CHEMICAL AND BACTERIOLOGICAL EVALUATION OF SOME SEA FOODS

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A B S T R A C T

Ninety samples of seafood (30 each of shrimp, crab and gandofli) were collected from some local markets at Menoufia and Qaliobia Governorates for chemical and bacteriological examination. The chemical examinations cleared that the mean values of protein and fat percentage were 22.2 and 3.2 for shrimp, 19.5 and 2.4 for crab and 20.4 and 1.8 for gandofli respectively. While calcium and phosphorus were (68.7, 192.8) and (82.3, 159.4) and (70.3, 226.5) respectively. However, pH, TVBN, TBA and Histamine levels were (6.85, 9.6, 0.30 and 20.8); (6.68, 7.85, 0.25 and 14.91) and (6.38, 8.66, 0.17 and 16.92) respectively. The levels of vitamins A and D were (27.6, 5.6) and (15.2, 4.2) and (7.8, 3 ug/g) in the examined samples of shrimp, crab and gandofli respectively. Bacteriological examinations recorded that the mean values of total aerobic plate count in shrimp, crab and gandofli were (2.2 $x10^2$, 3.5 $x10^2$ and 3.7 $x10^2$ cfu/gm) respectively. However, the mean values of coliform were (206.92 x 10^5, 133.13 x 10^6, 177.12 x 10^7 MPN/gm) respectively. The mean values of *staphylococcus aureus* count of were (0.7 x 10^2, 1.5 x 10^2 and 3.2 x 10^2 cfu/gm) respectively. Salmonellae were detected in the examined shrimp, crab and gandofli at rates of 6.7%, 6.7% and 10% respectively. While, the isolated *E. coli* in the examined were 0%, 3.3% and 3.3% respectively. Salmonellae isolates were belonged to serotypes S. *typhimurium, S. anatum, S. muenster and S. enteritidis*. While isolates *of E. coli* belonged to O55:H7 and O111:H4 for crab and gandofli.

Keywords: shrimp, crab, gandofli, seafoods, *staphylococcus aureus, E. coli*

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1. INTRODUCTION

Sea food are an important part of a healthful diet where they contain high quality protein and other essential nutrients can be low in saturated fatty acids and may contain omega -3 fatty acids . In fact, a well-balanced diet that include a variety of seafood can contribute in good heart health and children growth and development and safety. In addition, they contain high quality protein, which is easier to digest than other muscle, since it has little connective tissue; also it is rich in vitamins, minerals and other nutrients (FDA 2009 and Okonko et al., 2009). Seafood consumption has been increased in the recent years. In the future, seafood will be one of the important sources of animal protein for human consumption in many parts of the world (WHO, 1999 and Speedy (2003). Sea food are prone to contamination at various stages of handling and processing Raw sea food products ,water and utensils used are highly prone to contamination (Inabo et al., 2000), Processing and packaging are done mainly by uneducated workers with poor sanitary conditions (Oranusi et al., 2003). TBA factor is responsible for rancid flavor, off odors, colors as well as texture deterioration (Nawar, 1996). 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 (Edema et al., 2005; Acha and Szyfres,1991 and Gracey et al., 1999, Varnam and Evans, 1991, FDA, 1995, Ekholm and Hirshfield, 2001). Therefore, the present study was aimed to evaluate the nutrient composition and the bacteriological quality of some seafood with estimation of TBA.

2. MATERIAL AND METHODS

2.1. Samples

A total number of 90 samples of seafood (30 samples from shrimp, crab and gandofli) randomly collected from some local markets at Menoufia and Qaliobia Governorates . The samples were placed in small sterile polyethylene plastic bags in icebox and transferred immediately to the laboratory. Each samples was divided into two parts, the first was used for bacteriological examination and the other part kept frozen at -18 ºC until the chemical analysis.

2.2. Chemical analysis.

a. Determination of protein contents according to AOAC (2000): the samples were estimated by the Kjeldahl method.
b. Determination of fat contents according to APHA (1985): the fat % of samples were estimated by soxhlet extraction.
c. Determination of vitamins (A and D) by HPLC according to Brubacher et al., (1985) and Dias et al., (2003).
d. Determination of calcium and phosphorus %. (Digestion procedure according Stanek et al., 2013).
e. Preparation of blank and standard solutions (Andreji et al., 2005).
f. Determination of pH according to Pearson (1984):
g. Determination of total volatile basic Nitrogen (TVBN) according to FAO (1980).
h. Determination of thiobarbituric acid number (TBA) according to Vyncke (1970).
i. Determination of histamine according to Moret and Conte (1996).

