

## Keeping Quality Improvement of Some Smoked Fish Products With Special Reference to Herring Fillets With Caviar

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### Abstract

The microbiological quality of three commercial cold smoked herring products including, vacuum-packed herring fillets (product I), vacuum-packed herring roe (product II) and herring fillets with caviar (product III) was assessed. Physically, most of the collected samples were of normal appearance, while some jars of product III showed clear bulging of lids and gas formation indicating spoilage. Bacteriological examination of the three products revealed high levels of aerobic, anaerobic plate count  $>10^6$  -  $>10^7$  CFU/g; and relatively lower values of thermophilic count ( $>10^4$  to  $>10^5$  CFU/g). Examination of raw materials formulated with herring products revealed that the bacterial flora of cold smoked herring fillets and roe were ranged from  $>10^4$  to  $>10^5$  CFU/g. Moreover, the bacterial quality of different types of spices were variable, low values of aerobic plate count (APC), anaerobic plate count (AnPC) and thermophilic plate count (ThC) ( $>10$  -  $>10^3$ ) were recorded for pepper, cumin and cardamom; while chillies, allspice and coriander samples were the most highly contaminated ( $>10^4$  -  $>10^6$  CFU/g). The microflora associated with cold smoked herring fillets and roe consisted of staphylococcus and micrococcus spp., while those of spices were dominated by *Bacillus* spp. On the other hand, dipping treatments of herring roe and fillets in lactic acid or/and potassium sorbate significantly reduced the mean populations of aerobic plate count, anaerobic plate count and thermophilic plate count. Furthermore, heat treatment of spices reduced initial counts by approximately 1.0–2.0 logarithms. The study concluded that the use of 1.5% Lactic acid and 0.20 % Potassium sorbate dipping treatments, with heat treated spices followed by packaging and storage at 4 °C was the most suitable for improvement of the keeping quality and extending shelf-life (1-2 months) of cold-smoked herring products.

### Introduction

Fish production is one of the major economic activities in Egypt. Smoked products are traditionally consumed, even nowadays when fresh fish is more popular in supermarkets and seafood shops. One of the most common smoked products is herring. Cold smoked herring and other ready-to-eat fish products could be naturally contaminated with different microorganisms, therefore, they are good carriers of pathogenic bacteria (9;

41) which could represent a potential hazard for susceptible individuals or YOPI" (young, old, pregnant and immuno-compromised people). The cold-smoking process does not destroy the natural microbial population or thermophilic pathogens of the raw fish, and not decrease the water activity enough to inhibit post-process microbial growth (23; 25). Therefore, ready to eat fish products must be stored at chilled temperatures (25; 19).

The process used for cold smoking of fish are not exceptionally rigorous; thus, there is of great concern that some foodborne pathogens, if present, could survive. In addition to contributing to pathogen survival, the extensive handling of products following the cold smoking process provides ample opportunities for other foodborne pathogens such as, *Salmonellae*, *Shigellae*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio parahaemolyticus* and *Vibrio cholerae* to contaminate and survive in the products if insufficient attention is given to Good Manufacturing Practices (GMPs), Sanitation Standard Operating Procedures (SSOPs) and hygienic practices of plant employees (61).

Smoked herring undergoes many processing stages that can increase the risk of microbial contamination (59). Short shelf-life is exacerbated by fluctuating storage temperature during thawing, transport and sale. Together with the low salt concentration of the fish product and the fact that it is eaten without further heating, these aspects highlight the need for research into microbiological development in cold smoked herring products. Very limited work has been carried out on investigating microbiological aspects during storage of these products.

## Materials and methods

### Product description and sampling

Ten samples of each of the following three commercial products were collected after 2-3 months from production.

**Product I** Vacuum-packed cold smoked herring fillets, storage at 4°C for 3 months.

**Product II** Vacuum-packed cold smoked herring roe, storage at 4°C for 3 months.

**Product III** Plastic jars containing cold smoked herring fillets with caviar, storage at ambient temperature for 5 months. All the jars contained cold

smoked herring fillets, caviar (herring roe pasted with spices in the form of pellets), sodium chloride, oil, vinegar, spices and citric acid as preservatives. According to the manufacturer, the salt concentration of each sample was not less than 2.5%.

The products were produced by a single company, manufactured from different lots of cold smoked herring and they were expected to maintain good quality during their shelf-life at the recommended storage temperature.

