Microbiological and Chemical Studies on Some Seafood Served in Port -Said Restaurants

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Abstract

Twenty seven ready to eat seafood nine samples of each grilled mullet, boiled shrimps and fried squid (four samples from up-scale and five from popular restaurants) were collected from seafood restaurants in Port- Said. The samples were examined for bacteriological and chemical indices. The mean values of total bacteria count in grilled mullet, boiled shrimp and fried squid in up- scale and popular restaurants were (1.8×10^3, 1.5×10^3; 1. 5×10^3, 1.2×10^3 and 1.45×10^3 , 0.94×10^3cfu/gm) ; the mean value of the total coliform count were 0.85×10^3, 0.7×10^3 ; 0.62×10^3 , 0.56×10^3 and 0.7×10^3 , 0.5×10^3 MPN/gm), respectively. The incidence of *E. coli* species were 25%, 0.20%; 0.0%; 0.20%, and 0.25%, 0.20% respectively. While the incidence of *Listeria* species were 25%, 0.20%; 0.50%, 0.40% and 0.0%, 0.0%, respectively. The average value of protein, fat, ash and moisture content in grilled mullet from up- scale and popular restaurants were 18.7%, 19.5%; 13.4%, 19.1%; 2.2%, 3.2% and 65.7%, 57.9% respectively. While in boiled shrimps the results were 14.0%, 19.6%; 14.0%, 11.4%; 2.1%, 2.2% and 69.8%, 67.4% respectively. The results in the examined fried squid were 12.4%, 11.3%; 25.8%, 33.2%; 3.0%, 2.8% and 55.1%, 49.2% respectively. The mean values of the TVB.N were 13.8, 12.0; 22.9, 27.8 and 19.7, 13.4 mg / 100g, respectively. The mean values of TBA were 3.7, 3.3; 3.9, 4.6 and 3.2, 3.3 malonaldiahyde mg / kg, respectively. The mean values of FFA were 8.8, 12.6; 8.9, 7.5 and 19.3, 24.9%, respectively.

Key words: Bacteriological & chemical evaluation - Seafood - ***E. coli*** - ***Listeria*** spp.

Introduction

Fish and seafood have become more popular food component for a large section of world population ***(Ozcan et al., 2013 and Varadharajan et al., 2013)***. The consumption of seafood products has increased recently due to increase consumer

awareness of nutrition and food quality. Nutritionists recommend seafood because of its high nutritional value and considered an excellent source of high-quality protein in addition it contain lipids with high levels of unsaturated fatty acids, which are claimed to reduce the risk of cardiovascular disease. Seafood is tender, easily digested, and a good source of many important vitamins and minerals ***(Ghanbari et al., 2013)***.

Methods of processing such as deep fat-frying, oven-baking, grilling and microwave cooking could affect the fish nutritive value, specially the fatty acids. ***(Gladyshev et al., 2006)***. The microbial quality of seafood is closely related to both environmental and processing conditions ***(Cortesi et al., 2009)***. *Listeria monocytogenes* and *E. coli* species were the most pathogenic micro-organisms which contaminate the seafood ***(Feldhusen, 2000)***. Thermal processing techniques are the best way to improve eats quality, safety of food products and to extend the products shelf life ***(Alizade et al., 2009)***. The quality of fish and seafood can be estimated by microbial methods and chemical methods such as measuring volatile compounds and lipid oxidation ***(Gulsun et al., 2009)***. Total volatile basic nitrogen (TVB-N) act as important characteristic for the assessment the quality of seafood products and it is the most common chemical indicators for spoilage of marine fish ***(Amegovu et al., 2012; Bechtel, 2008)***.

The present study aimed to examine the grilled mullet, boiled shrimps and fried squid for their quality and safety through bacteriological examination as determination of the total bacteria count, total coliform count and detection of ***E. coli*** and ***Listeria*** species, as well as to determine chemical indices as (protein, fat, ash , moisture, total volatile basic nitrogen ,thiobarbituric acid and free fatty acids) to assure seafood quality, nutritive value and its role as a public health hazard.

Material and methods

2.1. Samples collection

Twenty seven ready to eat seafood nine samples of each grilled mullet, boiled shrimps and fried squid (four samples from up-scale and five from popular restaurants) were collected from seafood restaurants in Port- Said. The samples were examined for bacteriological and chemical indices. Each sample was kept in a separate strip plastic bag and transferred to the laboratory in ice box within minimum of delay to be examined in the laboratory.