2.3. Bacteriological examination:

Ten grams from each sample was aseptically placed into sterile homogenizer flask containing 90 ml of sterile peptone water 0.1% and homogenized for 2.5 minutes, serial ten fold dilution were prepared, then the following analysis were done:1- Indicator organisms (total aerobes, coliform and *Staphylococcus aureus* counts) were enumerated by the methods of APHA 1992. 2- Isolation, identification and serological tests of *Salmonella* were adopted according to FAO (1992), Kauffman (1974) and Harvey and Price (1981). Isolation, identification and serological tests of *E. coli* were carried out according to APHA (1992) and Kok et al., (1996).

3. RESULTS

In this study, table (1) revealed that the mean values of the protein and fat in the examined samples of shrimp, crab and gandofli were (22.2, 3.2); (19.5, 2.4) and (20.4, 1.8) respectively. Table (1) also revealed that the mean value of mineral compound such as ca. and ph.mg % are (68.7, 192.8); (82.3, 159.4) and (70.3, 226.5 mg %) respectively. The mean value of vit. A and D ug/g. of shrimp, crab and gandofli were (27.6, 5.6) ; (15.2 , 4.2) and (7.8, 3), respectively. PH values of gandofli, shrimp and crab were 6.38, 6.85 and 6.68, respectively. While the mean values of TVBN for shrimp, crab and gandofli were 9.6, 7.85 and 8.66 mg /kg respectively. The mean value of TBA of shrimp, crab and gandofli were 0.30, 0.25 and 0.17 mg /kg respectively. Table (2) revealed that the mean

aerobic bacterial counts of the examined shrimp ,crab and gandofli samples, were ($2.2x10^2$, $3.5\ x10^2$ and $3.7\ x10^2$ cfu/gm) respectively. In the same table, the mean values of coliform counts for shrimp, crab and gandofli were ($206\ x\ 10^5$, $133.13\ x10^6$ and $177.12\ x\ 10^7$). The mean values of *s. aureus* counts for shrimp, crab and gandofli samples were ($0.7\ x\ 10^2$, $1.5\ x10^2$ and $3.2\ x10^2$Cfu/gm) respectively. Table (3) revealed that the isolation of salmonellae was detected in the examined shrimp, crab and gandofli in a percentage of (6.7%, 6.7% and 10%), respectively. Serology of salmonellae were *S. typhimurium S. anatum, S. enteritidis and S. Muenster.* The isolation of *E. coli* was detected in the examined samples of shrimp, crab and gandofli with a percentage of (0%, 3% and 3%) respectively. Regarding the serological identification of the isolated *E. coli* strains one isolate was O111:H4 Isolated from samples of crab and one isolate O55:H7 isolated from samples of gandofli.

Table (1): Chemical components and quality indices of seafood samples. (n=30 samples) for each.

Types of sea food		Protein %	Fat %	Ca mg %	Ph mg %	pH	TVBN mg %	TBA mg %	Histamine mg %	Vit A µg/g	Vit D µg/g
Shrimp	Min.	21.1	2.5	44	165	6.64	7.41	0.13	6.5	17	4.3
	Max.	23.6	4.1	81	221	7.13	15.08	0.42	41.9	41	7.7
	Mean	22.2	3.2	68.7	192.8	6.85	9.6	0.30	20.8	27.6	5.6
Crab	Min.	18.4	1.9	63	139	6.46	4.95	0.10	4.1	9	3.6
	Max.	21.3	3.3	107	187	6.97	11.38	0.41	28.5	23	5.1
	Mean	19.5	2.4	82.3	159.4	6.68	7.85	0.25	14.91	15.2	4.2
Gandofli	Min.	19.2	1.4	56	196	6.12	5.92	0.06	4.7	4	1.9
	Max.	21.4	2.3	89	258	6.69	12.19	0.29	33.1	12	4
	Mean	20.4	1.8	70.3	226.5	6.38	8.66	0.17	16.92	7.8	3

Table (2): Statistical analytical results of bacterial counts (cfu/gm) of the examined seafood samples (n=30 samples for each).

