

Improving of chemical and bacteriological parameters of smoked herring

Mohamed A. Hussein, Abdallah F. Mahmoud, Nanis S. Elnagar and Adel Ibrahim El-Atabany

Department of Food Control and Technology, Faculty of Veterinary Medicine
Zagazig University, Zagazig 44511, Egypt

Abstract:

Twenty kilograms of herring were divided into four batches group one five kilogram of *Clupea harengus* mixed with 10% table salt (CG), the 2nd group five kilogram of eviscerated *Clupea harengus* mixed with 10% table salt (GG), the 3rd group five kilogram of *Clupea harengus* mixed with 10% table salt and 3% *Curcuma longa* and the 4th group five kilogram of eviscerated *Clupea harengus* mixed with 10% table salt and 3% *Curcuma longa* all groups introduced to traditional smoking. All the prepared groups after processing were kept under refrigeration storage at 4°C then examined after 1st, 4th, and 8th weeks. On the 8th week of storage the organoleptic score became 3.24 ± 0.1 , 3.12 ± 0.12 , 4.16 ± 0.14 and 3.31 ± 0.11 . The pH became 6.81 ± 0.21 , 6.65 ± 0.23 , 6.59 ± 0.25 and 6.53 ± 0.24 . The trimethylamine (TMA) 14.41 ± 0.54 , 11.24 ± 0.42 , 10.24 ± 0.39 and 9.85 ± 0.38 mg/100g. The thiobarbituric acid (TBA) became 4.98 ± 0.31 , 3.85 ± 0.32 , 3.12 ± 0.24 and 2.54 ± 0.23 mg malondialdehyde/Kg. The histamine became 22.1 ± 1.56 , 17.32 ± 1.49 , 16.42 ± 1.45 and 13.95 ± 1.35 mg/kg. Enterobacteriaceae count became $3.5 \times 10^5 \pm 2.1 \times 10^5$, $4.5 \times 10^4 \pm 1.6 \times 10^4$, $3.6 \times 10^4 \pm 0.45 \times 10^4$ and $1.21 \times 10^4 \pm 0.09 \times 10^4$ CFU/g and Staphylococci count became $17.5 \times 10^5 \pm 3.2 \times 10^5$, $22.48 \times 10^5 \pm 8.2 \times 10^5$, $2.3 \times 10^5 \pm 0.35 \times 10^5$ and $2.8 \times 10^5 \pm 0.24 \times 10^5$ CFU/g in (CG), (GG), (CLG), and (CLGG), respectively.

Key words: Smoked herring – Vacuum packaged herring- Staphylococci – Histamine – Enterobacteriaceae.

1.Introduction:

Smoking is one of the traditional processing methods for preservation and quality improvement of fish. In the past decades, up to 70% of the total fish catch in developing countries was preserved by smoking, a process through which volatiles from thermal combustion of wood penetrate into fish flesh (Ward, 1995). In contrast, in industrialized countries, smoking serves primarily as a tool to enhance the flavor and texture of fish, often producing value-added products. Smoking usually extends the shelf life of fish due to the reduced moisture content and the antimicrobial or antibacterial activities imparted by the phenolic compounds of smoke. In Egypt Smoked, products are traditionally consumed, and one of the most common smoked products is smoked herring. Safety of fish products and their quality assurance is one of the main problems of food industry today. The presence or absence of foodborne pathogens in a fish product is a function of the harvest environment, sanitary conditions, and practices associated with equipment and personnel in the processing environment (FDA, 2001). The handling of fish products during the manufacturing process involves a risk of contamination by *S.aureus*, a Gram-positive microorganism causing foodborne human intoxication. These bacteria are salt-tolerant and therefore can contaminate all cured preparations such as cold smoked fish, caviar, and fish-

based preserves. *Staphylococcus aureus* can contaminate food products during preparation and processing, so it is still a major cause of foodborne diseases. It inhabits the nasal passages, the skin, and hair of warm-blooded animals. Up to 30-50% of the human population is carriers. The most virulent factors of *S. aureus* is the production of heat-stable enterotoxins implicated in food-borne intoxications. The enterotoxins are highly stable, resist inactivation by proteolytic enzymes, such as pepsin or trypsin, and also resist chymotrypsin, rennin and papain, so they remain active in the digestive tract after ingestion (**Le Loir et al., 2003**). The use of natural preservatives, such as turmeric, garlic, and other spices, for marine seafood preservation has received immense interest among researchers. Moreover, the seafood industry is searching for natural preservatives to avoid adverse effects on fish, meat and to extend shelf life (**Gul & Bakht, 2015**). Turmeric (*Curcuma longa*) is one of the spices most widely used as a preservative and as a color, antiseptic, anticancer, wound healing, and antibacterial agent in biological systems worldwide. The first paper describing the biological action of curcumin was its antibacterial activity against various bacteria: *S. aureus*, *Trichophyton gypseum*, *Salmonella paratyphi*, and *Mycobacterium tuberculosis* (**Schraufst tter and Bernt, 1949**). So the current study conducted to evaluate the effect of *Curcuma longa* powder 3% in processing of smoked herring in order to control *Staphylococci* and delay formation of trimethylamine, thiobarbituric acid, and histamine.

2. Materials and methods:

Twenty kilograms of herring divided into four batches group one five kilogram of *Clupea harengus* mixed with 10% table salt, group two five kilogram of eviscerated *Clupea harengus* mixed with 10% table salt, group three five kilogram of *Clupea harengus* mixed with 10% table salt and 3% *Curcuma longa* and group four five kilogram of eviscerated *Clupea harengus* mixed with 10% table salt and 3% *Curcuma longa* all groups introduced to traditional smoking. All the prepared groups after processing were kept under refrigeration storage at 4°C then examined after 1st, 4th, and 8th weeks.

