Faculty of Veterinary Medicine

Veterinary Medical Journal-Giza (ISSN 1110-1423)
Faculty of Veterinary Medicine,Cairo University
Accredited from national authority for Quality Assurance and Accreditation
Giza, 12211, Egypt

Cairo University

Sensory, chemical and microbiological evaluation of locally produced smoked herring

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Abstract

In this study a grand total of 180 samples of locally produced smoked herring and herring eggs (90 of each) were collected from different markets in Giza Governorate, Egypt, the samples were subjected to organoleptic examination (skin condition, skin colour, taste of fish, odor of flesh and condition of the belly), chemical examination (pH, TBA and TVBN) and microbiological examination (Total bacterial count, MPN, *Staph. aureus* count, Total mold & Yeast count, Proteolytic count, Lipolytic count, isolation of *Salmonella*, *E. coli* and *L. monocytogenes*). The study proved that, the percentage organoleptically of accepted samples was 95.5%. The mean values of pH, TBA and TVBN in muscles of smoked herring samples were 5.95%, 8.042 and 22.195 respectively while in herring eggs they were 5.49%, 7.952 and 22.698 respectively. The mean count log_{10} cfu/g of TBC, MPN, *Staph. aureus* and total mold & yeast count in muscles of smoked herring samples were 4.33, 0.698, 0.126 and 2.905 respectively and in herring eggs they were 4.29, 0.640, 0.472 and 2.70; respectively.

Key words: Smoked herring, microbes, *Salmonella*, *E. coli*, *L. monocytogenes*, pH, TBA and TVBN.
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Introduction

Fish had long been regarded as a nutritious and highly desirable food due to their contribution of high quality proteins, richness in calcium, phosphorus and generous supply of vitamins **(Abdelhamid, 2003).** Herring is a kind of smoked fish which may be held in brine and freshened prior to smoking. Herring may be subjected to many risks of contamination either primary contamination from skin, inadequately cleaned fish cases and the hands of those preparing and handling the fish or post processing contamination or incorrect storage after processing. Such contaminants may render smoked fish either unfit for human consumption or even harmful to consumers **(Turnbull and Gilbert, 1982, Vanden Broek *et al.*, 1984 and Saddik *et al.*, 1985).**

Smoking permits lengthily preservation by removing moisture, which is essential for bacteriological and enzymatic spoilage. Smoked fish can be a source of microbial hazards including *L. monocytogenes*, *Salmonellae spp.* and *Clostridium botulinum* **(Heintiz, M.L and Johnson, 1998).** In Egypt, locally produced smoked herrings are mainly prepared from imported frozen herrings which was mostly stored for long time. They may reveal a rancid flavor, they may be previously contaminated with toxigenic molds which produce toxins not affected with heat treatment during smoking process (**Kasem *et al*, 1985**), the smoking process retards the microbial activity of fish by showing a slight

decrease in the bacterial counts. The action of smoke and dehydration however is not sufficient to reduce the bacterial counts significantly (**Deng *et al*, 1974**). A very large reduction in the aerobic plate count reaching more than 99% after smoking due to antibacterial effect of smoke (**Hammad,1985**).

So, the objective of the present work was to study the organoleptic, chemical & microbiological quality of locally produced smoked herrings collected from markets in Giza Governorate.

Materials and Methods

Preparation of samples:

A grand total of 180 smoked herring samples and herring eggs (90 of each) were randomly collected from different markets in Giza Governorate. The samples were directly transferred to the laboratory under complete aseptic condition without undue delay and subjected to organoleptic, chemical and microbiological examinations.

Organoleptic examination of smoked herrings: (Connell, 1990).

- Skin condition

The skin was examined by naked eye for any defects and was judged as intact, macerated, or very macerated.

- Skin color

The color was examined by naked eye and by the aid of hand lens. The color of the skin was judged as characteristics golden brown color, deposited by visible mold give dark brown in color.

- Taste of the flesh

The fish flesh was tasted twice, the first (at normal room temperature) and after being heated on flame as usually consumed. The taste possibilities were the specific smoked fish salty or bitter taste.

- Odor of the flesh

The smell of the flesh was tested twice, the first at normal room temperature and the second after being heated on flame. Two important odor possibilities were noted, the first one was characteristic smoke fishy odor and the second was unaccepted musty odor.

- Condition of the belly

It was inspected and being noted as intact, swollen or burst.

Organoleptic examination of herring eggs for colour and odor: (ESS, 2005):

Colour of herring eggs:

The colour of herring eggs was examined by naked eye by aid of hand lens, the colour was judged as.