### **Microbiological examination of the collected samples**

**Sample processing** A 10 g portion of each product sample (N=10) described above was aseptically weighed into 90 ml of 0.9% NaCl and 0.1% peptone water in a sterile plastic bag, and then blended in a Stomacher 400 Lab Blender (Seward Medical, London, UK) for 30 seconds. Ten-fold serial dilutions were used for microbiological analyses.

**Bacterial counts:** The spread plate technique was used to prepare duplicate plates for the determination of aerobic plate counts (APC), anaerobic plate counts (AnPC) and thermophilic count (ThC) according the procedures of the Compendium of Methods for the Microbiological Examination of Foods (2). After incubation, duplicate agar plates of colonies were counted.

### **Examination of raw materials and application of treatments**

**Evaluation of raw materials before treatment** five samples were collected from each raw materials used in manufacturing of the three products including herring fillets, herring roe, and spices, then evaluated bacteriologically for aerobic, anaerobic plate and thermophilic count. Moreover, isolation and identification of microorganisms were carried out according to **Bagge-Ravn et al. (3)**.

### **Antimicrobial treatment of herring roe and fillets**

The following antimicrobial treatments were applied:

1. No treatment (control)
2. 1% Lactic acid (LA1; El-Nasr Pharmaceutical Chemicals Co, Egypt)
3. 1.5% Lactic acid (LA2; El-Nasr Pharmaceutical Chemicals Co, Egypt)
4. 2% Lactic acid (LA3; El-Nasr Pharmaceutical Chemicals Co, Egypt)
5. 0.10 % Potassium sorbate (KS1; Bell Food Co, Egypt)
6. 0.15 % Potassium sorbate (KS2; Bell Food Co, Egypt)

7. 0.20 % Potassium sorbate (KS3; Bell Food Co, Egypt)
8. 1% Lactic acid followed by 0.10 % Potassium sorbate (LA1KS1)
9. 1% Lactic acid followed by 0.20 % Potassium sorbate (LA1KS3)
10. 1.5% Lactic acid followed by 0.20 % Potassium sorbate (LA2KS3)

All treatments were applied by dipping of the raw material (herring fillets and roe) for 2 minutes, followed by draining for approximately 1 minute. After the various treatments of herring fillets and roe, experimental product samples (similar to the original products) were produced, and then vacuum packaging and storage at 4°C for 3 months. Bacterial analysis (N=5) of each sample was carried out at the end of storage period for determination of aerobic, anaerobic plate and thermophilic count.

#### ***Decontamination of spices***

Heat treatment of different types of spices (herbs, seeds, dry powder) was carried out by autoclaving (121°C at 15 psi) for 15 minutes. Changes of aerobic, anaerobic plate and thermophilic count before and after processing of spices were recorded.

#### **Assessment of the final products after application of treatments**

The manufacturer formulated each product with heat-treated spices and the most effective dipping treatments (LA2KS3), followed by packaging and storage at 4°C for 6 months. Five samples of each product were obtained on 1, 2, 3, 4, 5 and 6 months for physical and bacteriological analysis of aerobic, anaerobic plate and thermophilic count.

## Results

**Table (1): Mean values of aerobic, anaerobic plate and thermophilic (CFU/g) count of three commercial cold smoked herring products after 2-3 months from production (N=10).**

Plant	Aerobic Plate Count			Anaerobic Plate Count			Thermophilic Count		
	Min	Max	mean <sup>±</sup> SE	Min	Max	mean <sup>±</sup> SE	Min	Max	mean <sup>±</sup> SE
Product I	2.8x10 <sup>4</sup>	4.7 x10 <sup>6</sup>	2.4 x10 <sup>6</sup> ± 8.4 x10 <sup>5</sup>	1.7x10 <sup>3</sup>	7.5 x10 <sup>6</sup>	3.3 x10 <sup>6</sup> ± 6.5 x10 <sup>7</sup>	2.8 x10 <sup>4</sup>	6.7 x10 <sup>5</sup>	2.7 x10 <sup>5</sup> ± 1.2 x10 <sup>5</sup>
Product II	5.3 x10 <sup>5</sup>	5.7 x10 <sup>7</sup>	1.5 x10 <sup>6</sup> ± 2 x10 <sup>5</sup>	4.1 x10 <sup>5</sup>	6.7 x10 <sup>7</sup>	1.8 x10 <sup>7</sup> ± 1.2 x10 <sup>6</sup>	3.2 x10 <sup>4</sup>	5.7 x10 <sup>6</sup>	1.5 x10 <sup>5</sup> ± 6.6 x10 <sup>4</sup>
Product III	7.5 x10 <sup>4</sup>	5.1 x10 <sup>8</sup>	2.3 x10 <sup>7</sup> ± 8 x10 <sup>6</sup>	1.6 x10 <sup>6</sup>	2.7 x10 <sup>7</sup>	7 x10 <sup>6</sup> ± 2.3 x10 <sup>5</sup>	4.6x10 <sup>3</sup>	5.7 x10 <sup>6</sup>	7.5 x10 <sup>5</sup> ± 6.6 x10 <sup>4</sup>