2.1. Organoleptic examination: Evaluation of salted fish quality was carried out by five postgraduate students in Food Hygiene Department, Faculty of Vet. Medicine, Zagazig University. Quality attributes studies included appearance, juiciness, saltiness, rancidity, flavor and general acceptability. Panel members scored all factors on a 5-point hedonic scale according to **Ikeme (1986)**

2.2. Chemical examination: pH value was determined according to **Suvanich et al., (2000)** by using pH meter after homogenization of each 10g fish muscle sample in 100ml distilled water by stomacher. The pH meter was calibrated using pH 4 and pH 10. Thiobarbituric acid (TBA) was determined using a previously published spectrophotometric method (**Vyncke, 1970**). Evaluation of trimethylamine (TMA) by steam distillation of trichloroacetic acid (TCA) extract modified method of **Malle and Tao (1987)**. Determination of Histamine in meat samples by utilizing the chemical connected immunosorbent measure (ELISA, catalyst immunoassay) is an immunological strategy like RIA yet utilizes a protein coupled to antigen or counter acting agent as opposed to a radioactive isotope. The histamine concentration in mg/kg (ppm) comparing to the absorbance of every specimen is read from the calibration curve and multiplied by the dilution factor (6) for herring.

2.3. Staphylococcal enumeration: Preparation of fish samples according to APHA, (2002). A quantity of 0.1 ml of each previously prepared dilution was spread over the dried surface of duplicate Baird –parker agar plates supplemented with egg – yolk tellurite using glass spreader. Inoculated plates and control one were incubated at 37°C for 24 hours. Suspected colonies (jet black, shining, convex colonies, 1-1.5 mm in diameter with a narrow white margin and surrounded by a wide clear area with a one Opacity around them) were recoded counting was repeated after reincubated at 37°C for a further 24 hours. Staphylococcal count/cm² was calculated as presumptive count (ISO, 1999). Enumeration of *Enterobacteriaceae* was carried out on violet red bile glucose agar (VRBG) agar. The agar was inoculated by spreading 0.1 ml of the decimal dilution onto the surface. Plates were inverted and incubated at 37°C for 24 hours under aerobic condition. the crystals violet and agar component bile salt, largely inhibit the gram positive during neutral red act as an indicator of pH of the agar, and glucose in the formation of acid. *Enterobacteriaceae* recovered, the glucose utilization lead to a drop in pH value, which translate into a red in the visible colonies, accordingly in evaluating the plates, only red or pink colonies with or without the same precipitate were considered (ISO, 2004).

2.4. Statistical analysis: Statistical significance was evaluated using either tukey-kramer honestly significant difference tests with $p < 0.05$ considered as significant. Correlation analyses were performed using JMP program (SAS Institute, Cary, NC, USA).

3. Results and discussion:

Effect of *Curcuma longa* 3% on the organoleptic score of smoked herring groups stored at 4°C.:

The illustrated data in table 1 declared that the final organoleptic score of smoked *Clupea harengus* after 1st week was 4.9 ± 0.16 , 4.88 ± 0.18 , 4.92 ± 0.17 and 4.89 ± 0.18 in control group (CG), gutted group (GG), *curcuma longa* group (CLG) and *curcuma longa* gutted group (CLGG), respectively.

The mean values of organoleptic score decreased at the 4th week of storage 4.45 ± 0.13 , 4.48 ± 0.13 , 4.78 ± 0.14 and 4.32 ± 0.12 in (CG), (GG), (CLG), and (CLGG), respectively. Finally, at the 8th week of storage the organoleptic score became 3.24 ± 0.1 , 3.12 ± 0.12 , 4.16 ± 0.14 and 3.31 ± 0.11 in (CG), (GG), (CLG), and (CLGG), respectively.

Table (1): Effect of *Curcuma longa* 3% on the organoleptic score of smoked herring groups stored at 4°C.

Weeks		(CG)	(GG)	(CLG)	(CLGG)
1 st week	Min- Max	4.7 - 5	4.7 - 4.9	4.8 - 5	4.8 - 4.9
	Mean \pm SE	4.9 ± 0.16^a	4.88 ± 0.18^a	4.92 ± 0.17^a	4.89 ± 0.18^a
4 th week	Min- Max	4.4 - 4.7	4.3 - 4.5	4.5 - 4.9	4 - 4.5
	Mean \pm SE	4.45 ± 0.13^a	4.48 ± 0.13^a	4.78 ± 0.14^a	4.32 ± 0.12^{ab}
8 th week	Min- Max	3 - 3.5	3 - 3.3	4 - 4.5	3 - 3.5
	Mean \pm SE	3.24 ± 0.1^b	3.12 ± 0.12^b	4.16 ± 0.14^a	3.31 ± 0.11^{ab}

Score 5 considered excellent, score 4 good, score 3 fair, score 2 and 1 bad and very bad considered as unfit for human consumption.

(a,b,c) Means carrying different superscript small letters on the same rows are significantly different (< 0.05). CG): Control group (GG): Gutted group (CLG) : *Curcuma longa* group (CLGG): *Curcuma longa* gutted group

There was a significant increase in organoleptic score of (CLG) which attributed to the effect of *Curcuma longa* treatment that improved the color, appearance, and flavor of the smoked products. The result of the study was in agreement with that of **Akteret al. (2013)**, who demonstrated that application of turmeric powder was effective in preserving the quality of sun-dried tengra for a period of 8 months during ambient storage. Moreover, *Curcuma longa* treatment has slowed down the rate of deterioration in the smoked samples and extended the shelf life by 1 week (**Pankyamma et al. (2016)**). **Arulkumar et al. (2017)** found that treatment with turmeric extract was highly effective in delaying the spoilage indices and extending the shelf life.