2.2. Bacteriological examination

2.2.1. Preparation of samples: (APHA, 1992).

Twenty five grams from each sample were aseptically excised from back muscles (above the lateral line) of grilled mullet, homogenized with 225 ml of 0.1% sterile peptone water in laboratory

blender for one minute to form a dilution of 1:10,from which tenth fold dilutions were accomplished up to 10^6.

2.2.2. Total Bacteria count (TBC):
One ml of each prepared serial dilution was separately inoculated into sterile duplicate Petri dishes. About 12 to 15 ml of tempered melted Standard plate count agar (cooled to 44 - 46 °C) were added to each inoculated plates, then thoroughly and uniformly mixed with the inoculums and left to solidify. After solidification, the inoculated plates were incubated at an inverted position at 30 °C ± 1 °C for 72 ± 3 hours. The number of countable colonies in selected duplicate plates of the same dilution was enumerated to obtain the total aerobic colony count per gm.
(FDA, 2009).

2.2.3. Total Coliform count (TCC):
One ml of each prepared serial dilution was separately inoculated into sterile duplicate Petri dishes. About 12 to 15 ml of Violet Red Bile Agar (VRBA) (Oxoid CM107) was proved. After agar solidifying 10 ml VRBA were added over layer and let agar solidify, then incubated for 24 h at 35ºC ***(FDA, 2013)***.

2.2.4. Detection and Identification of *Escherichia coli* (*E. coli*):
Loop full of previous preparation samples of each type of sample was inoculated separately onto sheep blood agar and on Eosin Methyl Blue media then incubated at 37°C for 24h. Suspected colonies were picked up and streaked on slope nutrient agar for further biochemical identification ***Konemann et al., (1993)***.

2.2.5. Detection, identification of *Listeria* species:
Twenty-five grams of each ready to eat sea food (grilled mullet, fried sepia and boiled shrimps) samples were homogenized in a stomacher for 2 min in 225 ml of *Listeria* enrichment broth (Difco), and incubated at 30 °C for 48 hrs. After incubation, one loop-full was sub cultured on *Listeria* Oxford medium base. The plates were incubated at 35°C for 24-48 hours. Suspected colonies were transferred from Listeria Oxford medium base to trypticase soy agar with yeast extract for purification and further chemical identification as Gram-stain, Catalase test, motility test, biochemical tests and Christie-Atkins, Munch- Petersen; test of haemolysis (CAMP Test). Further confirmation of ***Listeria*** spp. the isolates were inoculated into 10% aqueous stock solution of Manitol, L. Rhamnose and D. Xylose, ***(ISO, 2011)***.

2.3. Chemical indices:
2.3.1. Determine of moisture, crude protein, crude fat and ash were carried out in all samples as follows according to the method of ***A.O.A.C. (2000).***
2.3.2. Determine of total volatile base nitrogen (TVBN) mg/100gm and thiobarbituric acid (TBA) mg malonaldiahyde / kg sample

according to the methods of ***Egyptian Standard Specification (ESS) (1993).***

2.3.3. Determine of free fatty acid (FFA) % sample was determined as described **by *Takagi et al. (1984).***

Results

Table (1): *Total Bacteria Count and Total Coliform Count in the examined seafood samples.*

Samples	Grilled Mullet				Boiled Shrimp				Fried Squid			
	TBC		TCC		TBC		TCC		TBC		TCC	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Up-Scale restaurants	1.85×10^3	$\pm0.60\times10^3$	0.85×10^3	$\pm0.15\times10^3$	1.55×10^3	$\pm0.71\times10^3$	0.62×10^3	$\pm0.28\times10^3$	1.45×10^3	$\pm0.33\times10^3$	0.70×10^3	$\pm0.28\times10^3$
Popular restaurants	1.50×10^3	$\pm0.45\times10^3$	0.70×10^3	$\pm0.25\times10^3$	1.24×10^3	$\pm0.50\times10^3$	0.56×10^3	$\pm0.24\times10^3$	0.94×10^3	$\pm0.30\times10^3$	0.50×10^3	$\pm0.22\times10^3$

*Values expressed as mean ± SD.

