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Original Research Article

Chemical and bacteriological evaluation of some crustaceans

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ABSTRACT

Forty samples of crustaceans, 20 shrimp (local, imported, peeled and non-peeled) and 20 local chilled samples were collected from Beni-Suef markets. Samples were evaluated by physiochemical deteriorative criteria (pH, TVB-N) and bacteriological quality including APC at 35°C (mesophils), APC at 7°C (psychrotrophs), coliforms (MPN), fecal coliform (MPN), *E. coli* (MPN), *S. aureus* count and isolation and identification of specific pathogens (*E. coli*, and *S. aureus*). All fresh and frozen seafood samples were judged as safe food from the microbiological point of view. The total mesophilic, psychrotrophic and *S. aureus* of all examined seafood samples lied within the standard permissible limits.

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1. Introduction

Seafood is an important part of a healthful diet containing high quality protein and other essential nutrients. They are of low saturated fatty acids and may contain omega-3 fatty acids. In fact, a well-balanced diet including a variety of seafood can contribute in the good heart health, children growth, development and safety. Moreover, they contain high quality protein, which is easier to digest than other muscles, since it has a little connective tissue and rich in vitamins, minerals and other nutrients (Okonko et al., 2009). In the recent years, seafood consumption has been increased. In the future, seafood may be one of the important sources of animal protein for human consumption in many

parts worldwide (WHO, 1999; Speedy, 2003). Processing and packaging are mainly done by uneducated workers with poor sanitary conditions (Oranusi et al., 2003). Shrimp are the most important items and exported marine among the range of sea foods in the global fishery trade (Csavas, 1991). Shrimp are highly susceptible to both microbiological and chemical deterioration due to its high water content, neutral pH and relatively large quantities of free amino acids and naturally presence of autolytic enzymes producing bacteria. The number and type of bacteria found in freshly caught shrimp is influenced by a number of factors such as water, temperature, harvesting area, type of sediment and size of shrimp (Jeyasekaran et al., 2006). In the United States, competition from crab meat imports has adversely impacted the fresh crab meat industry.

The Virginia Marine Resources Commission estimates that Virginia's yearly crab harvest has been decreasing since 1995 (Vaughn, 2007). The bacterial flora on crabs reflects the environment from which they were harvested; the flora may change from season to season depending on the water quality, water temperature and harvest location (Cockey et al., 2006). The flora are also influenced by environmental factors such as temperature, packaging and duration of storage (Cockey et al., 2006). Furthermore, fresh crab meat is a perishable product that will undergo spoilage and flavor loss within 10–14 days or less during storage (Ward et al., 1977). Under refrigeration, spoilage of seafood occurs because of growth of psychrotrophic bacteria such as *Pseudomonas* spp. (Suklim et al., 2008). The shelf life of crab meat depends on several contributing factors, including initial microbial counts and container integrity (Rippen et al., 1989). The quality deterioration of shrimp can be assessed by sensory evaluation (Ramachandran et al., 1997), chemically by pH (Chen et al., 1990), as well as bacteriologically by enumerating spoilage microorganisms especially Coliforms, *Pseudomonas* (Al-Dagal et al., 1999), isolation and identification of pathogenic microorganisms (Zuberi et al., 1981). Several reports recorded that contamination of seafood with *Staphylococcus aureus*, salmonellae, coliform bacteria and others lead to health risks ranging from allergy reaction, stomach and intestinal growths, a general degeneration of peripheral cellular tissues, to gradual break down of the digestive and excretive system, abdominal cramps, vomiting, chills and fever (Acha et al., 1991; Varnam et al., 1991; Gracey et al., 1999; Ekholm et al., 2001; Edema et al., 2005). Therefore, the present study was aimed to evaluate the nutrient composition and the bacteriological quality of some seafood with estimation of TVB-N.

2. Materials and methods

2.1. Collection of samples

Forty samples of crustaceans, 20 shrimp (local, imported, peeled and non-peeled) and 20 local chilled samples were collected from Beni-Suef Governorate markets. Collected samples identified and directly transferred in an insulated ice box to the laboratory with minimum of delay to be examined within 6 hours from collection.

2.2. Chemical examination

The pH was determined according to the method recommended by Korkeala et al. (1986). Total volatile basic nitrogen 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 muscle maceration technique recommended by ICMSF (1986) as follows: Ten grams of a sample was removed using sterile scalpel and forceps and transmitted to a sterile homogenizer flask containing 90 ml of 0.1% sterile peptone water (DM185D, 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 of the flask 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 in a sequential manner by ten folds serial dilution up to 10^{-6} .

Aerobic plate count (APC) at 35 °C and coliforms MPN (3 tubes method) were performed according to the techniques recommended by AOAC (1990). For *Staphylococcus aureus*, samples were spread plated on Baird parker agar (Himedia) and incubated at 35 °C for 48 h. Typical colonies were confirmed using tube coagulase test (AOAC, 1995).

3. Results

In the present study, it has been found that the mean values of pH in the examined samples of shrimp and crab were 6.47 and 6.62, respectively, while the mean value of TVB-N in the examined samples of shrimp and crab were 23.07 and 23.78 mg/100g, respectively (Table 1). Meanwhile, the mean aerobic bacterial counts at 35°C and 7°C of the examined shrimp samples were 2×10^4 and 1.9×10^5 cfu/g, respectively (Table 2). Moreover, the mean values of coliforms (MPN), fecal coliform (MPN) and *E. coli* (MPN) for shrimp were 3.53×10^2 , 2.56×10^2 and 1.1×10^2 m.os/g, respectively, while the mean values of *S. aureus* counts for shrimp was 7.22×10^2 cfu/g (Table 2). On the other hand, the mean value of APC at 35°C and APC at 7°C of examined crab samples were 1.8

$\times 10^5$ and 9.22×10^4 cfu/g, respectively. The mean values of coliforms (MPN), fecal coliform (MPN) and *E. coli* (MPN) for examined crab samples were 2.63×10^2 , 1.27×10^2 and 5.65 m.os/g respectively, while the mean values of *S.*

aureus counts in crab was 6.58×10^2 cfu/g (Table 3).