**Table (2): Mean values of aerobic, anaerobic plate and thermophilic (CFU/g) count on raw materials used in processing of cold smoked herring products (N=5).**

Raw material	Aerobic Plate Count			Anaerobic Plate Count			Thermophilic Count		
	Min	Max	Mean <sup>±</sup> SE	Min	Max	Mean <sup>±</sup> SE	Min	Max	Mean <sup>±</sup> SE
Herring fillets	8.8x10 <sup>3</sup>	4.7 x10 <sup>5</sup>	1.4 x10 <sup>4</sup> ± 8.4 x10 <sup>3</sup>	1.7x10 <sup>3</sup>	2.5 x10 <sup>3</sup>	3.3 x10 <sup>4</sup> ± 6.5 x10 <sup>3</sup>	3.7 x10 <sup>3</sup>	4.2 x10 <sup>3</sup>	2.8 x10 <sup>3</sup> ± 2.3 x10 <sup>3</sup>
Herring roe	3.3 x10 <sup>4</sup>	5.7 x10 <sup>5</sup>	1.5 x10 <sup>5</sup> ± 2 x10 <sup>4</sup>	4.1 x10 <sup>4</sup>	6.7 x10 <sup>4</sup>	5.8 x10 <sup>4</sup> ± 1.2 x10 <sup>4</sup>	2.1x10 <sup>4</sup>	5.7 x10 <sup>4</sup>	3.5 x10 <sup>4</sup> ± 6.6 x10 <sup>4</sup>

**Table(3): Isolation and identification of microorganisms on raw materials used in processing of cold smoked herring products (N=5).**

Raw material	Isolated microorganisms
Herring fillets	<i>Staphylococcus</i> spp., <i>Micrococcus</i> spp.
Herring roe	<i>Staphylococcus</i> spp., <i>Micrococcus</i> spp.
Pepper	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Escherichia coli</i> , <i>Klebsiella</i> spp.
Cumin	<i>Bacillus</i> spp., <i>Staphylococcus aureus</i> , <i>Aspergillus</i> spp.
Chillies	<i>Bacillus</i> spp., <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Shigella</i> , <i>enterococci</i>
Allspice	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp., <i>Klebsiella</i> spp., <i>Clostridium</i> spp., <i>Aspergillus</i> spp., <i>Penicillium</i> spp.
Coriander	<i>Bacillus</i> spp., <i>Escherichia coli</i> , <i>Klebsiella</i> spp., <i>Aspergillus</i> spp.
Cardamom	<i>Bacillus</i> spp., <i>Staphylococcus</i> spp.

**Table (4): Mean counts (log CFU/g) of aerobic, anerobic plate and thermophilic count on experimental product samples, formulated with and without antimicrobials, 3 months after using antimicrobial substances, followed by packaging and storage at 4 °C (N=5).**

