Al-Mukhtar Journal of Sciences 32 (1): 46-53, 2017

Effect of the Seasons and Salt Concentrations on Microbial Load of Wet-Salted Fermented Product (Fassiekh)

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Received: 26 Februray 2017 / Accepted: 25 July 2017
Doi: https://doi.org/10.54172/mjsc.v32i1.93

Abstract: The study aims at investigating the microbial load of wet-salted fermented product (a traditional fermented product in Sudan named locally; fassiekh) and determination of total viable bacterial count, according to salt concentrations (20%, 25% and 30% of the fish weight) and seasons (summer, autumn and winter) variations. Fessiekh processed from popular fessiekh fish species (*Hydrocynus* spp, local name: Kass).The isolation and identification of bacteria and mould were examined for the microbial quality of fassiekh. The total viable count of bacteria in fresh fish used as raw materials in fessiekh preparation (*Hydrocynus froskalii*), ranged from $2x10^3$-$5.5x10^3$cfu/g. The concentrations of salt had direct effect on the microbial quality of salted *Hydrocynus froskalii*. It was observed that the total bacterial count was increased during the first five days in summer and autumn while at winter season it showed increases till the tenth day, followed by remarkable decreasing. The counts began to decrease as salting proceeded. Five *Staphylococcus spp* (*Staph.aureus*, *Staph.rostri*, *Staph.lentus*, *Staph.epidermidis* and *Staph.pyogen*) were isolated from all samples of salted fish and appraises about 46.77% of total isolates. Also three species of *Micrococcus* were isolated (*Micro.leuteus*, *Micro.roseus* and *Micro.lactis*), and *Aerococcus viridans*, and they represented 36.17% & 12.9% of all isolated samples respectively. The viable bacteria counts of commercial fessiekh were significantly higher ($p<0.05$) when compared to the experimentally salted fish at the same salt concentrations. No yeasts or fungi were detected in tested samples.

Key words: Salt concentration, Seasons, Microbiological analysis, Fessiekh.

INTRODUCTION

Food safety is everybody's concern, and it is difficult to find anyone who has not encountered an unpleasant moment of food borne illness at least once in his lifespan. Food borne illnesses may result from the consumption of food contaminated by microbial pathogens, toxic chemicals or radioactive materials (Macachor, 2016). Fish are highly perishable food items as they start to spoil as soon as they are harvested. So, processing and storage methods are vital factors in fish consumption. During transportation from point to markets, there is a great chance for the fish to be contaminated by bacteria (Clucas and Ward 1996). Preservation of fish by salt is an old age technology. This method of preservation still has popularity in many developing countries due to its simplicity and low cost of processing (Takagi *et al,*. 1984).Sudan has a number of large water reservoirs, which contains a huge wealth of fish of several types, and the estimated wealth was about 110 thousand tons. The main sources of fish in Sudan are the Blue Nile, White Nile, River Nile, lake reservoirs behind dams and irrigation

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canals, as well as the Red Sea. In Sudan, nearly 70% of the total fish landings are consumed in forms of fresh fish. The rest is cured either by salting, fermentation or sun-drying. The process of fish salting and fermentation is termed locally as "Fessiekh" making. Fessiekh is a wet salted product, soft in texture with a strong pungent smell and a shiny silvery appearance. It can be stored for more than three months (FAO, 1992).Fessiekh is not a truly indigenous Sudanese food product but it is the major fermented product from fish in Sudan. This product has an immense popularity in Egypt and is also familiar with other regions of the Middle East, (Makie *et al*., 1971). It is consumed after cooking together with additives such as peanut paste and some spices, which made it more acceptable and delicious. According to (Osman *et al.,* 2012), the process of fassiekh making is about a century old, introduced to Sudan from Egypt during the Turko-Egyptian rule (1821-1885), and then transferred and established traditionally within families or through non-formal training(Sulieman and Khamis 2011).