Effect of *Curcuma longa* 3% on the potency of hydrogen ion (pH) of smoked herring groups stored at 4°C.:

Fresh fish is close to neutral pH and gradually rising during storage with pH values above 7.1 is sign of decomposition. The pH value of all samples slightly increased during cold storage as a function of time, because of the production of basic amines due to decomposition of nitrogenous compounds caused primarily by microbial activity. **Özyurt, et al., (2009)** reported pH as an index, which is important in determining the quality of fish, and it can be used as a guide.

The illustrated data in table 2 declared that the pH of smoked *Clupea harengus* after 1st week was 5.95 ± 0.23 , 6.18 ± 0.21 , 5.69 ± 0.21 and 6.02 ± 0.22 in control group (CG), gutted group (GG), *curcuma longa* group (CLG) and *curcuma longa* gutted group (CLGG), respectively.

The mean values of pH increased at the 4th week of storage 6.23 ± 0.25 , 6.27 ± 0.24 , 5.92 ± 0.26 and 6.23 ± 0.25 in (CG), (GG), (CLG), and (CLGG), respectively. Finally at the 8th week of storage the pH became 6.81 ± 0.21 , 6.65 ± 0.23 , 6.59 ± 0.25 and 6.53 ± 0.24 in (CG), (GG), (CLG), and (CLGG), respectively. Generally all treated groups around the the maximum acceptable limit of pH value (6.5) in fish and fish products recommended by **ES (2005)**.

Table (2): Effect of *Curcuma longa* 3% on the pH values of smoked herring groups stored at 4 °C .

Weeks		(CG)	(GG)	(CLG)	(CLGG)
1 st week	Min- Max	5.7-6.1	6.05- 6.3	5.6- 5.8	5.9 – 6.2
	Mean \pm SE	5.98 ± 0.23^b	6.18 ± 0.24^a	5.69 ± 0.21^c	6.02 ± 0.22^b
4 th week	Min- Max	6.1- 6.32	6.2 – 6.35	5.84 – 6.2	6.1- 6.38
	Mean \pm SE	6.23 ± 0.25^a	6.27 ± 0.24^a	5.92 ± 0.26^b	6.23 ± 0.25^a
8 th week	Min- Max	6.65 – 6.92	6.52 – 6.82	6.49 – 6.64	6.47 – 6.78
	Mean \pm SE	6.81 ± 0.21^a	6.65 ± 0.23^a	6.59 ± 0.25^b	6.53 ± 0.24^b

(a,b ,c) Means of the same rows carrying different superscript small letters are significantly different (< 0.05).

Nearly similar finding obtained by **Pankyamma et al.(2016)** they found that pH of turmeric treated samples has shown a reduction and thereafter remained more or less constant throughout the storage time.

There was a significant decrease in pH of (CLG) and (CLGG) which attributed to the effect of *Curcuma longa* treatment that reduce fish spoilage bacteria responsible for volatile base compounds such as ammonia and trimethylamine as well as other biogenic amines (**Ruiz-Capillas et al., 2005**).

Effect of *Curcuma longa* 3% on the trimethylamine (TMA-N) mg/100g of smoked herring groups stored at 4°C.:

TMA is derived from trimethylamine oxide (TMAO) and is essential for osmoregulation in marine fish. During spoilage, TMAO is reduced to TMA by enzymatic activity (Cai et al., 2014; Viji et al., 2015). TMA-N is considered as a valuable tool in the evaluation of fish quality because of its rapid accumulation in muscle under refrigerated conditions (Gökodlu et al., 1998).

Table (3): Effect of *Curcuma longa* 3% on trimethylamine mg/100g of smoked herring groups stored at 4°C.

Weeks		(CG)	(GG)	(CLG)	(CLGG)
1 st week	Min- Max	6.5 – 8.5	4.8- 6.9	5.2 – 6.7	4.6 – 6.7
	Mean ± SE	7.25 ± 0.34 ^a	5.61 ± 0.24 ^a	5.94 ± 0.35 ^a	5.28 ± 0.27 ^b
4 th week	Min- Max	8.4 – 10.5	6.5- 9.5	6.4 – 8.4	6.5 – 8.2
	Mean ± SE	9.54 ± 0.42 ^a	7.85 ± 0.37 ^b	7.54 ± 0.31 ^b	7.26 ± 0.32 ^b
8 th week	Min- Max	13.2 – 15.8	9.8- 12.4	8.9 – 11.8	8.4 – 10.7
	Mean ± SE	14.41 ± 0.54 ^a	11.24 ± 0.42 ^b	10.24 ± 0.39 ^{bc}	9.85 ± 0.38 ^c

(a,b ,c) Means of the same rows carrying different superscript small letters are significantly different (< 0.05).

During storage table 3, the TMA value decreased significantly ($p < 0.05$) from 7.25 ± 0.34 mg/100 g in (CG) to final values of 5.94 ± 0.35 and 5.28 ± 0.27 mg/100 g in (CLG) and (CLGG), respectively, after first week of storage. The gradual increase in TMA in all groups became 9.54 ± 0.42 , 7.85 ± 0.37 , 7.54 ± 0.31 and 7.26 ± 0.32 mg/100g in (CG), (GG), (CLG) and (CLGG), respectively after 4th week. Finally the values were 14.41 ± 0.54 , 11.24 ± 0.42 , 10.24 ± 0.39 and 9.85 ± 0.38 mg/100g in (CG), (GG), (CLG), and (CLGG), respectively after 8th week. TMA contents in all treated groups remained below the critical values (10–12 mg per 100 g) established as indicator of spoilage in smoked fish (European Commission, 1995).