Type of sea food	count	Aerobic bacteria (cfu/gm|)	Coliform (MPN/gm)	*staphylococcus aureus* (cfu/gm)
Shrimp	Min.	$1.1x10^2$	$7x10^4$	$1x10^2$
	Max	$6x10^2$	$150x10^6$	$1.5x10^2$
	Mean	$2.2x10^2$	$206x10^5$	$0.7x10^2$
crab	Min.	$1.2x10^2$	$7x10^4$	$1.02x10^2$
	Max.	$1.02x10^3$	$150x10^7$	$3x10^2$
	Mean	$3.5x10^2$	$133.13x10^6$	$1.5x10^2$
Gandofli	Min.	$1.2x10^2$	$4x10^6$	$1.5x10^2$
	Max.	$9.4x10^2$	$1100x10^7$	$9x10^2$
	Mean	$3.7x10^2$	$177.12x10^7$	$3.2x10^2$

Table (3): Incidence and serotypes of salmonellae and *E. coli* isolates in the examined sea food samples (n=30)

Pathogen	Salmonellae			*E coli*		.
Sea food	No. of $^{+}$ve samples	% of $^{+}$ve samples	Serotypes	No. of $^{+}$ve samples	% of $^{+}$ve samples	Serotypes
Shrimp	2	6.7	*S. typhimurium* *S. enteritidis*	0	0	0
Crab	2	6.7	*S.muenster* *S. enteritidis*	1	3.3	O111:H4
Gandofli	3	10	*S. typhimurium* *S. enteritidis* *S.anatum*	1	3.3	O55:H7

4. DISCUSSION

Table(1) indicated protein and fat % similar to those reported by (Emi Lin Renitta ,2005) and El-Leboudi –Sohad (2010).Also, table (1)revealed that the mean mg % of mineral compound such as calcium and phosphorus mg % are (68.7, 192.8); (82.3,159.4) and (70.3, 226.5 mg %) respectively. These results are nearly with those recorded by Erkan and Ozkan (2008) and Santha et al., (2014). Concerning vit. A and D in shrimp, crab and gandofli was (27.6, 5.6); (15.2, 4.2) and (7.8, 3) respectively. The obtained results are more or less similar to those reported by Dias, et al., (2003). The pH values of gandofli was 6.38, which was within the permissible limit 6.5 recommended by ES (2005a). However, pH value in shrimp and crab was 6.85 and 6.68 respectively, which exceeded the permissible limit (6.5) recommended by ES (2005b). The obtained results were agreed with Gehad et al., 2010) and Gimenez and Dalgaard (2004). The mean values of TVBN for shrimp ,crab and gandofli were(9.6, 7.85 and 8.66 mg /kg) respectively which were within the permissible limit (30 mg /100gm) recommended by ES (2005a) .,these results also similar nearly with Gehad et al 2010 ; Kyrana and Lougovois 2002) . In the present study the mean values of TBA of shrimp ,crab and gandofli were 0.30 , 0.25 and 0.17 mg /kg ,respectively ,which were within the permissible limit (3 mg /kg) recommended by ES (2005a), These results were similar with Nawar (1996) and Saritha et al., (2014). TBA is widely used for the assessment of degree of secondary lipid oxidation (Nishimoto, 1985). TBA factor is responsible for rancid flavour, off odor, color as well as texture deterioration (Nawar, 1996). The mean values of histamine for shrimp, crab and gandofli were 20.8, 14.91 and 6.92 mg percentage respectively. Histamine Fish Poisoning (HFP) is a chemical intoxication that occur after eating bacteriologically contaminated fish of particular species. Its incidence has been under estimated because of it is frequently mild nature, lack of mandatory reporting and misdiagnosis (as seafood allergy). The fish are non-toxic when caught, but increase in histamine content as bacterial numbers increase. Fish may look and smell normal and cooking does not destroy the histamine although the condition is caused by histamine intoxication, it is pathogenesis is not fully understood and other toxins or potentiators may be involved