The fermented fassiekh, normally produced in houses or in small-scale sectors by covering fish with salt in alternate layers for up to 7 days or less, depending on temperature, then transferred to be fermented with additional salt and left to fermentation up to 10 days (Dirar 1993, Anihouvi *et al.,* 2012, El Hag *et al.,* 2012) reported that the prevalence of traditional preservation methods employed throughout Sudan are defective and need efforts pertaining to their improvement and development. Therefore, the objective of the present study was to examine the microbial load present in salted fassiekh in order to evaluate the hygienic practices of fassiekh processing.

MATERIALS AND METHODS

Collection of samples

Samples of fresh fish namely Kass (*Hydrocynus forskalii*), were brought from local fish markets and weighed about 71kg. These samples were kept in polyethylene bags with crushed ice and transported to the Fisheries Research Center, where microbiological investigations were immediately carried out.

Processing

Fresh fishes were washed, eviscerated, washed again and transferred to baskets to dry up while covered by a thin cloth to prevent insect's invasions. Fish were weighed to the nearest gram using a dial balance (KRUPS type 875) and for the purpose of salting, divided into three equal groups. The first group was subjected to a total weight of salt amounting to 20% of the fish weight, the second to 25% and the third to 30%. The procedure used is called dry-salting. In this method, salt was applied by hand and brushing off the fish surface, the inner lining of eviscerated abdominal cavity and the gills chambers. This process was conducted by separating the fish layers by coarse salt mattresses inside a plastic container. When the salt penetrates the flesh, it extracts the fluids through plasmolysis. The extracted fluid (pickle) was allowed to drain continuously. Used salt is removed from the fish surfaces and the fish restocked with new dry salt between the layers once during the ripening process. During processing of fessiekh in the laboratory, sampling was carried out every five days for 7 times (about a month), the first sample took place after the treated fish became as a fessiekh product.

The steps applied above were repeated three times according to the seasons of the year: summer, autumn, and winter (average air temperatures 37, 30, 27^{o}C respectively). Commercial fessiekh samples (used for comparison) were obtained from Central Vegetables and Fruit market, south of Khartoum.

Microbiological examination

Appropriate serial dilution was made by using a desired amount of samples (20g) and transferred to a sterile bottle containing 180 ml of Peptone water (0.1% w/v) to give 10^{-1} dilution, then 1ml from the bottle was transferred to a tube containing 9 ml of Peptone water to give 10^{-6} dilutions; then further dilutions were made in a similar manner. A total viable count was enumerated by pouring plate method using Plate

ISSN: online 2617-2186 print 2617-2178

Count Agar (PCA) at (37 ± 1° C, 48 h) and Mannitol Salt Agar (MSA) at (37 ± 1° C 36 - 48h) was used as a selective and differential characteristic medium for identification of *Staphylococcus* and *Micrococcus* spp. as described in (Harrigan 1998). Pure colonies of staphylococci isolates were differentiated by conducting coagulase test as well as biochemical tests such as Urea test, Voges–Proskauer (VP) test, and Sugar fermentation as described in(Barrow GHandFeltham 1993, Harrigan 1998). Potato dextrose agar was used for counting mold and yeast (22±1°C, 5 days).

Statistical analysis

Data obtained were analyzed as a completely randomized design and the means were compared by T- tests described by SPSS software (Version 13), with 0.05 level of significance.

RESULTS AND DISCUSSION

The total viable count of bacteria in fresh fish used as raw material in Fessiekh preparation (*Hydrocynus froskalii*) was presented in (Table 1). From the results, the total viable count of bacteria in fresh fish (whole) ranged between $3x10^3$ -$5.5x10^3$cfu/g. The number of bacterial counts could be explained on the basis of contamination of fish during catching, handling, transportation, and exposure to the surrounding environment. It could be noticed that the total bacterial plate counts of the samples from gills, viscera and whole fish were slightly different. (Shewan 1977) and Gram, (1989) noted that the bacterial flora on newly caught fish depends on the environment in which it was caught rather than on the fish species. Among fish parts (Table 1), the viscera contained the highest bacterial counts. The numbers of microorganisms in the gastrointestinal tract of fish were far higher than in the surrounding water. This indicated the presence of a favorable ecological niche for the microorganisms (FAO, 1995).