The (CG) was significantly higher ($p < 0.05$) than (GG), (CLG) and (CLGG). Thus, the results indicated that both of gutting and addition of *Curcuma longa* 3% were effective at inhibiting the decarboxylation of TMAO to TMA by the action of bacteria. Nearly similar findings obtained by Vaz-Pires et al. (2008) and Arulkumar et al. (2017) found that treatment with turmeric extract was highly effective in delaying the reduction of TMA-N.

Table (4): Effect of *Curcuma longa* 3% on Thiobarbituric (TBA) acid mg MDA/kg of smoked herring groups stored at 4°C.

Weeks		(CG)	(GG)	(CLG)	(CLGG)
1 st week	Min- Max	1.2 – 1.8	1.1- 1.62	0.9 – 1.37	0.72 – 1.18
	Mean ± SE	1.84 ± 0.10 ^a	1.34 ± 0.09 ^a	1.19 ± 0.08 ^a	0.98 ± 0.08 ^b
4 th week	Min- Max	2.14 – 2.85	1.52- 2.17	1.42 – 1.95	1.23 – 1.62
	Mean ± SE	2.45 ± 0.07 ^a	1.83 ± 0.07 ^{ab}	1.52 ± 0.09 ^b	1.41 ± 0.09 ^c
8 th week	Min- Max	3.54 – 6.63	2.94- 4.95	2.74 – 3.49	1.95 – 3.14
	Mean ± SE	4.98 ± 0.31 ^a	3.85 ± 0.32 ^{ab}	3.12 ± 0.24 ^b	2.54 ± 0.23 ^c

(a,b ,c) Means of the same rows carrying different superscript small letters are significantly different (< 0.05).

Lipid in fish muscle typically consists of high percentage of polyunsaturated fatty acids and is consequently prone to oxidative reaction, which is the major cause of a shortened shelf life of fish. The TBA index is a measure of malonaldehyde (MDA) content, one of the degradation products of lipid hydroperoxides, formed during the oxidation process of polyunsaturated fatty acids (Gomes et al., 2003). Bensid et al., (2014) declared that MDA, a secondary product of lipid oxidation, and considered as a suitable indicator of fish meat freshness. TBA also is considered as spoilage indicator with microbiological and organoleptic examination in fish during storage period.

The higher fat content in *Clupea harengus* compared to other aquaculture fishes makes it more vulnerable to lipid oxidation during storage. Lipid oxidation and quality deterioration in fish products can be controlled by the addition of preservatives having antioxidant activities.

The illustrated data in table 4 declared that the TBA of smoked *Clupea harengus* after 1st week was 1.84 ± 0.10 , 1.34 ± 0.09 , 1.19 ± 0.08 and 0.98 ± 0.08 mg MDA/kg in control group (CG), gutted group (GG), *curcuma longa* group (CLG) and *curcuma longa* gutted group (CLGG), respectively.

The mean values of TBA increased at the 4th week of storage 2.45 ± 0.07 , 1.83 ± 0.07 , 1.52 ± 0.09 and 1.41 ± 0.09 mg MDA/kg in (CG), (GG), (CLG), and (CLGG), respectively. Finally at the 8th week of storage the TBA became 4.98 ± 0.31 , 3.85 ± 0.32 , 3.12 ± 0.24 and 2.54 ± 0.23 in (CG), (GG), (CLG), and (CLGG), respectively.

Concerning the permissible limit of TBA value in fish and fish products (4.5 mg MDA/kg) recommended by ES (2005); neither treated groups nor control one exceeded such limit in any occasion of examination.

There was a general trend toward an increase in TBA values. This observation is in agreement with the result reported by other authors (Maqsood and Benjakul 2010). The (CG) was significantly higher ($p < 0.05$) than (CLG) and (CLGC). Thus, the results indicated that addition of *Curcuma longa* 3% were effective at inhibiting lipid oxidation. These results coincide to findings of Arulkumar et al. (2017) found that treatment with turmeric extract was highly effective in delaying formation of TBA. Moreover, Basniwal et al. (2011) declared the preservative action of turmeric extract could be due to the presence of essential oils, curcumins, curcuminoids, turmeric oil, turmerol, and valeric acid, which are the major phenolic compounds in turmeric.

Effect of *Curcuma longa* 3% on histamine mg /kg of smoked herring groups stored at 4°C.

Biogenic amines are present in a wide range of food products including fish products, meat products, dairy products, wine, beer, vegetables, fruits, nuts and chocolate (Brink et al., 1990). In virtually, all foods that contain proteins or free amino acids and are subjected to conditions enabling microbial or biochemical activity, biogenic amines formation can be expected in them, the total amount of the different amines formed strongly depends on the nature of the food and count and type of microorganisms present (Brink et al., 1990).

The illustrated data in table 5 declared that the histamine of smoked *Clupea harengus* after 1st week was 9.55 ± 0.98 , 8.31 ± 0.89 , 8.53 ± 0.97 and $7.13 \pm$

0.92 mg /kg in control group (CG), gutted group (GG), *curcuma longa* group (CLG) and *curcuma longa* gutted group (CLGG), respectively.