Table (2): *Detections of E. coli in examined seafood samples***.**

Type of seafood	NO.	Positive		No. of accepted samples**%
		No.	%*	
Grilled mullet from Up-Scale restaurants	4	1	25	75
Grilled mullet from Popular restaurants	5	1	20	80
Boiled Shrimp from Up-Scale restaurants	4	0	0	100
Boiled Shrimp from Popular restaurants	5	1	20	80
Fried Squid from Up-Scale restaurants	4	1	25	75
Fried Squid from Popular restaurants	5	1	20	80

** Percentage in relation to total number of sample in each row.*

Accepted: refused samples according to* ***EEC, 2005.

Table (3): *Detections of Listeria ssp. in examined seafood samples.*

Type of seafood	NO.	Positive		No. of accepted samples**%
		No.	%*	
Grilled mullet from Up-Scale restaurants	4	1	25	75
Grilled mullet from Popular restaurants	5	1	20	80
Boiled Shrimp from Up-Scale restaurants	4	2	50	50
Boiled Shrimp from Popular restaurants	5	2	40	60
Fried Squid from Up-Scale restaurants	4	0	0	100
Fried Squid from Popular restaurants	5	0	0	100

** Percentage in relation to total number of sample in each row.*
***Accepted: refused samples according to EEC, 2005.*

Table (4): *Chemical composition of the "Grilled Mullet, Boiled Shrimp and Fried Squid "from Up-Scale and Popular in Port- Said restaurants*.*

Samples	Grilled Mullet				Boiled Shrimp				Fried Squid			
	Protein %	Fat %	Ash %	Moisture %	Protein %	Fat %	Ash %	Moisture %	Protein %	Fat %	Ash %	Moisture %
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Up-Scale restaurants	18.77 ±0.7	13.49 ±0.5	2.29 ±0.03	65.7 ±0.2	14.0 ±0.6	14.0 ±0.6	2.1 ±0.7	69.8 ±3.2	12.4 ±0.5	25.8 ±1.1	3.0 ±0.09	55.1 ±2.5
Popular restaurants	19.53 ±0.6	19.15 ±0.8	3.20 ±0.09	57.9 ±0.2	19.6 ±0.9	11.4 ±0.4	2.2 ±0.8	67.4 ±3.0	11.3 ±0.4	33.2 ±1.5	2.8 ±0.06	49.2 ±2.2

**Values expressed as mean ± SD*

Table (5): *Concentration of TVB. N, TBA and FFA in "Grilled Mullet, Boiled Shrimp and Fried Squid "from Up-Scale and Popular in Port- Said restaurants.*

*Total volatile bases estimated as mg/100 mg sample.

Samples	Grilled Mullet			Boiled Shrimp			Fried Squid		
	TVB.N*	TBA**	FFA***	TVB.N*	TBA**	FFA***	TVB.N*	TBA**	FFA***
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Up-Scale restaurants	13.81 ±0.59	3.78 ±0.18	8.84 ±0.32	22.96 ±0.91	3.96 ±0.11	8.94 ±0.33	19.70 ±0.69	3.26 ±0.9	19.35 ±0.86
Popular restaurants	12.06 ±0.48	3.31 ±0.12	12.60 ±0.57	27.83 ±0.12	4.64 ±0.11	7.52 ±0.21	13.44 ±0.48	3.39 ±1.0	24.9 ±1.00