The accepted colour of herring eggs of smoked fish is brown.

The unaccepted colour of herring eggs of smoked fish is dark brown due to deposits by visible mold growth.

Odor of herring eggs: by smelling

First normal odor was accepted smoke fishy odor.

The second was unaccepted musty odor.

Chemical quality indices:

- Measurement of pH value. **(Goulas and Kontominas,2005)**

Ten grams of sample were homogenized in 100 ml of distilled water and the mixture was filtered. The pH of filtrate was measured using a pH meter.

- Measurement of TBA-Reactive Substances. **(Du and Ahn, 2002)**

Five grams of sample were weighed into a 50 ml test tube and homogenized with 15 ml of deionized distilled water using a homogenizer (Lab-Blender 400) for 10 seconds at the highest speed. One milliliter of the meat homogenate was transferred to a disposable test tube (3 x 100 mm), and butylated hydroxyanisole (50 ul, 7.2%) and TBA-trichloroacetic acid (TCA) (15 mM TBA-15% TCA; 2 ml) were added.

The mixture was vortexed, incubated in a boiling water bath for 15 min to develop color, cooled in cold water for 10 minutes, vortexed again, and centrifuged

for 15 min at 2500 x g. The absorbance of the resulting supernatant was determined at 531 nm against a blank containing 1 ml of deionized distilled water and 2 ml of TBA-TCA solution. The amount of TBA-reactive substances (TBARS) were expressed as milligrams of malonaldehyde per kilogram of meat.

• Determination of total volatile basic nitrogen. **(Ronald and Ronald, 1991):**

Ten grams of samples were macerated with 100 ml tap water and washed into a distilling flask with 200 ml tap water. Then 2 grams magnesium oxide and an antifoaming were added. To a 500-700 ml receiving flask, 25 ml of 2% bone acid solution and few drops of methyl-red indicator (0.016 gram methyl red and 0.083 gram bromocresol green in 100 ethanol) were added. A macro-Kjeldhal distillation apparatus was connected to the distillation flask with the receiver tube was dipped below the liquid. The mixture was boiled in exactly 10 minutes and the distillation was continued for 25 minutes using the same heating rate. The condenser was then washed with distilled water and the distillate was titrated with 0.05 M (0. 1N) sulphoric acid. The Total volatile base nitrogen (rug/100 gram sample) was calculated as the titration multiply by 14.

Microbiological examination:

Preparation of samples "homo-genate" **(ICMSF, 1978).**

Twenty five grams of smoked fish were taken aseptically and mixed into a sterile blender jar with 225 ml of sterile buffered peptone water (Oxoid). Samples were blended for 2.5 min. Sample homogenate was mixed by shaking, one ml was aseptically taken and transferred into a tube containing 9 ml sterile buffered peptone water and tenth-fold serial dilution was prepared. The microbiological examinations including: enumeration of aerobic plate count **(APHA, 1992),** enumeration of Coliforms (MPN/ g) **(AOAC, 2000),** determination of *Staph. aureus* count **(AOAC, 2000),** total mold and yeast count **(APHA, 1985),** enumeration of lipolytic bacteria **(Smith and Haas, 1992),** enumeration of proteolytic bacteria **(Lee and Kraft, 1992).**

Isolation of *E. coli.* (AOAC, 1990):

A loopful from each positive *Escherichia coli* broth tube (with turbidity and gas formation) was streaked onto plates of Eosin methylene blue (EMB) (Oxoid CM69) and incubated at 37°C for 24 hours. Typical colonies (greenish metallic with dark purple center) were picked up and transferred to nutrient agar slants and incubated at 37°C for 24 hours, for further identification.

Isolation of *Salmonellae.* (AOAC, 2000):

• Pre-enrichment:

Twenty five gms from sample were added to 225ml peptone water and homogenized by shaking for 2.5 minutes; the homogenate was incubated at 37°C for 24 hrs.

• Enrichment:

• One ml of pre-enrichment culture was transferred to 10 ml Rappapot Vassiliadis broth and incubated at 43°C for 24 hrs. Loopfull from enrichment sample in MacConkey broth previously incubated for 24 hours at 37°C was streaked onto the service of MacConkey agar plates. Inoculated plates were incubated at 37°C for 18 – 24 hours. Typical pink colored and colorless colonies were picked up and purified then cultured on slope agar for further identification.