Ex. P		Blank	LA1	LA2	LA3	KS1	KS2	KS3	LA1KS1	LA1KS3	LA2KS3
Product sample I	APC	6.1 ± 0.2 A*	5.7 ± 0.3 AB	4.7 ± 0.3 CD	4.1 ± 0.1 DE	5.2 ± 0.2 BC	5.0 ± 0.2 C	4.2 ± 0.1 DE	4.0 ± 0.1 CD	3.7 ± 0.2 E	3.1 ± 0.2 F
	AnPC	6.2 ± 0.3 A	4.9 ± 0.5 BC	4.6 ± 0.1 BCD	3.9 ± 0.2 DE	5.3 ± 0.3 B	4.2 ± 0.3 CDE	4.1 ± 0.3 DE	4.1 ± 0.1 DE	3.6 ± 0.1 EF	2.9 ± 0.2 F
	ThC	5.1 ± 0.2 A	4.4 ± 0.3 BC	4.6 ± 0.1 AB	3.0 ± 0.1 C	4.9 ± 0.3 AB	4.4 ± 0.3 BC	3.0 ± 0.1 C	4.6 ± 0.1 AB	3.2 ± 0.1 D	2.4 ± 0.1 E
Product sample II	APC	6.7 ± 0.3 A	5.2 ± 0.4 BC	4.7 ± 0.2 C	4.6 ± 0.1 C	5.6 ± 0.1 B	5.2 ± 0.2 BC	4.7 ± 0.2 C	4.6 ± 0.2 C	4.6 ± 0.1 C	3.2 ± 0.4 D
	AnPC	6.0 ± 0.3 A	5.0 ± 0.3 B	4.6 ± 0.2 B	4.5 ± 0.1 D	5.6 ± 0.1 BC	4.9 ± 0.3 CD	4.6 ± 0.1 D	4.2 ± 0.3 B	4.5 ± 0.2 D	2.9 ± 0.3 E
	ThC	5.5 ± 0.4 A	4.7 ± 0.3 B	4.4 ± 0.4 BC	4.1 ± 0.1 BCD	4.3 ± 0.3 BCD	3.0 ± 0.2 BCD	3.5 ± 0.3 B	3.6 ± 0.2 CD	3.6 ± 0.1 CD	2.4 ± 0.2 E
Product sample III	APC	0.5 ± 0.2 A	5.7 ± 0.2 A	5.7 ± 0.1 B	5.1 ± 0.2 CD	6.1 ± 0.3 B	5.1 ± 0.2 CD	4.9 ± 0.2 CD	5.5 ± 0.3 BC	4.7 ± 0.1 B	3.4 ± 0.1 E
	AnPC	0.0 ± 0.3 A	6.5 ± 0.3 B	5.0 ± 0.3 DE	5.0 ± 0.1 DE	6.3 ± 0.2 BC	5.2 ± 0.3 BE	4.4 ± 0.3 E	5.6 ± 0.2 CD	4.0 ± 0.3 DE	2.9 ± 0.3 F
	ThC	7.1 ± 0.2 A	5.1 ± 0.1 B	4.5 ± 0.2 C	3.5 ± 0.2 E	4.6 ± 0.2 C	4.2 ± 0.2 CD	3.0 ± 0.2 DE	4.4 ± 0.1 C	3.7 ± 0.1 DE	2.6 ± 0.1 F

\*Means with the different letter are significantly different.

APC= aerobic, plate count, AnPC= anaerobic plate count, ThC= thermophilic plate count.

Ex.P=experimental product, Blank =No treatment, LA1=1% Lactic acid, LA2=1.5% Lactic acid, LA3=2% Lactic acid KS1=0.10% Potassium sorbate, KS2=0.15 % Potassium sorbate, KS3=0.20 % Potassium sorbate LA1KS1=1% Lactic acid followed by 0.10 % Potassium sorbate, LA1KS3=1% Lactic acid followed by 0.20 % Potassium sorbate, LA2KS3=1.5% Lactic acid followed by 0.20 % Potassium sorbate,

**Table (5): Mean counts of aerobic, anerobic plate and thermophilic count (CFU/g) before and after heat treatment of spices (N=5).**

Spice	Before autoclaving			After autoclaving		
	APC	AnPC	ThC	APC	AnPC	ThC
Pepper	$1.5 \times 10^3$	$1.3 \times 10^3$	$2 \times 10^3$	$2.7 \times 10^2$	$7 \times 10^2$	$1.3 \times 10^2$
Cumin	$5.5 \times 10^2$	$6.6 \times 10^2$	$1.5 \times 10^2$	$1 \times 10^2$	$6 \times 10^2$	$1.8 \times 10^1$
Chillies	$4.6 \times 10^5$	$3.2 \times 10^5$	$4.2 \times 10^4$	$9.8 \times 10^3$	$3 \times 10^3$	$7 \times 10^2$
Allspice	$5.3 \times 10^6$	$2.2 \times 10^6$	$2 \times 10^5$	$2.7 \times 10^3$	$4.5 \times 10^3$	$7 \times 10^2$
Coriander	$4.3 \times 10^5$	$8.3 \times 10^4$	$2 \times 10^4$	$8.7 \times 10^3$	$2.5 \times 10^2$	$7 \times 10^2$
Cardamom	$3.6 \times 10^2$	$6.6 \times 10^1$	$1.8 \times 10^2$	$1.6 \times 10^1$	$1.1 \times 10^1$	$1.3 \times 10^2$

C= aerobic, plate count, AnPC= anaerobic plate count, ThC= thermophilic plate count.

