

Quality and Safety Evaluation of Salted Fish

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Abstract

There are many types of salted fish in Egypt such as Feseekh (prefermented salted mullet), salted sardine and Meloha. Many cases of food poisoning are occurred caused by these salted products, so, the purpose of the present study was to get a view of the quality of the salted fish sold in the Egyptian market. A total of 105 samples including Feseekh, salted sardine and Meloha (35 of each). All collected samples were microbiologically and chemically analysed. Samples were examined microbiologically for determination of Aerobic Plate Count (APC), *Staphylococcal aureus* counts (log₁₀cfu/g), direct extraction of *Staphylococcus aureus enterotoxins*, coliform and *Escherichia coli*. Isolation and identification of *salmonella*, *Vibrio parahaemolyticus* and Sulphur reducing bacteria. The bacteriological examination revealed that the mean values of APC in the examined fish samples were 2.57±0.13, 3.53±0.13 and 3.73±0.23 for sardine, meloha and feseekh, respectively.

The *Staphylococcal* counts were 1.82±0.07, 2.34±0.11 and 2.53±0.12 for examined salted fish samples, respectively. Prevalence of *Staph. aureus enterotoxins* directly extracted from examined positive samples that contained more than 10⁴cfu/g as enterotoxin A detected in one sample of meloha and type D from feseekh. Mean values of coliform count were 2.23±0.10, 2.94±0.14 and 3.11±0.14 in examined samples, respectively, and *E.coli* average count was 1.2±0.05, 1.21±0.12 and 1.28±0.03. The incidence of food poisoning organisms (*Salmonella*, *Vibrio parahaemolyticus* and Sulphur reducing bacteria) also was investigated and one of *Vibrio parahaemolyticus* was isolated in sardine and meloha samples with a percentage of 2.86 % and 2.86 %, respectively. The chemical analysis of salted fermented fish showed that mean values of histamine levels, sodium chloride and pH were 10.83±0.44, 11.03±1.02 and 15.85±0.9, 17.35±0.21, 15.56±0.08 and 11.90±0.33, 6.20 ±0.03, 6.18 ±0.04 and 6.28±0.04 in sardine, meloha and feseekh, respectively. The public health of isolated bacteria was recorded and discussed.

Keywords: Ecoli; Histamine; Salmonella; Salted Fish

Introduction

Salted fish products are popular in many countries around the globe, as these have been proven to be safe for millions, even in the developed countries [1]. NaCl is added to foods for its effects on sensory, functional and preservation properties. NaCl inhibits microbial growth by restriction of the available water in the meat and fish products. However, its pro-oxidant activity is reported to accelerate the development of lipid oxidation in marinated and salted fatty fish products [2]. Numerous kinds of microorganisms such as bacteria, yeast and mould can be present in salted fish due to the spontaneous fermentation that occurs in the process. The microorganisms in salted fish can be originated from the fish itself and the salt also used in the manufacture. Bacteria are found in many parts of fresh fish, i.e. in body surface, in gill and in intestine. In studies of sea food borne pathogens, four major pathogens have emerged as being of significant importance in terms of human health and disease.

These include *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, and *Salmonella spp.* [3]. *V. parahaemolyticus* is a human pathogen that occurs naturally in the marine environments and is frequently isolated from a variety of seafood including fish, shrimp, crab, lobster, scallop, and oyster [4]. This pathogen is a common cause of foodborne illnesses in many Asian countries, including Taiwan, China, and Japan, and is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States [5]. One of the health risks is histamine and other biogenic amines (BAs) that can form during handling and storage of raw material or during subsequent processing (Particularly ripening) and storage steps.

BAs are formed through the decarboxylation of specific free amino acids by exogenous decarboxylases released from the microbial population associated with the seafood [6,7]. The growth of microorganisms makes food organoleptically unacceptable for

consumption because of changes in colour, odor and texture [8]. The problem is that many cases of food poisoning are occurred caused by these salted products. So, the purpose of the present study was to get an objective overview of the quality of the prefermented salted mullet, salted sardine and meloha sold in the Egyptian market.