Table (1): Total Viable Bacterial counts (cfu/g) of fresh *Hydrocynus forskalii*

Seasons	Whole	Gill	Viscera
Summer	3.5×10^3	2×10^3	4×10^3
Autumn	5.5×10^3	2×10^3	8.5×10^3
Winter	3.5×10^3	2.5×10^3	4.5×10^3

On the contrary, authors believed that the microflora of the gastrointestinal tract was merely a reflection of the environment and the food intake. Liston, (1980) stated that the total number of organisms fall in the range of 10^2-10^7cfu/cm^2 on the skin surface. Microbial contents of species after salting with different concentrations varied with time (Table 2).

Table (2): Total Viable Bacterial counts (cfu/g) of salted samples during different seasons

Salt concentration	20%			25%			30%		
Seasons	Summer	Autumn	Winter	Summer	Autumn	Winter	Summer	Autumn	Winter
0 day	2×10^3	4×10^3	12.5×10^3	5×10^3	2×10^3	7.5×10^3	3×10^3	1×10^3	8.5×10^3
5	1.5×10^3	4.5×10^3	15×10^3	3.5×10^3	3×10^3	12.5×10^3	2.5×10^3	2×10^3	7.5×10^3
10	1×10^3	4.5×10^3	14.5×10^3	1×10^3	1×10^3	10×10^3	0.5×10^3	1×10^3	7.5×10^3
15	4.5×10^3	2.5×10^3	14×10^3	3.5×10^3	1×10^3	5×10^3	4.5×10^3	0.5×10^3	4.5×10^3
20	3.5×10^3	1×10^3	10×10^3	1.5×10^3	2×10^3	5×10^3	1.5×10^3	0.5×10^3	2.5×10^3
25	1.5×10^3	0.5×10^3	3.5×10^3	0.5×10^3	2×10^3	3.5×10^3	0.5×10^3	0.5×10^3	1.5×10^3
30	0.5×10^3	0.5×10^3	2×10^3	0.5×10^3	1×10^3	1.5×10^3	0.5×10^3	0.5×10^3	1.5×10^3

There was an increase in the case of total viable bacterial counts during the first five days (average temperatures 30°C, and 37°C) and ten days at temperatures 27°C. (Tsai *et al.,* 2005), found that

ISSN: online 2617-2186 print 2617-2178

total viable bacterial count of mackerel fish salted and sold in Taiwan markets ranged between $4.3x10^2$ and $5x10^5$cfu/g. On the other hand, (Gun *et al*., 1996) reported that bacterial counts were $4.7x10^3$cfu/g and $5x10^5$cfu/g on the beginning and the end of storage period respectively, at storage period of trout preserved by salt for 9 weeks. The findings of this study are confirming the findings of (Gun *et al*., 1996; (Tsai *et al.,* 2005).

The decreasing of the total viable bacterial counts with time could be explained on the basis that in a short processing period as that in the case of Fessiekh, it is hard to believe that the substrate for microbial growth comes from the degradation of the protein. It is more likely the microbial growth occurs as a result of attacking proteinaceous and other soluble nitrogenous compounds contained in the fish juice. This is substantiated by the observation that the growth occurs during the zero and ten days after the salted fish became a product, and then the counts drop steadily, this occurs with salt penetration inside the muscle and that sodium chloride has been used as a preservative for a long time. Also, the early increase occurred while fish were wet and the provision of salt promoted the growth of halotolerant and halophilic bacteria in fish. As the fish became drier, there was a decrease in water activity and this together with the accumulated salt in the flesh resulted in suppression of bacterial growth. Birch *et al*., (1986) reported that when moisture content or water activity was lowered, the amount of water available for supporting microbial growth was reduced. The primary objectives of high levels of salt used in fish fermentation are to select the halophilic organisms, which will affect the degradative process on the organic compounds in the fish muscle to bring about the desired flavors in the product (FAO, 1992). Similarity, (Dirar 1993) mentioned that the salt and the mats used in the fessiekh fermentation process might contribute important halotolerant strains. (Eltom 1989) reported that after the addition of the salt in Fessiekh fermentation, the viable bacterial count rose to 1.8×10^8cell/g on the fourth day followed by decreasing to 8.6×10^5 cell/g on day twelve. This pattern of rising and fall in the microbial count was observed during fish fermentation by different researchers (Hamed *et al*., 1973;(Ahmed *et al.,* 2010, El Hag *et al.,* 2012).The bacterial genera isolated from salted fish (*Hydrocynus froskalii*) were *Micrococcus* spp (38.17) and *Aerococcus* spp (12.9%) while *Staphylococcus* spp was found in all samples of salted fish with a record about (46.77%) and *Staphylococcus aureus* was the most dominant species isolated as shown in (Table 3).

