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Original Research Article

Physicochemical and bacteriological status of retail-marketed shrimps and crabs in Beni-Suef, Egypt

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ABSTRACT

This study aimed at investigating the physicochemical and bacteriological status of retail-marketed crustaceans in Beni-Suef Governorate, Egypt. Therefore, 120 samples of crustaceans, 60 samples of imported unpeeled shrimp, and 60 samples of locally harvested chilled crab were collected from different fish markets in Beni-Suef Governorate. Collected samples were examined through assessing the physicochemical deterioration criteria in terms of pH and total volatile basic nitrogen (TVB-N), and the bacterial load including mesophilic count, psychrophilic count, *Staphylococcus (Staph.) aureus* count and most probable number (MPN) of coliforms, faecal coliforms and *E. coli*. As well as isolation and biochemical identification of pathogenic *E. coli* and *Staph. aureus*. The obtained results revealed that high percentages of examined crustaceans had pathogenic *E. coli* and *Staph. aureus*, however, total mesophilic, psychrophilic and *Staph. aureus* counts in most examined crustacean samples lie within the recommended permissible limits by national and international organizations. Similarly, pH and TVB-N mean values were within acceptable limits. We conclude that crustaceans marketed in Beni-Suef are subjected to inadequate hygienic measures during processing, time/temperature abuse, inappropriate handling and unsatisfactory personnel hygiene.

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1. Introduction

Seafood, including crustaceans, represent an important part of a healthful diet due to their high-quality protein content, vitamins, minerals and high levels of polyunsaturated fatty acids, named omega-3 (Okonko et al., 2009). Additionally, seafood proteins are easily digestible because of their low connective tissue content (FDA, 2009). On the other hand, seafood including crustaceans are highly perishable due to less acidic muscle pH, highly active muscular enzymes and high levels of unsaturated fatty acids, consequently, they are susceptible to microbiological, chemical, and physical changes during postmortem storage (Nirmal and Benjakul, 2010). As well as they could be subjected to different sources of contamination during various stages of handling and processing. Also, raw ingredients, handlers, water, equipment and utensils used in seafood processing may lead to contamination of seafood (Inabo et al., 2000). Because processing and packaging are done mainly by workers with poor personal hygiene and low sanitary measures (Oranusi et al., 2003).

The quality and safety of seafood can be assessed through sensory evaluation, physicochemical analysis by measuring pH and total volatile basic nitrogen (TVB-N), and bacteriologically by enumerating spoilage microorganisms especially coliforms, *Pseudomonas*, and identification of pathogenic microorganisms (Chen et al., 1990; Ramachandran et al., 1997 and Al-Dagal et al., 1999). In this regard, several reports recorded that contamination of seafood with *Staphylococcus aureus*, coliforms and other pathogenic bacteria can lead to serious health risks ranging from allergic reactions, mild gastrointestinal disturbances, a general degeneration of peripheral cellular tissues, to a gradual breakdown of the digestive and

excretive systems, abdominal cramps, vomiting, chills and fever (Acha and Szyfres, 1991; Varnam and Evans, 1991; Gracey et al., 1999; Ekholm and Hirshfield, 2001).

So far, there have been many studies on the microbiological quality of crustaceans (Ray et al., 1976; Ward et al., 1977; Wents et al., 1985; Ingham et al., 1990; Segner, 1992; Chung and Cadwallader, 1993; Chen et al., 1996; Gimenez and Dalgaard 2004; and Fath El-Bab et al. 2010). However, a little attention has been paid to the quality and safety of crustaceans in Beni-Suef governorate. Therefore, the present study was conducted to evaluate the physicochemical quality (pH and TVB-N) and bacteriological status of shrimps and crabs retail-marketed in Beni-Suef Governorate.

2. Materials and methods

2.1 Collection of samples:

For the objectives of this study, 120 crustacean samples were collected from different fish markets in Beni-Suef Governorate included 60 shrimp samples (30 peeled shrimp and 30 unpeeled shrimp) and 60 locally chilled crab samples. The collected samples were identified, individually wrapped and directly transferred to the laboratory in a clean and sanitized insulated icebox with a minimum of delay to be examined within 6 hours from collection.

2.2 Physicochemical quality parameters:

The pH was determined in 10 g flesh added to 10 mL of distilled water using a pH meter according to the method recommended by Korkeala et al. (1986). While TVB-N (mg N/100 g) was measured according to the method recommended by FAO (1986).