• Selective platting:

Salmonella Shigella (SS)agar and Xylose lysine desoxycholate (XLD) agar plates were inoculated by enriched cultures then incubated at 37°C for 24 hrs. Suspected colonies were creamy with or without black centers on S.S and red in color with without black centers on XLD.

Isolation of *L. monocytogenes.* (AOAC, 2000):

• Enrichment:

Twenty-five grams of the sample were added to 225 ml *Listeria* Primary Enrichment medium (UVMI) in stomacher bag (stomacher blender 400 – BA 7021 Seward medical, England) and blended for 2 min. Inoculated UVM1 was incubated at 30°C. After 24 hours, 0.1 ml of the inoculated UVM1 was transferred to l4m1 *of Listeria* Secondary Enrichment medium (UVM2) and incubated at 30°C for 24 hours.

• Plating:

After 24 hours, a loopful was streaked onto PALCAM agar plates. The plates were incubated at 37°C for 48 hours, suspected colonies (2mm in diameter, grey-green in color with a black sunken centre and a black halo) were picked up and plated onto trypticase soy agar with 0.6 % yeast extract and incubated at 37°C for 24 hours then put in refrigerator for further identification.

Statistical analysis (S.A.S, 2001):

Numerical data collected were statistically analysed for analysis of variance and least significant difference. Chi-square, t. test and correlation were calculated when required.

Result and Discussion

Table (1): Organoleptic criteria of smoked herring:

Item	Character	No	%
Skin condition	Intact	88	97.7
	Macerated	2	2.3
	Very macerated	-	-
Skin color	Golden yellow	88	97.7
	Moldy growth	2	2.3
Taste of fish	Smoke fishy taste	89	98.8
	Bitter taste	1	1.2

Organoleptic examination of smoked herring showed that 95.5% of samples were accepted. The obtained results were relatively as reported by **El-Sayed, (1995)** who mentioned that the organoleptic examination of smoked fish revealed that the percentage of accepted and rejected samples were 96 and 4%, while lower values were reported by **Abed El-Daem F. Wafaa, (1999)** who found that, the percentage of accepted and rejected smoked fish samples were 80 and 20%.

Table (2): Chemical evaluation of smoked herring:

Value / Statistics	pH		TBA		TVBN	
Mean value	Muscle	Herring eggs	Muscle	Herring eggs	Muscle	Herring eggs
	5.95	5.49	8.042	7.952	22.195	22.698

Results in table (2) showed that, the mean values for pH, TBA & TVBN for smoked herring flesh were 5.95, 8.042 & 22.195 respectively while for herring eggs they were 5.49, 7.952 & 22.698 respectively. From these results it was shown that the mean value of pH and TVBN within the acceptable limit. While TBA value was exceeded the acceptable limit establishing to **E.SS, (2005)** No. (288). **Nilgun Kaba, (2013)** reported that, the TBA value and TVBN value were stayed after smoking and canning within the acceptability limit values, while **(Joseph *et al.* 1987)** found that, the total volatile basic nitrogen was increased after cold smoking (at 45 °c) for 5 hours.

Table (3): Microbiological counts (log_{10} cfu/g) smoked herring.

Count / Statistics	TBC		Coliforms (MPN)		Staph. aureus		Total mold and yeast	
Mean count log	Muscle	Herring eggs	Muscle	Herring eggs	Muscle	Herring eggs	Muscle	Herring eggs
	4.33	4.29	0.698	0.640	0.126	0.472	2.945	2.705

The mean count of TBC of muscles of smoked herring was 4.33 log count and for herring eggs was 4.29 log count, lower value of TBC was reported by **Hammad, (1985)**. In smoked fish, the maximum permissible limit recommended by **E.SS, (2005)** was 1×10^5 cfu/g, i.e. mean count in this study was not exceed the permissible limit. The coliforms count (MPN) of muscles of examined smoked herring samples was 0.698 and for herring eggs was 0.640. The coliform group is one of the indicator microorganisms in microbial criteria for food safety which indicates the possible faecal contamination and therefore, the potential presence of enteric pathogens **(Jay, 2000)**. The mean count of *Staph. aureus* log_{10} cfu/g for muscles of smoked herring samples was 0.126 and for herring eggs was 0.472, the lower count of *Staph. aureus* in this study may attributed to the effect of the phenolic acids which formed from smoking process are lethal for *Staph. aureus* and many other pathogens. The mean count of total mold & yeast for muscles of smoked herring samples was 2.959 and for herring eggs was 2.705, these results were relatively higher than those reported by **El-Shater,**

(1994), **El-Sayed, (1995)** who reported that, the smoked fish with mold & yeast count was 1.53×10^4 with a minimum value of 1×10^2 and maximum value of $1.8p \times 10^8$. Mold and yeast count is used to indicate quality of different food products as smoked fish. In this study *Salmonellae, E. coli* and *L. monocytogenes* failed to be isolated from any sample, this agree with results reported by **Gbogbalomo, (2012)**.