Table (3): Bacterial groups isolated from salted samples

Days	Microorganisms
0	*Staphylococcus aureus+ Staphylococcus pyogenes+ Staphylococcus rostri*
5	*Staphylococcus aureus+Staphylococcus lentus+Micrococcus roseus+ Micrococcusleuteus*
10	*Staphylococcus aureus+ Staphylococcus pyogenes + Micrococcus roseus+ Micrococcus leutus*
15	*Staphylococcus aureus+Micrococcus leutus +Aerococcusviridans Staphylococcus epidermidis*
20	*Staphylococcus aureus + Aerococcusviridasn*
25	*Staphylococcus aureus+ Micrococcus roseus+Micrococcus leutus*
30	*Staphylococcus aureus+Micrococcus leuteus+ Micrococcus lactis*

ISSN: online 2617-2186 print 2617-2178

The obtained result was in disagreement with (El Hag *et al.,* 2012) who found that *Staphylococcus xylosus* species were the dominant bacteria

isolated from salted Kawara fish (*Alestes* spp) during storage. (Goja 1993) reported that *Staphylococcus saccharolyticus* was predominant species of *Staphylococci* isolated from the salted fassiekh produced in Ed Dueim city. *Staphylococcus* spp can reach high levels ($>10^5$cfu/g) in products prepared with hands under bad conditions and can cause food poisoning (Varnam & Evans, 1991). Also, (Vishwanath *et al.,* 1998) reported that *Staphylococcus aureus* grew well in salted food and in low water activity. Although (Hernandez-Herrero *et al.,* 1999) reported that, *Staphylococcus aureus* was not identified as an indigenous flora of fish culture and in fish hunting from clean water. (Eltom 1989) found that the most commonly encountered bacterial genera in Fessiekh fermentation were *Bacillus*, *Staphylococcus* and *Micrococcus*. *Bacillus* and *Micrococcus* have also been reported to be present in fermented fish in many countries of South East Asia (Saisithi *et al.,* 1966, Goja 1993, Ahmed *et al.,* 2010) Mackie *et al*., 1971; Hassan *et al*., 1972; Goja, 1993; Ahmed *et al.* , 2010; Goja, 2013)

The viable bacteria counts of commercial Fessiekh presented in (Table 4), were significantly higher ($p<0.05$) when compared to the laboratory prepared samples at different salt concentrations (Table 5).

Table (4): Total viable bacterial counts (cfu/g) and commonly isolated bacteria from commercial fessiekh

Days	Viable bacterial counts (cfu/g)	Dominant microorganisms
0	$3x10^4$	*Micrococcus luteus*
5	$21x10^3$	*Micrococcus luteus*
10	$21x10^3$	*Micrococcus luteus*
15	$20x10^3$	*Staphylococcus aureus*
20	$15x10^3$	*Micrococcus luteus*
25	$12x10^3$	*Staphylococcus aureus*
30	$12x10^3$	*Micrococcus luteus*

Table (5): Comparison between viable bacterial counts (cfu/g) of commercial and experimental fessiekh at different salt concentrations during the study.