2.3 Bacteriological examination

Preparation of collected samples was done according to the technique recommended by ICMSF (1986) as follows. Ten grams of samples were removed using a sterile scalpel and forceps and transmitted to a sterile homogenizer flask containing 90 mL of 0.1% sterile peptone water (MAST, UK). The contents were homogenized at 2000 rpm for 2.5 minutes using a sterile homogenizer (MPW 302, Universal Laboratory Aid, made in Poland). The homogenate was allowed to stand for about 15 minutes at room temperature. The contents represented the dilution 10^{-1} were thoroughly mixed by shaking, one mL was aseptically transferred using a sterile pipette into a sterile test tube containing 9 mL of 0.1 % sterile peptone water to be diluted sequentially by ten-fold serial dilution up to 10^{-6} .

Aerobic plate counts (APC) at 35 °C for mesophilic count and 7°C for psychrophilic count were carried out using a standard plate count agar. As well as coliforms MPN (3-tube method) was performed on lauryl sulphate broth with inverted Durham’s tubes at 35 °C for 48 h, fecal coliforms MPN on E.C. broth Durham’s tubes at 44 ± 0.5 °C for 48 h, and *E. coli* MPN using eosin methylene blue plates at 35 °C for

24 h according to the technique recommended by AOAC (1990).

Subsequently, typical *E. coli* colonies were confirmed using IMVC methods. Samples for *Staphylococcus aureus* were spread plated on a Baird Parker agar and incubated at 35 °C for 48 h. Typical colonies were confirmed using catalase and tube coagulase tests (AOAC, 1995).

3. Results

The mean values of pH in the examined samples of unpeeled shrimp and crab were 6.47 and 6.62, respectively, while the mean values of TVB-N in the examined samples were 23.07 and 23.78 mg/100g, respectively (Table 1). The mean APC values at 35°C & 7°C of the examined unpeeled shrimp samples were 1×10^4 and 8×10^4 CFU/g, respectively. The mean values of coliforms (MPN), fecal coliform (MPN) and *E. coli* (MPN) in shrimp samples were 4×10 , 17 and 4 cells/g, respectively. While The mean values of *S. aureus* count for imported unpeeled shrimp was 2×10^3 CFU/g (Table 2).

Table (1): Statistical analysis of the chemical criteria of examined crustacean samples (n= 60).

Crustaceans	Number of Samples	Criteria	Minimum	Maximum	Mean	Standard error
Shrimp	60	pH	6.08	6.70	6.47	0.05
		TVB-N mg/100 mg	14.0	28.60	23.07	0.87
Crab	60	pH	6.08	7.81	6.62	0.089
		TVB-N mg / 100mg	18.33	29.06	23.78	0.69

Additionally, Table (3) revealed that the mean values of APC at 35°C and 7°C in examined

crab samples were 2×10^5 and 6×10^4 CFU/g, respectively. Moreover, the mean values of

coliforms (MPN), fecal coliform (MPN) and *E. coli* (MPN) in examined crab samples were 3×10^2 , 4×10^2 and 4 cells/g, respectively. While the mean value of *S. aureus* count in crab was 2×10^3 CFU/g. Moreover, based on the biochemical identification of *E. coli* and *Staph.*

aureus colonies, the incidences of *E. coli* type I in examined samples were 45 and 55 % in shrimp and crab, respectively. while coagulase positive *Staph. aureus* was detected in 75 and 35 %, respectively (Table 4).

Table (2): Statistical analysis of bacterial counts (CFU or cells/g) in examined imported unpeeled shrimp samples (n= 60).

Criteria	Minimum	Maximum	Mean	Standard error
Mesophilic count	10^3	6×10^4	1×10^4	4×10^3
Psychrophilic count	10^3	9×10^5	8×10^4	4×10^4
Coliforms (MPN)	3	150	4×10	10
Fecal coliforms (MPN)	3	93	17	6.
<i>E. coli</i> (MPN)	3	23	4	1
<i>S. aureus</i> count	$<10^2$	7×10^3	2×10^3	5×10^2

Table (3): Statistical analysis of bacterial counts (CFU or cells/g) in examined crab samples (n= 60).

Criteria	Minimum	Maximum	Mean	Standard error
Mesophilic count	1×10^3	7×10^5	2×10^5	3×10^4
Psychrophilic count	10^2	3×10^5	6×10^4	1×10^4
Coliforms (MPN)	3	1.1×10^3	3×10^2	65.360
Fecal coliforms (MPN)	3	1.1×10^3	4×10^2	72.866
<i>E. coli</i> (MPN)	3	23	4	0.6804
<i>S. aureus</i> count	$<10^2$	8×10^3	2×10^3	4×10^2

Table (4): Incidence of pathogenic bacteria in examined samples (n= 60)

Pathogens	Shrimp		Crab	
	No.	%	No.	%
<i>E. coli</i> type I	27	45	33	55
Coagulase + <i>Staph. aureus</i>	45	75	21	35

5. DISCUSSION

The obtained data showed that the mean pH values of examined samples of unpeeled shrimp, and chilled crab were within the permissible limit (6.5 - 7) recommended by FDA (2007). Similar results were reported by Fath El-Bab et al. (2010); and Gimenez and Dalgaard (2004). In this regard, Özyurt et al. (2009) found that the rate of pH change in fish mainly depends on the storage temperature, however, pH values above 7.1 being an indicative of decomposition. There is a relationship between the increase in pH and the deterioration of food material. Because basic nitrogenous compounds produced by microbial growth and enzymatic autolysis could elevate the pH (Huss et al., 2000).