** *Thiobarbituric acid estimates as mg malonaldiahyde / kg sample.*

*** *Free Fatty Acid estimates as% sample.*

Discussion

The results given in Table (1) revealed that the mean values of total bacteria count in grilled mullet, boiled shrimp and fried squid in up- scale and popular restaurants were (1.8×10^3, 1.5×10^3; 1.5×10^3, 1.2×10^3 and 1.45×10^3 , 0.94×10^3cfu/mg) ; the mean value of the total coliform count were 0.85×10^3, 0.7×10^3 ; 0.62×10^3, 0.56×10^3 and 0.7×10^3 , 0.5×10^3 MPN/mg), respectively. These results were lower than those suggested by ***Hassanien et al., (2014)*** who found that a mean value of TBC ranged from $6.3 \times 10^3 \pm 0.94 \times 10^3$ in fried squid and ***Hatha et al., (1998)*** who found that a mean value of TBC ranged from 1.0×10^2 to 6.4×10^4 under 1000 per g in cooked shrimp. Our results were lower than the results obtained by ***Vigano et al., (2007)*** who found that a mean value of coliform count in the examined fried M. cephalus, Saurus fish, S. pharaonis, Shrimp were $2.03 \times 10^2 \pm 0.20 \times 10^2$, $2.26 \times 10^2 \pm 0.14 \times 10^2$, $2.62 \times 10^2 \pm 0.26 \times 10^2$, $2.41 \times 10^2 \pm 0.19 \times 10^2$ respectively. Meanwhile, they disagreed with those of ***(Altug and Bayrak, 2003)*** who cannot detect coliform in all examined fish samples. The difference in the mean value of TBC and TCC may attribute to the difference in localities, and the extent of fish contamination.

The results given in table (2) revealed that the incidence of *E. coli* species were 25%, 0.20%; 0.0%; 0.20%, and 0.25%, 0.20% respectively. ***Salim, (2008)*** disagreed with our results, who reported that all examined fried fish; shrimp were free from ***E. coli***. While, the results of ***Hassanien et al., (2014)*** were lower than our

results who detected of 12 isolate of ***E. coli*** among 140 samples with incident percent of 8.6% from fried seafood. Isolate Finally detection of *E.coli* in cooked dishes indicates bad hygienic measure in this restaurant. These results came in accordance with those obtained by ***Vigano et al. (2007)*** who studied the microbiological quality of ready-to-eat foods samples analyzed, *E. coli* was the most frequently isolated species from boiled or fried food stuffs that were faecally contaminated and the contamination was likely to have occurred after preparation and before consumption.

Table (3) showed the incidene of *listeria* species in grilled, boiled and fried fish samples were 25%, 0.20%; 0.50%, 0.40% and 0.0%, 0.0%, respectively. Our results were disagreeing with ***(Hastein et al., 2006)***. ***Younis (2013) and Hosein et al. (2008)*** who failed to isolate *L. monocytogenes* from ready to eat fish, shrimp, M. cephalus, Saurus fish while ***Aziz et al. (2013)*** could detect the *l. monocytogenes* in a percentage of 14.57% in ready to eat (RTE) seafood products. But ***Hassanien et al. (2014)*** results were higher than our results that detected *L. monocytogenes* in percent of 25.7% and 25.7% of fried sepia and shrimp respectively.

The results given in Table (4) and Tables (5) showed that the average value of protein, fat, ash and moisture content in grilled mullet from up- scale and popular restaurants were 18.7%, 19.5%; 13.4%, 19.1%; 2.2%, 3.2% and 65.7%, 57.9% respectively. While in boiled shrimps the results were 14.0%, 19.6%; 14.0%, 11.4%; 2.1%, 2.2% and 69.8%, 67.4% respectively. The results in the examined fried squid were 12.4%, 11.3%; 25.8%, 33.2%; 3.0%, 2.8% and 55.1%, 49.2% respectively. The mean values of the TVB.N were 13.8, 12.0; 22.9, 27.8 and 19.7, 13.4 mg / 100g, respectively. The mean values of TBA were 3.7, 3.3; 3.9, 4.6 and 3.2, 3.3 malonaldiahyde mg / kg, respectively. The mean values of FFA were 8.8 , 12.6 ; 8.9, 7.5 and 19.3 24.9%, respectively. These results indicated that fried squid absorb more of cooking oil during frying at protein restaurant. Our results were agree with ***Talab (2011)*** who reported that the high increase in fat content in fried fish may be attributed not only to water loss but also due to oil absorption during cooking process. ***Rosa et al. (2007)*** also said that the fat, protein and ash contents of grilled fish increased. This increase may be due to a concentration effect caused by moisture loss. On other hand ***Garcia-Arias et al. (2003)*** found that the chemical composition of mullet was significantly affected by all the cooking methods ($p<0.05$). The moisture content of the fish samples ranged from 79.5% to 51.5%, decreasing after cooking. The decrease in the moisture content has been described as the most prominent change that makes