**Table (6): Log mean values of aerobic, anaerobic plate and thermophilic (CFU/g) count on the final product samples formulated with heat-treated spices and LA2KS3, followed by packaging and storage at 4°C for 6 months.**

Final Product		One month	Two months	Three months	Four months	Five months	Six months
Product I (vacuum packed cold smoked herring fillets)	APC	$2.2 \pm 0.2$	$2.9 \pm 0.3$	$3.1 \pm 0.2$	$3.5 \pm 0.1$	$4.3 \pm 0.3$	$5.2 \pm 0.5$
	AnPC	$2.1 \pm 0.2$	$2.4 \pm 0.3$	$2.8 \pm 0.2$	$3.1 \pm 0.2$	$4.1 \pm 0.4$	$5.0 \pm 0.3$
	ThC	$1.9 \pm 0.1$	$2.1 \pm 0.1$	$2.4 \pm 0.1$	$2.9 \pm 0.1$	$3.6 \pm 0.2$	$4.5 \pm 0.6$
Product II (vacuum packed cold smoked herring roe)	APC	$2.2 \pm 0.4$	$3.1 \pm 0.4$	$3.4 \pm 0.4$	$3.8 \pm 0.1$	$4.7 \pm 0.5$	$5.4 \pm 0.6$
	AnPC	$2.1 \pm 0.3$	$2.3 \pm 0.3$	$2.8 \pm 0.7$	$3.5 \pm 0.1$	$4.2 \pm 0.7$	$5.3 \pm 0.4$
	ThC	$1.9 \pm 0.2$	$2.1 \pm 0.2$	$2.3 \pm 0.4$	$2.8 \pm 0.1$	$3.6 \pm 0.4$	$4.7 \pm 0.5$
Product III (cold smoked herring fillets with caviar)	APC	$2.8 \pm 0.1$	$3.1 \pm 0.1$	$3.5 \pm 0.1$	$3.7 \pm 0.2$	$4.1 \pm 0.3$	$4.7 \pm 0.2$
	AnPC	$2.2 \pm 0.3$	$2.4 \pm 0.3$	$2.8 \pm 0.3$	$3.3 \pm 0.1$	$3.6 \pm 0.2$	$4.4 \pm 0.3$
	ThC	$1.6 \pm 0.1$	$2.0 \pm 0.1$	$2.6 \pm 0.1$	$3.1 \pm 0.2$	$3.5 \pm 0.2$	$3.8 \pm 0.2$

APC= aerobic, plate count, AnPC= anaerobic plate count, ThC= thermophilic plate count.

### Discussion

Product shelf life must be established through microbiological analysis. Where previous data in support of shelf life is not available, especially for new products, this should be established using recognised industry standards. A microbiological sampling programme should be established for testing both raw materials and finished product.

All the collected samples of vacuum-packed cold smoked herring fillets (product I) and vacuum-packed cold smoked herring roe (product II) were of normal appearance, odour and texture. Some jars of product III (plastic jars containing cold smoked herring fillets with caviar) showed clear bulging of lids and gas formation indicating spoilage, while the other jars were of normal appearance. Visual examination of the herring from the bulging jars showed soft herring flesh, cloudy appearance and strong gas production. These changes were not seen in the unspoiled samples. At the time of analysis, there were 2-3 months of the expected shelf-life still remaining. Gaseous spoilage type, manifested by bulging of the lids of the jars after some storage weeks, has been associated with acetic acid fish preserves (38; 51) and vacuum-packaged cold-smoked fish (28; 29; 34; 35; 58). *Lactobacillus* species (7; 38; 51), *Enterobacteriaceae* (35), or yeasts (31; 54) have been the specific spoilage organisms detected in semi-preserved and marinated herring. Limited information about the microbial ecology associated with other types of spoilage in herring fish products was recorded.