Salted fish (fessiekh)	Mean of viable count	T- Test	Sign
Hydrocynusspp 20% salt	2714.29	-3.111	
Commercial fessiekh	13285.71		**
Hydrocynusspp 25% salt	1428.57	-3.550	
Commercial fessiekh	13285.71		**
Hydrocynusspp 30% salt	785.71	-3.741	
Commercial fessiekh	13285.71		**

** High significant differences

According to (Sugumar *et al.,* 2004)unhygienic handling is one of the main factors contributing to poor quality of fish in the retails; this could be due to the quality of fish used for Fessiekh, and the amount of salts added and the techniques used in commercial production. No yeast or mould was detected in our fresh and salted samples.

CONCLUSION

From the results, it may be concluded that the total viable bacterial counts of *Hydrocynus froskalii* after salting were decreased due to the course of salting. It was seen that the main factors affecting fessiekh quality are related to the amount of salt used for the process. The final

ISSN: online 2617-2186 print 2617-2178

quality can be largely attributed to the effect of various conditions upon the fermenting agents and activities. Commercial fessiekh samples contained higher microbial load than experimentally prepared products. Therefore, strict control measures are recommended to be applied for producers, and a good guidance should be provided for household producers.

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ISSN: online 2617-2186 print 2617-2178

تأثير المواسم وتركيزات الملح على الحمولة الميكروبية للفسيخ

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تاريخ الاستلام: 26 فبراير 2017 / تاريخ القبول : 27 جولاي 2017.

Doi:https://doi.org/10.54172/mjsc.v32i1.93

المستخلص : تهدف الدراسة إلى التعرف على الميكروبات الموجودة في السمك المملح والمخمر والذي يعرف محليا باسم الفسيخ، فقد تم تحديد العدد الكلي للبكتريا الموجودة في الأسماك المملحة بتراكيز مختلفة (20٪، 25٪، 30٪ من وزن الأسماك) وذلك خلال المواسم المختلفة (الصيف، الخريف والشتاء)، وتم إعداد الفسيخ لنوع من الأسماك والمعروف محليا في السودان باسم الكاس (*Hydrocynus froskalii*) تم عزل وتعريف البكتيريا وكذلك فحص خميرة العفن في الفسيخ لمعرفة نوعية المحتوى الميكروبي في الفسيخ . أظهرت النتائج أن إجمالي عدد البكتيريا الحيوي في الأسماك الطازجة (*Hydrocynus froskalii*) المستخدمة كمادة خام في إعداد الفسيخ. تراوحت من $2x10^3$–$5.5x10^3$ ثم تغير المحتوى الميكروبي لسمكة *Hydrocynus froskalii* بعد التمليح بتراكيزه المختلفة وذلك خلال فترة التمليح والمواسم. وكانت هناك زيادة ملحوظة في إجمالي العدد الحيوي البكتيري خلال الأيام الخمسة الأولى في الصيف والخريف والعشرة أيام الأولى في الشتاء. بدأ العد البكتيري في الانخفاض كلما تقدمت عملية التمليح. و عُزلت خمسة أنواع من جنس *Staphylococcus* spp (*Staph. aureus*, *Staph. rostri*, *Staph.lentus*, *Staph.epidermidis* & *Staph.pyogen*) والتي عزلت من جميع عينات الأسماك المملحة حيث بلغت حوالي (46.77٪)، وعُزلت أيضاً ثلاثة أنواع من جنس *Micrococcus* spp (*Micro.lactis* و *Micro.roseus*, *Micro .leuteus*) وصلت إلى (38.17٪) في حين وصلت بكتيريا *Aerococcus* spp إلى (12.9٪). العدد الحيوي البكتيري للفسيخ التجاري كان عالياً ($p<0.05$) بالمقارنة مع فسيخ التجربة. لم يُعزل أي نوع من الفطريات سواء من فسيخ التجربة أو التجاري.

الكلمات المفتاحية: تركيز الملح، الموسم ، التحليل الميكروبيولوجي، الفسيخ.

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