenate were spread- plated on Baird-Parkar agar and incubated at 37°C for 48 hours. Typical colonies were counted, purified and subjected to further coagulase tests (using bactiden coagulase) for final confirmation (Antony *et al*., 2002).

Crabmeat samples (25 g) were aseptically taken and homogenized with 225 ml of alkaline peptone water and incubated at 37°C for 8 h for enrichment. A loopful of enriched sample was streaked onto thiosulfate citrate bile sucrose agar plates and incubated at 37°C for 18 to 24 h to test the existence of *V. parahaemolyticus*. After incubation, the plates were checked for colony morphology. Typical colonies were subjected to biochemical tests for confirmation (Merck, 2005). Crab sample (25g) was taken aseptically and homogenized with 225 ml of buffered water and incubated at 37°C for 24 h for preenrichment. Pre-enriched samples (1 ml each) were transferred to 10 ml of tetrathionate broth and incubated at 37°C for 24 h for selective enrichment. Enriched sample was streaked onto xylose lysine deoxycholate and *Salmonella–Shigella* agar plates and incubated at 37°C for 48 hours for examination of *Salmonella*. Suspect colonies were subjected to various biochemical tests for confirmation by using the triple sugar iron agar and urea broth (Omar, 1998).

For *Listeria* spp. analysis, 25 g of sample was homogenized with ½-strength Fraser broth. The homogenate samples were incubated at 30°C for 24 hours and homogenates (0.1ml each) were inoculated in 10 ml of full strength Fraser broth culture. In the meantime, Palcam and Oxford agars were similarly smeared on the culture. Palcam and Oxford agars were incubated for 24 hours at 37°C, and Fraser broth were incubated for 48 hours at 37°C. Palcam and Oxford agar cultures were smeared with whole-powered Fraser broth culture at 24^{th} and 48^{th} hours (Merck, 2005).

After incubation, the plates were checked for typical colonies, and suspected colonies were further subjected to biochemical tests for confirmation.

All analysis were done in duplicate. All chemicals were obtained from E.Merck, Germany.

Statistical analysis

Microbiological results were given using the $\log_{10}$ transformations of the bacterial counts. The data was also expressed as mean ± SD and analyzed through oneway ANOVA test using statistical Software SPSS statistical programme for Windows (Omar, 1998; Arannilew *et al*., 2005).

RESULTS AND DISCUSSION

Average total aerobic mesophilic bacteria number in the living crab transferred to the plant was 4.11 $\log_{10}$CFU/g. Ray *et al*. (1976) reported the bacterial count as approximately 4.28 $\log_{10}$CFU/g of fresh crabmeat. The results of our study are compatible with the results of these findings. On the other hand, the maximum level for fresh crab bacterial counts recommended by FDA (2008) was not exceeded. While determining microorganism number in the fresh crabmeat could not be sorted according to form and size.

After the steaming stage, total aerobic mesophilic bacteria number in jumbo, backfin, special, claw and superclaw was found 1.85 $\log_{10}$CFU/g, 1.78 $\log_{10}$CFU/g, 1.38 $\log_{10}$CFU/g, 1.52 $\log_{10}$CFU/g and 1.08 $\log_{10}$CFU/g, respectively. Crab meats were sorted by their sizes and forms in

aseptic conditions. But after workers manually selected the meat from the shell according to size and form as jumbo, backfin, special, claw and superclaw, it was observed that the total aerobic mesophilic bacteria numbers increased to 4.86 $\log_{10}$CFU/g, 4.90 $\log_{10}$CFU/g, 5.50 $\log_{10}$CFU/g, 4.32 $\log_{10}$CFU/g and 3.55 $\log_{10}$CFU/g respectively. After weighing and plastic can closure, these figures were found to increase further, reaching up to 5 $\log_{10}$CFU/g, 5.30 $\log_{10}$CFU/g, 6.77 $\log_{10}$CFU/g, 5.32 $\log_{10}$CFU/g and 4.50 $\log_{10}$CFU/g, respectively. These increases were possibly caused by the microorganisms transmitted from personnel, particularly from their hands. In many foods, the number of viable microorganism can provide information about the condition of the production and processing. Samakupa *et al.* (2003) indicated that during handling and preparation, bacteria are transmitted by the hands of the food workers, which is of vital importance. Therefore, aerobic mesophilic bacteria count is accepted as an important indicator of hygienic quality for foods.

The reduction observed in the total aerobic mesophilic bacteria after the steaming and resulted in internal temperatures between 85°C and 100°C for 12–14 minutes. Heat application of 90°C for 1.5 min. in the center for mollusc and 99–100°C for 3–4 min. for shellfish were accepted as safe processes before consumption. However, these temperatures are only sufficient for the destruction of vegetative forms of the pathogens; therefore, they are not the only safety factors for the whole production process (Temelli *et al.,* 2006).