#### **Microbiological examination of the cold smoked herring products:**

Bacterial analysis of the three commercial cold smoked herring products had high levels of aerobic, anaerobic and thermophilic bacterial count. The mean values of total aerobic and anaerobic bacterial count for product I, II, and III were ranged from  $>10^6$  to  $>10^7$  CFU/g; while relatively lower values of thermophilic count ( $>10^4$  to  $>10^5$  CFU/g) were recorded for the same products (Table 1). Limited studies have been published on the initial bacterial counts of cold smoked herring products. However, the initial counts obtained in the present study are in agreement with previous studies reporting initial total viable count between  $10^5$  and  $10^6$  CFU/g during storage of low-salted herrings samples (17) or for different packaged (18;

46) and unpackaged (8; 56) not-heat-treated fish products other than herring. In contrast, Lyhs et al. (39) found total viable count below  $10^2$ - $10^3$  CFU/g for freshly produced vacuum-packaged 'gravad' rainbow trout. Higher initial bacterial counts as found in the present study are most probably associated with cross-contamination of the fish during the filleting process including different sources like house aerobic microflora, used utensils and the personnel (3; 8; 56).

Microbiological criteria for APC normally consider the same limits for fresh, frozen or cold smoked and gravad fish. Magnússon and Traustadóttir (41) reported high APC values and good scores from sensory panel for smoked herring, concluding that total bacterial count has a minor influence on quality assessment of vacuum packed smoked herring. This may be the case for most of the vacuum packed smoked fish. Further work should be conducted to develop a specific criterion, such as a chemical quality index based on spoilage of dominant spoilage micro flora as suggested by Jørgensen et al. (29) for cold-smoked salmon.

#### **Examination of raw materials and application of treatments**

*Evaluation of raw materials before treatment* Table (2) showed the bacterial flora of cold smoked herring fillets and roe. The mean values of APC, AnPC and ThC were ranged from  $>10^4$  to  $>10^5$  (CFU/g). Earlier studies have shown that the Initial total counts of smoked herring fillets were in the order of  $10^4$ /g rising to  $10^8$ /g after 7 weeks storage (41). Several factors influence the spoilage flora in vacuum-packaged cold-smoked fish products. Size and composition of the developing microflora depend on the quality of the raw material, the in-house flora of the processing plant and the production method (58). During production, the fish undergoes many processing steps enabling microbial contamination in all these steps. Truelstrup Hansen et al. (59) showed that hygienic conditions in the smokehouse have a great effect on the amount and composition of the microflora in the final cold-smoked product.

In the present study, the bacterial quality of different types of spices, before treatment, formulated in product III, were variable. Low values of aerobic plate count, anaerobic plate count and thermophilic plate count ( $>10$  -  $>10^3$ ) were recorded for pepper, cumin and cardamom, while chillies, allspice and

coriander samples were the most highly contaminated, with counts of aerobic, anaerobic and thermophilic microorganisms at levels of  $>10^4$  -  $>10^6$  CFU/g (Table 4). **Rauscher and Hildebrandt (47)** showed a high total variance in the aerobic plate counts in some lots of spices, the total count of aerobic mesophilic and thermophilic bacteria ranged from  $10^1$  to  $10^6$  cfu g<sup>-1</sup> and  $10^1$  to  $10^5$  cfu g<sup>-1</sup> (**64**). Black pepper, cumin, chillies and all-spice were the most contaminated spices, with counts of aerobic mesophiles and thermoresistant microorganisms at levels of  $>10^5$  -  $>10^7$  CFU/g (**30; 49;60; 63**). The presence of high bacterial counts, particularly thermophilic aerobic sporeforming bacteria, in spices has been recognized as a potential source of spoilage organisms for some time (**27; 33; 42; 43**).

Interestingly, in this study, an unusual spoilage phenomenon affecting some samples of product III (cold smoked herring fillets with caviar) is described. During a problematic manufacturing period, some plastic containers started to show bulging due to gas formation after 2–3 storage months. The products were manufactured in one processing plant and they were expected to maintain good quality during a shelf-life of 5 months at the recommended storage ambient temperature. Spoilage had affected only some production lots and occurred from time to time. According to the manufacturer, good quality raw fish had always been used for the product but the quality of some other ingredients, such as spices, had been called in question. After this study, the manufacturer started to pay closer attention to the spices quality and storage times for spices. It is now already a year since the last spoiled lot was detected. These results emphasize the fact that all ingredients, even used only as small amounts for decoration and spicing, play an important role in the hygiene of food manufacture.