The mean values of histamine increased at the 4th week of storage 15.59 ± 1.17 , 11.42 ± 1.08 , 10.24 ± 1.11 and 9.62 ± 1.21 mg /kg in (CG), (GG), (CLG), and (CLGG), respectively. Finally, at the 8th week of storage the histamine became 22.1 ± 1.56 , 17.32 ± 1.49 , 16.42 ± 1.45 and 13.95 ± 1.35 mg/kg in (CG), (GG), (CLG), and (CLGG), respectively. Nearly similar values for histamine obtained by **Mackie et al., (1997)** investigated the formation of biogenic amines in herring and mackerel stored in ice and in a chill room at 10°C, they found that the levels of histamine in mackerel and herring at the end of storage period (13 days) were below 10mg/100gm. Lower histamine value 4.4 ± 0.98 mg/kg obtained by **Hussein (2014)** in marketed smoked herring.

Table (5): Effect of *Curcuma longa* 3% on histamine mg /kg of smoked herring groups stored at 4°C.

Weeks		(CG)	(GG)	(CLG)	(CLGG)
1 st week	Min- Max	8.21 – 10.1 ^a	7.25- 8.92	7.53 – 9.13	7.09 – 7.21
	Mean \pm SE	9.55 ± 0.98^a	8.31 ± 0.89^b	8.53 ± 0.97^b	7.13 ± 0.92^c
4 th week	Min- Max	14.2 – 17.4	10.5- 13.6	9.48 – 12.91	8.14 – 10.78
	Mean \pm SE	15.59 ± 1.17^a	11.42 ± 1.08^b	10.24 ± 1.11^b	9.62 ± 1.21^c
8 th week	Min- Max	21.2 – 22.9	15.4- 19.2	14.75 – 18.42	12.54 – 15.24
	Mean \pm SE	22.1 ± 1.56^a	17.32 ± 1.49^b	16.42 ± 1.45^b	13.95 ± 1.35^c

(a,b ,c) Means of the same rows carrying different superscript small letters are significantly different (< 0.05).

Overall, significant differences in histamine levels were noticed between the (CG) and treated groups (GG, CLG, and CLGC) ($p < 0.05$). Thus, the results indicated that addition of *Curcuma longa* 3% were effective at inhibiting histamine formation. These results coincide to findings of **Basniwal et al. (2011)** and **Arulkumar et al. (2017)** found that treatment with turmeric extract was highly effective in histamine formation.

The Food and Drug Administration organization (**FDA, 1996**) established a defect action level (an amount that signifies some mishandling of the fish) of 50(mg/kg) for histamine in tuna and other fish species as an indication of potential health risk. While **ES (2005)** established 20 mg/kg as a permissible limit for histamine in smoked salted and frozen fish products, it appears that level of histamine in all examined groups not constitutes a hazard for human health. Histamine poisoning results from the ingestion of food containing unusually high levels of histamine, fish of the families Scombridae and Scomberesocidae are commonly implicated in incidents of histamine poisoning so it was called scombroid poisoning (**Taylor, 1986**). **Ienistea (1973)** reported the deleterious effects in relation to the amount of histamine ingested at one meal as follows: 8-40 mg histamine causes mild poisoning, while 70-1,000 mg histamine causes disorders of moderate intensity, and 1,500-4,000 mg histamine causes severe incidents. **Russell and Maretic (1986)** mentioned that severity of the symptoms can vary considerably with the amount of histamine ingested and the individual's sensitivity to histamine.

Effect of *Curcuma longa* 3% on total Enterobacteriaceae count CFU/g of smoked herring groups stored at 4°C.

The *Enterobacteriaceae* count has been considered as another index of fish quality (Gram & Huss, 1996) because this parameter is related to the treatments subsequently suffered by the fish in the fishing process (storage, washing and evisceration) and from this point of view it can indicate if these procedures have been performed following the respect of the HACCP norms (Hazard Analysis Critical Control Point) related to the hygiene and to the foods safety. *Enterobacteriaceae* were the dominant microbial group found throughout refrigerated storage and that biogenic amine forming bacteria found during storage at 0, 4, 10, 15, and 20°C but not at 25°C (Kim et al., 2009). The illustrated data in table 6 declared that the Enterobacteriaceae count of smoked *Clupea harengus* after 1st week was $11.2 \times 10^3 \pm 1.01 \times 10^3$, $3.9 \times 10^3 \pm 0.94 \times 10^2$, $4.2 \times 10^3 \pm 1.01 \times 10^3$ and $2.6 \times 10^3 \pm 0.28 \times 10^3$ CFU/g in control group (CG), gutted group (GG), *curcuma longa* group (CLG) and *curcuma longa* gutted group (CLGG), respectively. Nearly similar finding in smoked fillet 3.10 - 3.17 log₁₀ CFU/g, (Pankyamma et al., 2016).

Table (6): Effect of *Curcuma longa* 3% on total Enterobacteriaceae count CFU/g of smoked herring groups stored at 4°C.