Table 1. Statistical analysis of the chemical criteria of examined samples

Sample	Criteria	Minimum	Maximum	Mean	S.E.
Shrimp	PH	6.08	6.7	6.47	0.05
	TVB-N mg%	14	28.6	23.07	0.87
Crab	PH	6.08	7.81	6.62	0.089
	TVB-N mg%	18.33	29.06	23.78	0.69

Table 2. Statistical analysis of the bacterial count (m.os/g) of examined shrimp samples.

Value criteria	Minimum	Maximum	Mean	SE \pm
APC at 35°C (mesophils)	9.6×10^3	8.8×10^4	2×10^4	5.4×10^3
APC at 7°C (psychrotrophs)	1×10^4	1.9×10^5	9×10^4	5.8×10^4
Coliforms (MPN)	9.2	1.1×10^3	3.53×10^2	9.9×10
Fecal coliforms (MPN)	3	1.1×10^3	2.56×10^2	9.7×10
<i>E. coli</i> (MPN)	3	93	1.1×10	4.532
<i>S.aureus</i> count	$<10^2$	4.3×10^3	7.22×10^2	2.71×10^2

Table 3. Statistical analysis of the bacterial count (m.os/gm) of examined crab samples.

Value criteria	Minimum	Maximum	Mean	\pm SE
APC at 35°C (mesophils)	5×10^3	6.6×10^5	1.8×10^5	4.77×10^4
APC at 7°C (psychrotrophs)	5.2×10^3	2.8×10^5	9.22×10^4	2.21×10^4
Coliform (MPN)	3	1.1×10^3	2.63×10^2	8.8×10
Fecal coliform (MPN)	3	1.1×10^3	1.27×10^2	$.75 \times 10^2$
<i>E. Coli</i> (MPN)	3	23	5.65	1.31
<i>Staph. aureus</i> count	$<10^2$	3.4×10^3	6.58×10^2	2.45×10^2

4. Discussion

Table 1 revealed that the pH mean value in shrimp and crab was 6.47 and 6.62, respectively, which still within the permissible limit (6.5-7) recommended by FDA (2007). The obtained results were nearly similar to those obtained by Fath El-Bab et al. (2010), Gimenez and Dalgaard (2004) and Nada et al. (2014). The mean values of TVBN for shrimp and crab were 23.07 and 23.78mg/100g, respectively (within the permissible limit; 30 mg /100g as recommended by ES 2005 a). Such findings were more or less similar to those given by Fath El-bab et al. (2010), Kyrana and Lougovois (2002) and Nada et al (2014). Montgomery et al. (1970) reported that the maximum limit of acceptability of TVB content of peeled shrimp in Australia and Japan was 30 mg /100G. Whereas, Wibowo et al. (1992) considered a level of TVB-N was 30 mg/ 100g as a limit of acceptability for industrial purposes. Authors further 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 other hand, Yamagata and Low (1995) recorded that TVB-N level of 12.11 and 14.48 mg N% were sufficient to develop such a fishy and distinct ureal odor in shrimp samples, respectively. Previous literature concluded that TVB-N content of 30 mg N% is considered a specific limit of acceptability and 80% of the examined iced peeled shrimp in this study are considered acceptable, whereas, 100% of samples should be considered acceptable according to the specified permissible limit (65 mg N%) given in the ESS (516/1993). Tables 2,3 stated that for shrimp and crab the mean aerobic bacterial counts was (2×10^4 and 1.8×10^5 cfu/gm), respectively, which was within the permissible limit (10^6 cfu/g) as recommended by ES (2005a). Those results were nearly similar to what given by Fath El-bab et al. (2010). Consequently, the initial bacterial level at arrival to processing plant shall be considered a function of the quality and extent of storage of shrimp or prawn and a limit of up to 10^7 should be considered a critical limit at the arrival to the processing plant. In the same table, the mean values of coliforms count (MPN), fecal coliform count (MPN) and *E.coli* count (MPN) for shrimp were

3.53×10^2 , 2.56×10^2 and 1.1×10^1 for shrimp and 2.63×10^2 , 1.27×10^2 and 5.65 m.os/g for crabs, respectively. The permissible limit is 10^2 MPN/gm as recommended by ES (2005 a). The contamination of seafood by coliforms lead to clinical symptoms as diarrhea, nausea, vomiting, fever (Varnam and Evans, 1991). The mean values of *S. aureus* counts for shrimp and crab were 7.22×10^2 and 6.58×10^2 m.os/g, which was within the permissible limit of ES (2005a) which is not more (10^3). These results were nearly similar to data given by Nada et al. (2014), Fath El-Bab et al. (2010). The presence of *S. aureus* might be due to contamination of seafood from human sources, equipment during the handling and processing (Forbes et al., 1998).

5. Conclusion

Importance of seafood is due to it is a good source of protein, vitamins and minerals and it is easily digestible food. Handling, processing and human interfering may lead to contamination of seafood by different microorganisms. Moreover, PH and TVB-N can be used as indicator for fish deterioration

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