(Lehanel, L. 2000). Concerning the bacteriological examination, the results recorded in table (2) stated that shrimp crab and gandofli for the mean aerobic bacterial counts were ($2.2x10^2$, $3.5 x10^2$ and $3.7 x10^2$ cfu/gm) respectively, which were within the permissible limit (10^6 cfu/gm) as recommended by ES (2005a). These results agreed with that obtained by Gehad et al. (2010), Rodriguez–Jerez et al., (1994) and Nosier et al., (2009). Besides controlling specific hazards, freezing , icing cooking extends shelf life and enhances product safety (Puls Net USA , 2009) In the same table the mean values of coliform counts ($206 x 10^5$, $133.13 x 10^6$ and $177.12 x 10^7$) for shrimp, crab and gandofli respectively. The permissible limit (10^2 MPN /gm) recommended by ES (2005 a) .The contamination of seafood by coliform lead to clinical symptoms as diarrhea, nausea, vomiting, fever (Varnam and Evans, 1991). The mean values of *S. aureus* counts were ($0.7x10^2$,$1.5 x10^2$ and $3.2 x10^2$Cfu/gm) for shrimp ,crab and gandofli samples respectively, which was within the permissible limit of (ES 2005 a) which is not more (10^3). Presence of *S. aureus* may be due to contamination of seafood from human sources, equipment during the handling and processing (Forbes et al., 1998). The results in table (3) revealed that salmonellae was detected in the examined shrimp, crab and gandofli in a percentage of 6.7%, 6.7% and 10% respectively. Serotypes of salmonellae were *S. typhimurium S. anatum, S. enteritidis and S. Muenster*. Salmonellae are transmitted to man by improper cooked meat or by cross contamination from other food that are consumed without further cooking (Gracey et al., 1999). The isolation of *E. coli* were 0%, 3% and 3% respectively. *E. coli* is often preferred over the coliform group as an indicator of public health hazard. Regarding the serological identification of the isolated *E. coli*, two isolates were detected one from crab O111:H4 and one from gandofli O55:H7.

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, TBA can be used as indicator for fish deterioration

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التقييم الكيميائي والبكتريولوجي لبعض المأكولات البحرية

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الملخص العربي

تم تجميع تسعين (90) عينة عشوائية من الأطعمة البحرية بواقع (30) عينة من كل من الجمبري والكابوريا والجاندوفلي من أسواق الاسماك بمحافظة المنوفية والقليوبية. وقد تم فحص العينات كيميائيا وبكتريولوجيا ودلت نتائج الفحص الكيميائي أن متوسط النسبة المئوية للبروتينات والدهون كانت (3.2، 22.2)، (19.5 ,2.4)،(20.4 ,108) لعينات الجمبري و الكابوريا والجاندوفلي وكانت النسبة المئوية للكالسيوم والفسفور وهي (68.7 ,192.8) و(82.3 و159.3) و(70.3 و226.5) وكانت متوسطات نسبة فيتامينات أو د وهي بالميكروجرام لكل جرام (27.6 ,5.6)و(4.2،15.2)و(3,7.8) لكل من عينات الجمبري والكابوريا والجندوفلي علي التوالي . كانت متوسطات قيم الأس الهيدروجيني (6.85 ,6.68 ,6.38) لكل من الجمبري و الكابوريا والجاندوفلي علي التوالي . وكانت متوسطات النسبة المئوية للنيتروجين المتطاير الكلي مجم (8.66,7.850,9.6). وكانت متوسطات النسبة المئوية لحامض الثيوباربيتيوريك مجم |(0.30 ,0.25 ,0.17) وكانت النسبة المئوية للهستامين مجم كالتالي (20.8 ,14.9 ,16.92). وكانت نتيجة الفحص البكتريولوجي كالاتي متوسطات العد البكتيري الكلي للميكروبات الهوائية لعينات الجمبري والكابوريا والجاندوفلي وكانت في الحدود المسموح بها($2.2x10^2$,$3.5x10^2$,$3.7x10^2$) (خلية /جم). وكانت متوسطات العد الأحتمالي للميكروبات القولونية بالنسبة لعينات الجمبري و الكابوريا والجاندوفلي ($133.13x\ 10^6$,$206\ x10^5$) ($177.12\ x\ 10^7$ and. كانت متوسطات العد البكتيري للميكروب العنقودي الذهبي المكور ($0.7\ x10^2$, $1.5\ x10^2$, $3.2\ x10^2$ خلية /جم علي التوالي. كما تم عزل ميكروب السالمونيلا من عينات الجمبري و الكابوريا والجاندوفلي بنسبة (10,6.7,6.7) علي التوالي . تم تصنيف السالمونيلا سيرولوجيا كالتالي سالمونيلا تيفي ميوريم وانترتيدس وأنيتم ومنيستر. وكانت الأشريشيا كولاي المعزولة من الكابوريا والجاندوفلي فقط بنسبة (3.3) ولم يتم عزلها من الجمبري وفي النهاية تم تصنيف الميكروبات القولونية سيرولوجيا O111:H4 في الكابوريا وفي الجاندوفلي O55:H7.