Weeks		(CG)	(GG)	(CLG)	(CLGG)
1 st week	Min- Max	$14 \times 10^2 - 16 \times 10^3$	$8 \times 10^2 - 9 \times 10^3$	$9 \times 10^2 - 12 \times 10^3$	$7 \times 10^2 - 8 \times 10^3$
	Mean \pm SE	$11.2 \times 10^3 \pm 1.01 \times 10^3$ ^a	$3.9 \times 10^3 \pm 0.94 \times 10^2$ ^b	$4.2 \times 10^3 \pm 1.01 \times 10^3$ ^b	$2.6 \times 10^3 \pm 0.28 \times 10^3$ ^b
4 th week	Min- Max	$12 \times 10^3 - 15 \times 10^4$	$17 \times 10^2 - 3 \times 10^4$	$16 \times 10^2 - 5 \times 10^4$	$9 \times 10^2 - 17 \times 10^3$
	Mean \pm SE	$8.99 \times 10^4 \pm 2.15 \times 10^4$ ^a	$1.1 \times 10^4 \pm 0.12 \times 10^4$ ^b	$2.35 \times 10^4 \pm 0.42 \times 10^4$ ^b	$8.84 \times 10^3 \pm 1.98 \times 10^3$ ^c
8 th week	Min- Max	$2 \times 10^4 - 15 \times 10^5$	$7 \times 10^3 - 14 \times 10^4$	$11 \times 10^3 - 12 \times 10^4$	$4 \times 10^3 - 7 \times 10^4$
	Mean \pm SE	$3.5 \times 10^5 \pm 2.1 \times 10^5$ ^a	$4.5 \times 10^4 \pm 1.6 \times 10^4$ ^b	$3.6 \times 10^4 \pm 0.45 \times 10^4$ ^{bc}	$1.21 \times 10^4 \pm 0.09 \times 10^4$ ^c

(a,b,c) Means of the same rows carrying different superscript small letters are significantly different (< 0.05).

Gradual increases in Enterobacteriaceae counts at the 4th week of storage $8.99 \times 10^4 \pm 2.15 \times 10^4$, $1.1 \times 10^4 \pm 0.12 \times 10^4$, $2.35 \times 10^4 \pm 0.42 \times 10^4$ and $8.84 \times 10^3 \pm 1.98 \times 10^3$ CFU/g in (CG), (GG), (CLG), and (CLGG), respectively. Finally at the 8th week of storage the Enterobacteriaceae count became $3.5 \times 10^5 \pm 2.1 \times 10^5$, $4.5 \times 10^4 \pm 1.6 \times 10^4$, $3.6 \times 10^4 \pm 0.45 \times 10^4$ and $1.21 \times 10^4 \pm 0.09 \times 10^4$ CFU/g in (CG), (GG), (CLG), and (CLGG), respectively. Overall, significant differences in *Enterobacteriaceae* counts were noticed between the (CG) and treated groups (GG, CLG and CLGG) ($p < 0.05$). Thus, the results indicated that addition of *Curcuma longa* 3% were effective at inhibiting growth of Enterobacteriaceae. These results coincide to findings of Pankyamma et al. (2016) found that turmeric treatment has displayed an antibacterial effect on the growth of *Enterobacteriaceae* and Arulkumar et al. (2017) found that treatment with turmeric extract was highly effective in reducing *Enterobacteriaceae* population.

Effect of *Curcuma longa* 3% on total Staphylococci count CFU/g of smoked herring groups stored at 4°C.

Table (7): Effect of *Curcuma longa* 3% on total Staphylococci count CFU/g of smoked herring groups stored at 4 °C.

Weeks		(CG)	(GG)	(CLG)	(CLGG)
1 st week	Min- Max	$7 \times 10^2 - 13 \times 10^3$	$10 \times 10^2 - 17 \times 10^3$	$7 \times 10^2 - 6 \times 10^3$	$5 \times 10^2 - 12 \times 10^3$
	Mean \pm SE	$5.59 \times 10^3 \pm 2.12 \times 10^{3ab}$	$11.9 \times 10^3 \pm 3.65 \times 10^{3a}$	$3.12 \times 10^3 \pm 0.39 \times 10^{3b}$	$7.6 \times 10^3 \pm 1.25 \times 10^{3ab}$
4 th week	Min- Max	$8 \times 10^3 - 7 \times 10^4$	$16 \times 10^3 - 10 \times 10^4$	$4 \times 10^3 - 1 \times 10^4$	$9 \times 10^3 - 2 \times 10^4$
	Mean \pm SE	$2.4 \times 10^4 \pm 0.84 \times 10^{4ab}$	$8.4 \times 10^4 \pm 1.17 \times 10^{4a}$	$9 \times 10^3 \pm 3.45 \times 10^{3b}$	$5.36 \times 10^4 \pm 1.34 \times 10^{4a}$
8 th week	Min- Max	$6 \times 10^4 - 37 \times 10^5$	$12 \times 10^4 - 45 \times 10^5$	$4 \times 10^4 - 8 \times 10^5$	$6 \times 10^4 - 9 \times 10^5$
	Mean \pm SE	$17.5 \times 10^5 \pm 3.2 \times 10^{5ab}$	$22.48 \times 10^5 \pm 8.2 \times 10^{5a}$	$2.3 \times 10^5 \pm 0.35 \times 10^{5b}$	$2.8 \times 10^5 \pm 0.24 \times 10^{5b}$

(a,b,c) Means of the same rows carrying different superscript small letters are significantly different (< 0.05).

The illustrated data in table 7 declared that the Staphylococci count of smoked *Clupea harengus* after 1st week was $5.59 \times 10^3 \pm 2.12 \times 10^3$, $11.9 \times 10^3 \pm 3.65 \times 10^3$, $3.12 \times 10^3 \pm 0.39 \times 10^3$ and $7.6 \times 10^3 \pm 1.25 \times 10^3$ CFU/g in control group (CG), gutted group (GG), *curcuma longa* group (CLG) and *curcuma longa* gutted group (CLGG), respectively. Gradual increases in Staphylococci counts at the 4th week of storage $2.4 \times 10^4 \pm 0.84 \times 10^4$, $8.4 \times 10^4 \pm 1.17 \times 10^4$, $9 \times 10^3 \pm 3.45 \times 10^3$ and $5.36 \times 10^4 \pm 1.34 \times 10^4$ CFU/g in (CG), (GG), (CLG), and (CLGG), respectively. Finally at the 8th week of storage the Staphylococci count became $17.5 \times 10^5 \pm 3.2 \times 10^5$, $22.48 \times 10^5 \pm 8.2 \times 10^5$, $2.3 \times 10^5 \pm 0.35 \times 10^5$ and $2.8 \times 10^5 \pm 0.24 \times 10^5$ CFU/g in (CG), (GG), (CLG), and (CLGG), respectively.