Materials and Methods

Collection of Samples

A total of 105 random samples of salted fish represented by salted sardine, and meloha and feseekh (35 samples of each), were collected from different supermarkets in Aswan governorate, Egypt. The collected samples were kept in its original bags and aseptically transferred without delay, in an insulated ice box to the laboratory and subjected to the following examinations.

Bacteriological Examination

Preparation of Sample

Twenty five grams of the examined samples were aseptically transferred to a sterile stomacher bag and homogenized with 225 ml sterile buffered peptone water (0.1%) for 30-60 seconds to give an initial dilution of 1/10. One ml of the initial dilution was transferred by means of sterile pipette to another sterile tube containing 9 ml of sterile buffered peptone water (0.1%) then mixed thoroughly by using vortex for 5-10 seconds to obtain the next dilution (1:100). Repeat this operation to obtain further decimal serial dilutions up to 10⁶ according to APHA [9].

Determination of Aerobic Plate Count

according to APHA [9].

Enumeration and Isolation of Staphylococcus Aureus

Was done according to FDA [10].

Direct Extraction of Staphylococcus Aureus Enterotoxins from Salted Fishes

According to [11]. Ten gm from each sample was blended with 10 ml of physiological saline (0.85%). The blended sample was homogenized in a high-speed cooling centrifuge at speed 32,000 xg and temperature 4 °C for 30 minutes. The clear supernatant fluid was filtered through a 0.2 µm low protein binding membrane filter (Mintain plates 4/pk 0.2 µm, Millipore Corporation, Bedford). The clear filtrate was used for assay of toxin content in the sample.

Extraction, Detection and Typing of Enterotoxin

According to Oda, *et al.* [12] and Shingaki, *et al.* [13] the clear culture supernatant fluid was tested serologically by RPLA technique using SET-RPLA (Oxoid) (A kit for the detection of staphylococcal enterotoxins A, B, C and D). (Manufactured by Denka Sekeu LTD, Japan for Oxoid LTD)

Enumeration of Coliforms and detection of fecal coliforms (by Most Probable Number (MPN) according to FDA [14]

Enumeration, Isolation and identification of B-glucuronidase-positive *Escherichia coli* according to ISO 16649-2 [15].

Enumeration of sulfur reducing bacteria according to the method recommended by ISO: 15213 [16] Technically identical with ES: 6191 [17].

Detection of *Salmonella* species according to ISO 6579-1/ [18].

Chemical Analysis of Salted Fish Samples

Determination of pH Value

It was performed according to the method recommended by ES (63-11/) [19]. Ten gm of each salted fish samples was homogenized with 50 mL deionized water for 1 min. pH was measured at room temperature using a digital pH meter (Suntex TS-1, Taiwan) equipped with a probe-type combined electrode (Ingold) through direct immersion of electrode into the mixture.

Determination of Sodium Chloride Content (NaCl)

NaCl was measured according to AOAC [20]. Known volume of 0.1 M AgNO₃ solution enough to precipitate all Cl as AgCl in 10 g test sample, then 20 ml of HNO₃ was added. The mixture was boiled gently on hot plate for 15 min., cooled then 50 ml of H₂O and 5 ml indicator were added. The mixture was titrated with 0.1N of NH₄SCN solution until the color became permanent light brown. Amount of 0.1M H₄SCN used was subtracted from added amount of 0.1M AgNO₃ and difference was calculated as NaCl. Each ml of 0.1N AgNO₃ = 0.058% NaCl.

Determination of Histamine

RIDASCREEN Histamine (Art. No.: R1601, 96 wells / Art. No.: R1604, 48 wells) is a competitive enzyme immunoassay for the quantitative analysis of histamine in food.

Statistical Analysis

Statistical analyses were run in triplicate and results were reported as mean values ± standard deviation (SD). Data were subjected to analysis of variance (one-way ANOVA Excel 5.0). A p-value less than 0.05 ($p \leq 0.05$) was considered statistically significant [21].