E. coli, which is an indicator of fecal contamination and coliform, and *S. aureus* which is an indicator of a poor sanitation, were not found in the crab meat. Similarly, they were not found after the steaming process. However, after sorting meats by size and form, bacteria number of coliform type were found 1.32 $\log_{10}$MPN/g, 1.36 $\log_{10}$MPN/g, 1.62 $\log_{10}$MPN/g, 1.32 $\log_{10}$MPN/g and 1.36 $\log_{10}$MPN/g in jumbo, backfin, special, claw and superclaw, respectively, and as for *E.Coli,* its number were determined as 1.17 $\log_{10}$MPN/g, 1.36 $\log_{10}$MPN/g, 1.36 $\log_{10}$MPN/g, 0.96 $\log_{10}$MPN/g and 0.96 $\log_{10}$MPN/g respectively. After weighing and plastic can closure processes, bacteria number of coliform type were found to increase up to 1.97 $\log_{10}$MPN/g, 1.97 $\log_{10}$MPN/g, 2.32 $\log_{10}$MPN/g, 1.63 $\log_{10}$MPN/g and 1.63 $\log_{10}$MPN/g, respectively. Similar increases were observed in *E. coli* numbers, and they were found 1.63 $\log_{10}$MPN/g, 1.63 $\log_{10}$MPN/g, 2.18 $\log_{10}$MPN/g, 1.36 $\log_{10}$MPN/g and 1.36 $\log_{10}$MPN/g, respectively. Meanwhile, *S. aureus* numbers after sorting meat by size and form were 2 $\log_{10}$CFU/g, 2.84 $\log_{10}$CFU/g, 3.47 $\log_{10}$CFU/g, 3 $\log_{10}$CFU/g and 2.30 $\log_{10}$CFU/g, respectively. After weighing and plastic can closure process, similar increases were observed in these numbers as well, and they were 2.95 $\log_{10}$CFU/g, 3.41 $\log_{10}$CFU/g, 3.64 $\log_{10}$CFU/g, 3.44 $\log_{10}$CFU/g, respectively. Finding these organisms, which could not be determined initially, in later processes, provides an idea about the environmental and workers' hygiene. As mentioned above, it is thought that the contamination is caused mostly by workers, especially by the hands of workers and the places touched by foods. Analysis of microbial data obtained from sampling workers' hands, different crabmeat processing tools (plates, knives etc.) and various food contact surfaces indicated likely contamination sources (Table I). This situation proves the presence of a contamination caused by insufficient sanitation applications.

In the study, it was tried to determine the microbial content of the processing environment by taking air samples from three different locations of the cold storage and meat sorting and processing rooms. Mean total aerobic mesophilic bacteria and yeast and mold count were 2.01±0.90 $\log_{10}$CFU/plate and 1.11±0.42 $\log_{10}$CFU/plate, respectively. Presence of these elements in the environmental air during processing could influence the total count on the product negatively. No microbial risk was found in the analysis of the samples taken from the crab pasteurization boxes.

Results of the analysis made on the samples taken from the pasteurized crabmeat was quick-chilled then cold-stored and samples showed only the presence of aerobic mesophiles and no detection of coliforms, *E. coli, S. aureus* or yeast/molds.

As can be seen in Table II, it was found that microorganisms determined in the different production stages before pasteurization pose no risk after pasteurization, and total bacteria number was

Table I.- Microbial data obtained from sampling workers' hands, different crabmeat processing tools and various food contact surfaces.

Sampling area	Total aerobic mesophilic bacteria(log CFU/g)	Coliform (logMPN/g)	*E.coli* (logMPN/g)	*S.aureus* (logCFU/g)	Yeast and molds (logCFU/g)
Food contact surfaces	3.85±2.82	2.17±1.62	1.24±0.88	2.54±2.17	Nd
Process equipment	3.64±2.85	2.02±1.65	0.84±0.47	2.30±1.30	Nd
Workers' hands	4.18±3.65	2.93±2.54	2.21±1.69	1.40±0.44	0.77±1.23

Nd, not detected

Table II.- Microorganism count of the crab meat after pasteurization.

Crab meats	Total aerobic mesophilic bacteria(log CFU/g)	Coliform (logMPN/g)	*E.coli* (logMPN/g)	*S.aureus* (logCFU/g)	Yeast and m olds (logCFU/g)
Jumbo	1.37±0.78	Nd	Nd	Nd	Nd
Backfin	1.49±1.08	Nd	Nd	Nd	Nd
Special	1.64±0.96	Nd	Nd	Nd	Nd
Claw	1.72±1.16	Nd	Nd	Nd	Nd
Superclaw	1.88±1.5	Nd	Nd	Nd	Nd

Nd, not detected

decreased (P>0.05). *V. parahaemalyticus* which reportedly causes gastroenteritis generally can occur as a result of seafood consumption (Venugopal *et al*., 2001) and *Listeria* spp. and *Salmonella* spp. which are known to cause poisoning resulted from the consumption of some "seafood" and such bacteria were not found during any production stages.

Personnel's hands and the equipment were concluded as the primary contamination sources, and the environmental air was as the secondary contamination source among the control points examined in the production stages of Blue Crabmeat. The only way to avoid / eliminate these microorganisms is to apply proper cleaning and sanitation procedures. On the other hand, pasteurization stages in the blue crabmeat production process have positive effects on the microbiological quality of the final product. Pasteurization is a heat treatment application that kills vegetative pathogens and, though not completely, reduces food spoiling microorganisms.

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