Conclusion and Recommendations:

The smoked fish in Egypt is used mainly from herring fish which is imported from North Europe. Local fishes are not used for smoking processes. So, the problem of importation, storage processing, post processing may arise clearly in the finished products. The frozen imported fishes may reach to the factory partially thawed which gives rise to microbiological multiplication, fat oxidation. The drying step is the main and first source of molds, after smoking the sources of post-processing microbio-logical contamination may arise from bad storage or exposure in the open air in front of shops.

The fore mentioned studies were carried out on the examination of muscles and caviar. The study of sensory examination proved that 4.5% of the whole smoked herring samples were rejected. It was proved that, the microbiological mean count of muscle was higher than herring eggs.

The study failed to isolate *Salmonellae*, *E. coli* and *L. monocytogenes* in samples, and this study revealed that the mean value of pH & TVBN for muscles & herring eggs of smoked herrings were within the acceptable limit as in **E.SS, (2005)** no. 288 while TBA value for muscles and herring eggs of smoked herring sample was exceeded the acceptable limit establishing to **E.SS, (2005).**

From this study it was shown that, the smoked fish processing in Egypt based on the importation of raw material as herring fish which considered the fish of choice for smoking purpose followed by marcel and salmon.

It is recommended:

1. The validity of raw material must be controlled by the authority to avoid the smoking of spoiled fish which may musk the spoilage criteria of some fish.
2. GMPs (Good manufacture practice) must be applied by the application of food safety programs as HACCP system which prevents the presence of spoiled non detected smoked fish to safe the consumer public health.
3. Trials to safe subistuded local raw material for smoking purpose instead of the imported fish and the concerned problems of importation process including transportation, storage, time consuming and the public health hazards of arising fat oxidation process and protleolytic process of the imported fish as well as the microbiological risk hazards which may be included the process of importation.

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الملخص العربي

استهدفت هذه الدراسة التقييم الحسي والكيميائي والميكروبي وكذلك مدى تواجد بعض مسببات التسمم الغذائي في أسماك الرنجة المدخنة المحلية والتي قد تسبب خطراً على صحة المستهلك، وقد أجريت هذه الدراسة لفحص 90 عينة من لحوم الأسماك المدخنة وتسعون عينة من بطارخها.

وتبين من هذا الفحص أن نسبة العينات المقبولة حسياً كانت 95.5%، والمرفوضة كانت 4.5% وذلك حسب المواصفات القياسية المصرية عدد رقم 288 لسنة 2005.

أثبت الفحص الكيميائي أن متوسط الأس الهيدروجيني في عضلات الرنجة المدخنة كان 5.95% وفي البطارخ كان 5.49%، وبالنسبة لمتوسط حامض الثايوباربتيوريك في العضلات لنفس العينات كان 8.042 وفي البطارخ 7.952 مجم مالونالدهيد/كجم من العينة، وايضاً وجد متوسط قيم القواعد النيتروجينية الطيارة في العضلات 22.195 وفي البطارخ 22.698 مجم مالونالدهيد/100 مجم من العينة.

أظهر الفحص الميكروبي أن متوسط العدد الكلي للميكروبات الهوائية في العضلات كان 4.33 لوج وفي البطارخ 4.29 لوج وبالنسبة لمتوسط العدد الكلي للميكروبات القولونية (الكوليفورم) في العضلات كان 0.698 لوج وفي البطارخ 0.640 لوج، وكان متوسط العدد الكلي للفطريات والخمائر في العضلات لنفس العينات كان 2.905 لوج وفي عزلها في العضلات 0.126 لوج وفي البطارخ 0.472 لوج. بالإضافة إلى متوسط العدد الكلي للميكروبات المكورات العنقودية الذهبية التي تم البطارخ 2.705 لوج، واتضح أيضاً من الفحص الميكروبي للعينات أنه لم يتم عزل ميكروب *Salmonella* ، ميكروب *E. coli* ، وايضاً ميكروب *L. monocytogenes* وقد تم مناقشة النتائج ومقارنتها بالحدود القصوى للمواصفات القياسية المصرية، وتم بيان الأثر المرضي للميكروبات المعزولة بهذه الدراسة.