Overall, significant differences in Staphylococci counts were noticed between the (CG) and treated groups (GG, CLG and CLGC) ($p < 0.05$). Thus, the results indicated that addition of *Curcuma longa* 3% were effective at inhibiting growth of Staphylococci. These results coincide to findings of **Handayani et al. (2018)** found that the treated fish using combination of turmeric and tamarind at different ratio. The concentration of turmeric (0%, 2%, and 6%) gave great reduction on the *S. aureus* population. Generally evisceration process and using of *Curcuma longa* 3% have great effects on the spoilage parameters indices, histamine formation, Enterobacteriaceae count, and Staphylococci count which considered as a promising solution to produce high quality smoked herring.

4. References:

- Akter, T., Ahmed, A.T.A., Khaleque, M.A. and Begum, M. (2013):** Effect of drying on the quality of tengra (*Mystus vittatus*) treated with turmeric and salt. Uniq. Res. J. Bio. Sci. 1, 1–5.
- American Public Health Association "APHA" (2002):** Compendium of methods for the microbiological examination of foods 4th Ed., APHA, Technical committee on Microbiological Methods for foods. Washington, D.C., USA.
- Arulkumar, A., Ramachandran, K., Paramasivam, S., Palanivel, R., & Miranda, J. M. (2017):** Effects of turmeric (*Curcuma longa*) on shelf life

- extension and biogenic amine control of cuttlefish (*Sepia brevimana*) during chilled storage. *CyTA-Journal of Food*, 15(3), 441-447.
- Basniwal, R.K., Butter, H.S., Jan, V.K., & Jain, N. (2011):** Curcumin nanoparticles: Preparation, characterization and antimicrobial study. *Journal of Agricultural and Food Chemistry*, 59, 2056–2061.
- Bensid, A., Ucar, Y., Bendeddouche, B., & Özogul, F. (2014):** Effect of the icing with thyme, oregano, and clove extracts on quality parameters of gutted and beheaded anchovy (*Engraulis encrasicolus*) during chilled storage. *Food Chemistry*, 145, 681-686.
- Brink, B. ten, Damink, C., Joosten H.M.L.J. and Huisin't Veld, J.H.J. (1990):** Occurrence and formation of biologically active amines in foods. *Int. J. Food Microbial*. 11. 73:84.
- Cai, L., Wu, X., Li, X., Zhong, K., Li, Y., & Li, J. (2014):** Effects of different freezing treatments on physicochemical responses and microbial characteristic of Japanese sea bass (*Lateolabrax japonicus*) fillets during refrigerated storage. *LWT-Food Science and Technology*, 59, 122–129.
- Egyptian organization for standardization (EOS)(2005):** Standard specifications for chilled and frozen fish fillets (3494) and (2- 889). Egypt.
- Egyptian Standardization "ES" (2007):** Physical and chemical methods for examination of fish and fish products: salted fish. Egyptian Organization for Standardization and Quality Control No. 4-2760/2007.
- European Commission (EC). (1995):** Commission of the European Community, Decision 95/ 149/EC of 8 March 1995 fixing the total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used. Brussels: CEC.
- FDA., (2001):** Processing Parameters Needed to Control Pathogens in Cold Smoked Fish., 2001, p.979.
- Food and Drug Administration (FDA) (1996):** Decomposition and histamine in raw, frozen tuna and mahi-mahi, canned tuna, and related species. Compliance Policy Guides 7108.240, Section 525:540.
- Gökodlu, N.; Özden, Ö. & Erkan, N. (1998):** Physical, chemical and sensory analyses of freshly harvested sardines (*Sardinapilchardus*) stored at 4°C. *J. Aquat. Food Prod. Technol.* 7: 5 - 15.
- Gomes, H.A., Silva, E.N., Nascimento, M.R.L. and Fukuma, H.T. (2003):** Evaluation of the 2-thiobarbituric acid method for the measurement of lipid oxidation in mechanically deboned gamma irradiated chicken meat. *Food Chem.* 80, 433–437.
- Gram, L. & Huss, H.H. (1996):** Microbiological spoilage of fish and fish products. *Inter. J. of Food Microbiol.*, 33 (1): 121-137.
- Gul, P., & Bakht, J. (2015):** Antimicrobial activity of turmeric extract and its potential use in food industry. *Journal of Food Science and Technology*, 52, 2272–2279.
- Handayani, B. R., Dipokusumo, B., Werdiningsih, W., Rahayu, T. I., & Sugita, D. L. (2018):** Microbial quality of yellow seasoned “pindang” fish treated with turmeric and tamarind. In *IOP Conference Series: Earth and Environmental Science* (Vol. 102, No. 1, p. 012019). IOP Publishing.