Results

It is evident from the result recorded in Table 1 that APC in the examined samples varied from 2.00 to 4.83 with an average value of $2.57 \pm 0.13 \log \text{ cfu/g}$, 2.04 to 4.91 with an average value of $3.53 \pm 0.13 \log \text{ cfu/g}$ and 2.00 to 5.83 with an average value of $3.73 \pm 0.23 \log \text{ cfu/g}$ for the examined samples of sardine, and meloha and feseikh, respectively. Table 1 showed that the mean values of *Staphylococcus aureus* count ($\log \text{ cfu/g}$) of examined samples sardine, and meloha and feseikh were 1.82 ± 0.07 , 2.34 ± 0.11 and 2.53 ± 0.12 respectively. Results achieved in Table 1 indicated that coliform was isolated from 80%, 97.14%, and 100% of sardine, meloha and feseikh respectively. Table 1 showed that the mean values of *E. coli* count ($\log \text{ cfu/g}$) of examined samples were 1.2 ± 0.05 , 1.21 ± 0.12 , 1.28 ± 0.03 , respectively.

Type of fish	Statistical analysis of microbial counts (mean $\log_{10} \text{ cfu/g} \pm \text{SE}$)											
	APC			<i>Staph. Aureus</i>			Coliform count			<i>E. coli</i> count		
	Positive samples		Mean±SE	Positive samples		Mean±SE	Positive samples		Mean±SE	Positive samples		Mean±SE
	No	%		No	%		No	%		No	%	
Sardine	35	100	$2.57 \text{A} \pm 0.13$	27	77.14	$1.82 \text{A} \pm 0.07$	28	80	$2.23 \text{A} \pm 0.10$	9	25.71	$1.2 \text{a} \pm 0.05$
Meloha	35	100	$3.53 \text{a} \pm 0.13$	24	68.57	$2.34 \text{a} \pm 0.11$	34	97.14	$2.94 \text{a} \pm 0.14$	11	31.43	$1.21 \text{a} \pm 0.12$
Feseikh	35	100	$3.73 \text{a} \pm 0.23$	25	71.43	$2.53 \text{a} \pm 0.12$	36	100	$3.11 \text{a} \pm 0.14$	14	40	$1.28 \text{a} \pm 0.03$

Table 2: Microbial properties of salted fish samples (n= 35 of each)

NB:

1. Significance difference between means ($P < 0.05$) having Capital and small letters in the same column for each count separately.
2. Ten, 13 and 15 of Sardine, and Meloha and Feseikh samples were unaccepted as they contained *Staph. aureus* $> 100 \text{ cfu/g}$ samples according to (ES 1725/2005) concerning salted Feseikh, (ES 1725-2/2005) concerning salted Sardine and (ES 1725-3/2005) concerning salted Meloha

Type of examined samples	Types of <i>Staph. aureus</i> enterotoxins							
	A		B		C		D	
	No	%	No	%	No	%	No	%
Sardine	ND	0	ND	0	ND	0	ND	0
Meloha	1	50	ND	0	ND	0	ND	0
Feseikh	ND	0	ND	0	ND	0	1	50

Table 2: *Staph. aureus* enterotoxins in salted fishes

Type of examined samples	Isolated organisms					
	<i>Salmonella spp.</i>		<i>V. parahaemolyticus</i>		Sulfur reducing bacteria	
	No	%	No	%	No	%
Sardine	ND	0	1	2.86	ND	0
Meloha	ND	0	1	2.86	ND	0
Feseikh	ND	0	ND	0	ND	0

Table 3: Incidence of *Sal. Spp.*, *V. parahaemolyticus* and Sulfur reducing bacteria in salted fishes (n=35 of each)

Type of fish	Statistical analysis									
	pH			NaCl			Histamine			
	Accepted samples		Mean±SE	Accepted samples		Mean±SE	Accepted samples		Mean±SE	
	No	%		No	%		No	%		
Sardine	34	97.14	$6.20 \text{''A''} \pm 0.03$	35	100	$17.35 \text{''A''} \pm 0.21$	35	100	$10.83 \text{''a''} \pm 0.44$	
Meloha	29	82.86	$6.18 \text{''a''} \pm 0.04$	35	100	$15.56 \text{''aB''} \pm 0.08$	30	85.71	$11.03 \text{''a''} \pm 1.02$	
Feseikh	32	91.43	$6.28 \text{''a''} \pm 0.04$	35	100	$11.90 \text{''ab''} \pm 0.33$	31	88.57	$15.85 \text{''A''} \pm 0.9$	