On the second axis, Table (3) showed isolation and identification of microorganisms on raw materials used in processing of cold smoked herring products. The microflora associated with cold smoked herring fillets and roe consists of *Staphylococcus* and *Micrococcus* species. Earlier studies indicated that the initial flora of the herring fillets was dominated by *Alteromonas putrefaciens* and *Pseudomonas* species. (**44**), *LactoBacillus* spp (**41**), lactic acid bacteria and Enterobacteriaceae (**40**). On the other hand, the microbiological quality of spices demonstrated profiles of microorganisms, including *Bacillus* spp., *Staphylococcus* spp., *Escherichia coli*,

*Klebsiella* spp., *Shigella*, *Enterococci*, *Clostridium* spp., *Aspergillus* spp., and *Penicillium* spp. (Table 3). The prevalence of *Bacillus* spp. was 100% in all the examined spices, followed by *Staphylococci*, *Enterobacteriaceae*, *Aspergillus* spp., and other microorganisms, while *Salmonella* spp. and yeasts were not detected in any sample investigated. Microbiological assays of vegetable seasoning used in food are in harmony with our findings (4; 42). The prevalence of *Bacillus* spp. in food ingredients is of interest to the food microbiologist because of their potential importance as spoilage organisms in foods (21; 27; 57). Considerable variation in the occurrence of *Bacillus* species. in spices has been reported, between brands as well as among samples from the same brand. Several spices were reported to have high loads of *B. cereus* (22; 30; 36; 42).

**Antimicrobial treatment of herring roe and fillets** Table (4) showed the results of the dipping treatments of herring roe and fillets in antimicrobials followed by packaging and storage at 4°C for 3 months. In general, growth of the pathogen on experimental product samples formulated with antimicrobials was inhibited by all dipping treatments throughout storage. Dipping of samples in lactic acid or potassium sorbate significantly reduced the mean populations of APC from 0.4 to 2.0, 1.1 to 2.1, 2.4 to 3.6; and AnPC from 0.9 to 2.3 , 1.0 to 2.3, 1.5 to 3.6 log CFU/g on experimental product I, II and III respectively, while the ThC was reduced from 0.2 to 1.3 log CFU/g, 0.8 to 2.0 log CFU/g, 2.0 to 3.6 log CFU/g for the same product samples (Tables 3). Combining lactic acid and potassium sorbate resulted in an increased reduction in the mean populations of aerobic, plate count, anaerobic plate count, thermophilic plate count (log1). The most effective dipping treatments for products formulated with antimicrobials were LA2KS3 (1.5% Lactic acid followed by 0.20 % Potassium sorbate). On these samples, levels of contamination were decreased from >5-8 log CFU/g to >2-3 log CFU/g by the end of storage for the three product samples (Tables 4). Several reports have addressed the use of lactic acid and potassium sorbate to effectively suppress the growth of bacteria on, or in, various processed food products (1; 12; 20; 26; 45; 65).

**Decontamination of spices:** Autoclaving of spices for 15 min. reduced initial counts (aerobic, plate count, anaerobic plate count, thermophilic plate count) by approximately 1.0–2.0 logarithms, with the exception of cumin

which showed limited effect Table (5). Several sterilization methods on microbial loads of spices have been reported. For example, irradiation (13; 48; 52; 53), microwave treatments (10) and heat treatment. Although heat treatment was previously reported as a good process for microbial decontamination of seeds and food powders (5; 15; 50; 62), it causes more intensive colour loss of some spices (62).

**Assessment of the final products after application of treatments:**

Regarding to the physical examination of the final cold-smoked herring products formulated with LA2KS3 dipping treatments and heat treated spices during 6 months storage at 4 degrees C, the study revealed that all the examined samples were of normal characters, moreover, no signs of spoilage were observed for any sample during storage period.

The initial shelf-life of the commercial herring products recorded by manufacturer, was 3 months at 4°C for product I&II (vacuum-packed cold-smoked herring fillets and roe), while it was 5 months at ambient temperature for product III (herring fillets with roe); with an associate bacterial microflora of the three products in varying levels  $10^6$ - $10^7$  CFU/g for aerobic, plate count, anaerobic plate count, and 105 for thermophilic plate count (Table 1). The use of LA2KS3 (1.5% Lactic acid followed by 0.20 % Potassium sorbate) dipping treatments, heat treated spices and storage at 4 °C ( particularly for product III) increased the shelf-life to five months for product I&II and six months for product III. Moreover, these treatments limited the level of aerobic plate count , anaerobic plate count and thermophilic plate count to about 104, 104, 103 cfu/g, respectively, at the previously mentioned shelf-life periods (Table 6).