TVB-N is a good freshness index for the assessment of shellfish quality due to gradually increase in TVB-N with inclination to spoilage (Yamagata and Low, 1995). Herein, the mean values of TVB-N in the examined samples of unpeeled shrimp and crab were within the permissible limit (30 mg /100 g) recommended by the Egyptian Standards (ES, 2005). Also, these results were similar with that recorded by Fath El-bab et al. (2010), and Kyrana and Lougovois (2002). In this respect, Montgomery et al. (1970) reported that the maximum limit of acceptability of TVB content of shrimp in Australia and Japan was 30 mg /100 g. Similarly, Wibowo et al. (1992) considered a

level of TVB-N of 30 mg/ 100g as a limit of acceptability for industrial purposes. Furthermore, they added that fishy odor started to develop when the TVB-N was 30.44 mg N% and the development of putrefactive odor was accompanied by a value of 31.88 mg N%. On the contrary, Yamagata and Low (1995) recorded that TVB-N level of 12.11 and 14.48 mg N% were enough to develop such a fishy and distinct urea odor in shrimp samples, respectively. From previous data, it could be concluded that TVB-N content of 30 mg N% is considered a specific limit of acceptability, Also, 100% of the samples should be considered acceptable according to the specified permissible limit (65 mg N%) given in the ES (1993).

The mean mesophilic and psychophilic counts in the examined unpeeled shrimp and crab samples were within the permissible limit (10^6 CFU/g) recommended by ES (2005). These results were very close to that obtained by Fath El-bab et al. (2010) and Kyrana and Lougovois (2002). Conversely, higher results were obtained by El-desoky (2002) who reported that the mesophilic counts in fresh marketed shrimp ranged from 7×10^4 to 6×10^9 with a mean value of 3.5×10^8 CFU/g, while, in examined crab samples, they ranged from 7×10^4 to 2×10^9 with a mean value of 2.6×10^7 CFU/g. Mesophilic count remains one of the most helpful indicators of the microbiological status of seafood. High viable counts indicate contaminated raw materials, poor sanitation, time/temperature abuse during production or storage. In addition, high counts foretell the likelihood of spoilage, because most food materials contain about 10^6 to 10^8 CFU/g at the time when spoilage is evident (FAO, 1992). On the contrary, Shamshad et al. (1990) stated that high bacterial counts are unacceptable but do not always indicate a degree of quality loss or spoilage.

Coliforms, fecal coliforms and *E. coli* are widely used as an index of fecal contamination and presence of sewage-derived pathogens in shellfish and harvesting waters (Banwart, 1989). However, Wilson and Moore (1996) mentioned that indicator organisms such as coliforms and fecal coliforms are not sufficient alone to confirm the absence of bacterial pathogens, and examination for specific pathogens such as *Salmonella* and *E. coli* species should be conducted when determining the acceptability of seafood for human consumption. The permissible limit of coliforms (10^2 MPN /g) which is recommended by ES (2005) were exceeded by most of examined shrimp and crab samples. In addition, ICMSF (1986) recommended 4×10^2 MPN/g as an acceptability limit of fecal coliforms. Herein, we found that 15 % and 25 % of examined shrimp and crab, samples, respectively exceeded such permissible limit. The contamination of seafood by coliforms lead to clinical symptoms as diarrhea, nausea, vomiting, fever (Varnam and Evans, 1991).

The high incidences of pathogenic *E. coli* and *Staph. aureus* in examined samples could be attributed to the fecal contamination of either harvesting water and/or these samples, the unsanitary handling during harvesting, transporting and processing of crustaceans, and poor personal hygiene (Forbes et al., 1998).

6. Conclusions:

From the present study we conclude that crustaceans marketed in Beni-Suef are subjected to inadequate hygienic measures during processing, time/temperature abuse, inappropriate handling and unsatisfactory personnel hygiene. High percentages of examined crustaceans had pathogenic *E. coli* and *Staph. aureus*, however, their total bacterial loads were mostly within recommended limits.

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