Table 4: Mean pH, NaCl and Histamine in salted fishes

Table 2 illustrated that *Staph. aureus* enterotoxins types “A” and “D” were successfully extracted from one sample (50%) out of two samples each of meloha and feseikh, respectively. It is evident from the results recorded in Table 3 indicated the isolation of *V. parahaemolyticus* from one sample each of sardine and meloha (2.86 %), while examined feseikh samples were free from *V. parahaemolyticus*. Moreover, Salmonella and sulfur reducing bacteria were not detected in all types of examined salted fish samples. Table 4 illustrated Hydrogen ion concentration (pH) mean level was significantly higher ($P < 0.001$) in examined feseikh samples (6.28 ± 0.04) as compared with both sardine (5.87 ± 0.02) and meloha (5.9 ± 0.02).

While no significant difference between examined sardine and meloha ($P > 0.05$). Table 4 showed that sodium chloride mean level was significantly higher ($P < 0.001$) between all groups of examined salted fish samples in which sodium chloride content was higher in sardine (17.35 ± 0.21) followed by meloha (15.56 ± 0.08) and finally feseikh samples (11.90 ± 0.33). Table 4 showed histamine mean level was significantly higher ($P < 0.001$) in examined feseikh samples (15.85 ± 0.9) as compared with both sardine (10.83 ± 0.44) and meloha (11.03 ± 1.02). While no significant difference between examined sardine and meloha ($P > 0.05$).

Discussion

Micro-organisms are important causes of food spoilage because they break down food into Compounds that they can utilize. Therefore, food quality decrease as spoilage start and quality of food product relies on quantification of total number of micro-organisms. Hence, the growth of bacteria in fermented fish product may sometimes cause problems Aidam, et al. [22]. It is evident from the results recorded in Table 1 that the APC of sardine, meloha and feseikh were 2.57 ± 0.13 , 3.53 ± 0.13 and 3.73 ± 0.23 respectively these results mostly agreed with those obtained by AL-Asous [23] and Gabriel & Alano-Budeao [24]. On the other hand, disagreed with Wattimena, SC et al. [25], El-Dengawy, et al. [26] and El-Shorbagy [27] found that the mean colony counts in examined Feseikh samples was 51×10^6 , finally the mean colony counts in examined salted sardine samples was 15.75×10^6 .

Lower results were obtained by El-kewaiey [28] who revealed that the highest mean value of the total aerobic counts of feseikh sample was 1.3×10^4 and Rahman, et al. [29]. The results in Table 1 showed that the mean values of *Staph. aureus* count /g of sardine, meloha and feseikh were 1.82 ± 0.07 , 2.34 ± 0.11 and 2.53 ± 0.12 respectively. These results mostly agreed with those obtained by Gabriel & Alano-Budeao [24] found TPC significantly decreased from 4.51 log CFU/g in the fresh fish to 2.79 log CFU/g after the 24-h salting process. These results similar to with Edris, et al. [30] found mean values of *Staph. aureus* count /g of vacuumed packed feseikh, fseikh in jar were 6.1×10^2 , 2.1×10 respectively. On the other hand, disagreed with Ebied & Ibrahim [31] and Shafik, et al. [32], higher results were obtained by El-Shorbagy [27] who found that *S. aureus* count in feseikh samples was 15×10^3 /gm and in sardine samples was 4.25×10^3 /gm.