Vacuum atmosphere packaging in combination with chilled storage offers the fish industry and the consumer many advantages, such as extending the shelf-life of the products, longer transport distances of the product and reduced financial losses (6; 14; 49; 55). Storage temperatures below the growth optimum lead to extended generation times and lag times, and the growth rate decreases. (11). In the temperature range between 0 and 10 °C, minor changes have an enormous effect on bacterial growth (24; 32). Thus, to guarantee maximum antimicrobial effect, the storage temperature should be kept as low as possible (16; 14). However, refrigeration cannot

kill or completely eliminate spoilage bacteria, but will limit the spoilage to psychrotrophic microorganisms, which can grow in products chilled at a temperature below 7 °C (39; 40). Thus, a synergistic effect was observed between storage temperature and the application of chemical preservatives (19). A variety of chemical preservatives and additives can extend the shelf life of food and/or inhibit pathogens, either singly or in combination (37). Potassium sorbate treatment was most effective in controlling microbial quality and extended the shelf-life of the catfish samples (12).

Therefore, processors of cold-smoked fish should be concerned with the microbiological quality of the raw materials they receive from suppliers since product from contaminated sources may not be appropriate for production of cold-smoked products. In general, good sanitation procedures should be applied throughout harvest, transportation, storage, and post-harvest handling.

In conclusion, the present study has demonstrated that the use of 1.5% Lactic acid followed by 0.20 % Potassium sorbate dipping treatments, with heat treated spices and storage at 4 °C were found to be the most suitable for keeping quality and extending the shelf-life of vacuum packaging and non vacuum packaging cold-smoked herring products.

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## تحسين جودة بعض منتجات الأسماك المدخنة وبخاصة معلبات فيليه الرنجة مع الكفيار

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### الملخص العربى

أجريت هذه الدراسة لتقييم الجودة الميكروبية لثلاثة من منتجات الرنجة المدخنة وتشمل فيليه رنجة معبأة بالتفريغ، بطارخ رنجة معبأة بالتفريغ و فيليه رنجة مع الكفيار. أفاد الفحص الظاهرى أن معظم العينات كانت سليمة وذات مظهر طبيعى بينما لوحظ بعض مظاهر الفساد مثل إنتفاخ الغطاء مع تكوين فقعات غازية فى بعض برطمنات المنتج الثالث. وأظهر التحليل البكتريولوجى للمنتجات الثلاثة إرتفاع مستوى العد الكلى البكتري للميكروبات الهوائية واللاهوائية ( $10^6$  إلى  $10^7$  خلية بالجرام) بينما كان العد الكلى البكتيرى للميكروبات المحبة للحرارة أقل نسبيا ( $10^4$  إلى  $10^5$  خلية بالجرام). وقد أفاد فحص المواد الخام المستخدمة فى تصنيع منتجات الرنجة المدخنة أن معدل التلوث البكتيرى لفيلية و بطارخ الرنجة المدخنة قد تراوح بين ( $10^4$  إلى  $10^5$  خلية بالجرام) كما أظهر الفحص البكتريولوجى عن إختلاف معدلات التلوث البكتيرى لأنواع المختلفة من التوابل حيث سجلت قيم منخفضة للعد الكلى للبكتريا الهوائية واللاهوائية والمحبة للحرارة ( $10^3$  إلى  $10^4$  خلية بالجرام) للفلفل الأسود والكمون والحبهان، بينما كانت الشطة والبهارات والكسبرة أكثر تلوثا ( $10^4$  إلى  $10^6$  خلية بالجرام). وقد تم عزل ميكروب ستافيلوكوكس وميكروكوكس من فيليه و بطارخ الرنجة المدخنة بينما تم عزل ميكروب الباسليس بصفة أساسية من عينات التوابل. ومن ناحية أخرى فقد لوحظ أن معالجة فيليه و بطارخ الرنجة المدخنة بغمسها فى حمض الاكتيك أو سوربات البوتاسيوم عن إنخفاض معدلات التلوث بالبكتيريا الهوائية واللاهوائية و المحبة للحرارة. كما أفادت المعالجة الحرارية للتوابل عن إنخفاض العد الكلى البكتيرى بها. وقد خلصت الدراسة إلى أن إستخدام المواد الخام المغموسة فى 1,5% حمض لكتيك و 0,2% سوربات بوتاسيوم مع التوابل المعاملة حراريا لصنع معلبات الرنجة المدخنة وحفظها فى 4 درجات مئوية أدى إلى إطالة فترة صلاحية المنتجات (1-2 شهر) وتحسين جودتها.