the protein, fat and ash contents increase significantly in cooked fish fillets. The our results indicate that TVB.N is one of the most widely measurement of seafood quality and appear as the most common chemical indicator of fish spoilage , that agree with ***Zhong-Yi et al. (2010) and Amegovu et al. (2012).*** On other hand TBA content reflects the secondary lipid oxidation and low TBA values may reflect the freshness of seafood that agrees with ***(Hamid et al, 2011)***. Our results of FFA were higher than ***Surabhi and Das (2007)*** who recorded that the FFA of fried carp fish were recorded 6.12 and 2.14 respectively. Finally, our findings revealed that TBC, TCC, *E. coli* and *Listeria spp.* varied from up- scale and popular restaurants in (grilled mullet, fried squid and boiled shrimps) due to post-cooking contamination, additives, during packing. However, Good Manufacturing Practices (GMP) and Hazard Analysis Critical control Point (HACCP) application in the chain of food production and processing should be undertaken in order to minimize the contamination risk.

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دراسات ميكروبيولوجية وكيميائية على بعض الأكلات البحرية المقدم فى مطاعم بورسعيد

نفيسة حس البنا [١]، أشرف رفعت الزينى [٢]، صفاء عل الوصيف[٣]، ريم صالح الباجورى

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تم إجراء هذه الدراسة على سبعة وعشرين عينة عشوائية من المأكولات البحرية المعدة للأكل تسع عينات من كل البوري المشوي والروبيان المسلوق والحبارالمقلي (أربعة عينات من المطاعم الراقية وخمسة عينات من المطاعم الشعبية) جمعت من مطاعم المأكولات البحرية في بورسعيد، تم فحصالعينات بكتيريولوجيا وكيميائيا وقد كانت القيمة المتوسطة للعد الكلى للبكتيريا فى البورى المشوى والروبيان المسلوق والحيارالمقلي فى المطاعم الراقية و الشعب (١,٨ × ١٠[٣] ، ١,٥ × ١٠[٣]؛ ١,٥ × ١٠[٣]، ١,٢ × ١٠[٣]؛ ١,٤٥ × ١٠[٣]، ٠,٩٤× ١٠[٣] على التوالي، بينما العد الكلى لبكتيريا القولون ٠,٨٥ × ١٠[٣] ،٠,٧ × ١٠[٣]؛ ٠,٦٢ × ١٠[٣] ، ٠,٥٦ × ١٠[٣] ؛ ٠,٧ × ١٠[٣] ، ٠,٥× ١٠[٣] على التوالى و كانت النسبة المئوية لايشيرشيا كولاى ٢٥%،٢٠ %؛ ٠,٠%، ٢٠%؛ ٢٥%، ٢٠% علىالتوالى. أما النسب المئوية فى الليستريا كانت ٢٥%،٢٠ %؛ ٥٠%، ٤٠%؛ ٠,٠%، ٠,٠% على التوالى. وقد دلت نتائج الفحص الكيميائي انمتوسط النسبة المئوية للبروتينات والدهون والرماد والرطوبة فى البورى المشوى ١٨,٧%، ١٩,٥%؛ ١٣,٤% ، ١٩,١% ؛ ٢,٢%، ٣,٢%؛ ٦٥,٧%، ٥٧,٩% على التوالى، وفى الروبيان المسلوق ١٤,٤%، ١٩,٦%؛ ١٤,٠%، ١١,٤%؛ ٢,١%، ٢,٢%؛ ٦٩,٨%، ٦٧,٤% على التوالى ، أما الحبار المقلى 12.4%، ١١,٣%؛ ٢٥,٨، ٣٣,٢%؛ ٣,٠%، ٢,٨%؛ ٥٥,١%، ٤٩,٢% على التوالى وكانت نتيجة متوسط النيتروجين الكلى (١٢,٠،١٣,٨؛ ٢٢,٩، ٢٧,٨؛ ١٩,٧، ١٣,٤) مجم/١٠٠جم ، بينما متوسط حمض الثايوباربيوتريك (٣,٧، ٣,٣؛ ٣,٩، ٤,٦؛ ٣,٢، ٣,٣)مالونالدهيد مجم/كجم ، أما متوسط النسبة المئوية للأحماض الدهنية الحرة (٨,٨، ١٢,٦؛ ٨,٩ ،٧٠٥؛ ١٩,٣، ٢٤,٩) % على التوالى.