Comparing to the results recorded in Table 1 revealed that the mean value of coliforms in sardine, meloha and feseikh were 2.23 ± 0.10 , 2.94 ± 0.14 and 3.11 ± 0.14 respectively, nearly similar results were obtained by Edris, et al. [33] found mean value of coliforms count in mugil and sardine 2.10 ± 0.16 and 2.52 ± 0.11 respectively, disagree with Ginigaddarage, et al. [34] found total coliforms were absent in all locally produced samples and examined 46 imported samples, three samples were detected with total coliforms were less than 100 MPN/g in two samples. The results also disagreed with Suleiman & Mustafa [35] reported that indicator organisms like coliforms and pathogens like *Staphylococcus aureus* were absent in tested dried fish samples after processing. This results differ with that obtained by Gabriel & Alano-Budeao [24] found the total coliforms of the unprocessed fishes was 3.70 log CFU/g. Possible sources of this group of microorganisms include improperly cleaned and sanitized equipment and unhygienic handling practices Jay [36].

On the other hand, disagreed with Majumdar, et al. [37] found that total coliform count (TCC) in retail market and control samples ranged from 73.27 ± 16.74 to 94.03 ± 20.14 MPN/g and 20.11 ± 2.39 to 31.45 ± 5.74 MPN/g, respectively. Table 1 showed that the mean value of *E. coli* count (log cfu/g) of examined samples of sardine, meloha and feseikh were 1.2 ± 0.05 , 1.21 ± 0.12 and 1.28 ± 0.03 respectively. This results differ with that obtained by Suleiman & Mustafa [35] found all examined samples were negative for *E. coli*. Table 3 showed that the isolation of *V. parahaemolyticus* from one sample each of sardine and melouha (2.86 %). On the other hand, disagreed with Edris, et al. [33] found *V. parahaemolyticus* was 16.67%, 6.67%, 6.67% of feseikh, sardine and meloha, respectively and Baffone, et al. [38] isolated *V. parahaemolyticus* from 5% of the examined marine fish samples. Table 3 showed that examined feseikh samples were free from Salmonella and sulfur reducing bacteria in all types of examined salted fish samples. These results go hand to hand with those recorded by El-Dengawy [39] found that anaerobic spore formers producing H_2S were not detected in all examined samples.

It is evident from the result of pH recorded in Table 4 were nearly similar to those reported by Aidam, et al. [22] found pH level in two products collected from different factories; Jebel Al-aulia dam and Wadi Halfa 7.2 ± 0.01 and 6.7 ± 0.04 respectively and El-Shehaw, et al. [26] found pH level 6.03 ± 0.01 and 5.47 ± 0.01 for salted sardine and salted mullet respectively. Table 4 showed that the mean value of sodium chloride in examined samples of sardine; meloha and feseikh were 17.35 ± 0.21 , 15.56 ± 0.08 and 11.90 ± 0.33 respectively. These results go hand to hand with those recorded by El Sheikha, et al. [40] found that mean value of sodium chloride in feseikh 14 ± 0.21 . These results differ with that obtained by Kasozi, et al. [41] who found that mean value of sodium chloride in dry-salted *A. baremoze* samples was 13.8 ± 0.76 .

The results of histamine content recorded in Table 4 were 10.83 ± 0.44 , 11.03 ± 1.02 and 15.85 ± 0.9 in sardine; meloha and feseikh respectively. On the other hand, disagreed with Kasoz N, *et al.* [41] who found histamine content (mg/100g) in salted-fermented mullet fish flesh (*Mugil cephalus*) (feseikh) after storage for 45 and 60 day 3.28 and 3.413 respectively, Raslan & Hamed [42] found free amino acid and biogenic amines content of feseikh fish high was (1078 mg/kg) and in low salt (1799 mg/kg) increased significantly during long time of ripening and storage. These results are in line with those recorded by Edris, *et al.* [33] showed that the prevalence of unaccepted samples according to histamine content were 43.33%, 33.33 % and 20% in examined feseikh, sardine and meloha, respectively [43].

Conclusions

The present study concluded that salted fishes are contaminated with various types of bacteria; this due to neglected sanitary measures adopted during handling of fish during salting processes and could be attributed to improper sanitation during catching, handling, processing, storage, transportation, distribution and fish marketing. Therefore, a concerted effort should be made to maintain sanitary condition in processing, preparation and handling to decrease the contamination of the fish products to the minimum limits.

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