- Hussein, H. K. (2014) :** Biogenic amines in some fish products. M.V. Sc., Thesis (Meat Hygiene), Faculty of veterinary, zagazig university.
- Ienistea, C. (1973):** Significance and detection of histamine in food. In: Hobbs BC, Christian JHB, editors. The microbiological safety of foods. New York: Academic Press. P 327-43.
- Ikeme, A. I. (1986):** Extending the shelf-life of smoked mackerel. *FAO Fisheries Report (FAO)*.
- ISO (1999):** Horizontal method for the enumeration of coagulase positive *Staphylococcus aureus* and other species.
- ISO (2004):** Microbiology of food and animal feeding stuffs. Horizontal method for detection and enumeration of enterobacteriaceae, part 2: colony count method. Enumeration. ENISO, Geneva.
- Kim, M.K., Mah, J.H., & Hwang, H.J. (2009):** Biogenic amine formation and bacterial contribution in fish, squid and shellfish. *Food Chemistry*, 116, 87–95.
- Le Loir, Y.; Baron, F. and Gautier, M. (2003):** *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.*, 2(1): 63–76.
- Mackie, I.M.; Pirie, L.; Ritchie, A.H. and Yamanaka, H. (1997):** The formation of non-volatile amines in relation to concentration of free basic amino acid during postmortem storage of the muscle of scallop (*Pecten maximus*), herring (*Clupea harengus*) and mackerel (*Scomber scombrus*). *Food Chemistry*. 60. 291:295.
- Malle, P. & Tao, S.H. (1987):** Rapid quantitative determination of TMA using Steam distillation; *J. of Food Protection*; (9): 756 - 760.
- Maqsood, S. and Benjakul, S. (2010):** Synergistic effect of tannic acid and modified atmospheric packaging on the prevention of lipid oxidation and quality losses of refrigerated striped catfish slices. *Food Chem.* 121, 29–38.
- Özyurt, G.; Kuley, E.; Özkütük, S.; & Özoğul, F. (2009):** Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and gold band goat fish (*Upeneus moluccensis*) during storage in ice. *Food Chemistry*, 114: 505-510.
- Pankyamma, V., Somarajan, T., Ninan, G., Kuttanpillay Velayudhanelayodam, L., Abubacker Aliyamveetil, Z., & Puthanpurackal Kizhakkathil, B. (2016):** Effects of Turmeric Treatment and Smoking Duration on the Shelf Life of Ready to Cook Fillets from Sutchi Catfish during Chill Storage. *Journal of Food Process Engineering*, 39(5), 472-483.
- Ruiz-Capillas, C. & Moral, A. (2005):** Sensory and biochemical aspects of quality of whole big eye tuna (*Thunnus obesus*) during bulk storage in controlled atmospheres. *Food Chemistry*, 89(3), 347-354.
- Russell, F. E. and Maretic, Z. (1986):** Scombroid poisoning: Mini-review with case histories. *Toxicon*, 24.967:973.
- Schraufstätter, E., & Bernt, H. (1949):** Antibacterial action of curcumin and related compounds. *Nature*, 164(4167), 456.
- Suvanich, V.; Jahneke, M. & Marshall, D. (2000):** Changes in selected chemical quality characteristics of channel catfish frame mince during chill and frozen storage. *J. Food Sci.*, (65), 24-29.

- Taylor, S. L. (1986):** Histamine food poisoning: Toxicology and clinical aspects. Crit. Rev. Toxicol. 17. 91:128.
- Vaz-Pires, P., Seixas, P., Mota, M., Lapa-Guimaraes, J., Pickova, J., Lindo, A., & Silva, T. (2008):** Sensory, microbiological, physical and chemical properties of cuttlefish (*Sepia officinalis*) and broadtail shorfin squid (*Illexcoindetii*) stored in ice. LWT-Food Science and Technology, 41, 1655–1664.
- Viji, P., Binsi, P.K., Visnuvinayagam, S., Bindu, J., Ravishankar, C.N., & SrinivasaGopal, T.K. (2015):** Efficacy of mint (*Menthaarvensis*) leaf and citrus (*Citrus aurantium*) peel extracts as natural preservatives for shelf life extension of chill storage Indian mackerel. Journal of Food Science and Technology, 52, 6278–6289.
- Vyncke, W. (1970):** Direct determination of the thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. *Fette, Seifen, Anstrichmittel*, 72(12), 1084-1087.
- Ward, A.R. (1995):** Fish smoking in the tropics: A review. Trop. Sci. 35, 103–112.

تحسين الخصائص الكيميائية والبكتيرية فى الرنجة المدخنة

محمد عبدالله حسين، عبدالله فكرى محمود، نانيس سامى النجار و عادل ابراهيم العتبانى

قسم مراقبة الأغذية – كلية الطب البيطرى – جامعة الزقازيق – مصر – صندوق بريد ٤٤٥١١

فى هذا البحث تم تصنيع ٢٠ كيلوجرام من الرنجة المدخنة بأحد المصانع بمحافظة الشرقية والتي تم تقسيمها إلى أربعة مجموعات فى اثناء إعدادها للتدخين المجموعة الأولى الضابطة وتم تصنيعها عن طريق إضافة الملح فقط بنسبة ١٠% من وزن الأسماك، المجموعة الثانية وتم فقط إجراء عملية التجفيف للأسماك وإضافة ١٠% ملح، المجموعة الثالثة وتم إضافة الكركم تركيز ٣% وكذلك الملح تركيز ١٠% والمجموعة الرابعة تم تجفيف الأسماك وإضافة الكركم تركيز ٣% والملح ١٠% ثم تم إجراء عملية التدخين التقليدى فى الأفران المعدة لذلك. وتم تخزين الأربعة مجموعات فى ظروف التبريد عند ٤ درجة سليزية وفحص تلك المجموعات ظاهريا وكيميائيا وبكتيريا بعد مرور أسبوع واربعه أسابيع وثمانية أسابيع من التخزين. وبعد نهاية الأسبوع وجد أن متوسط قيم الفحص الحسى كانت ٣.٢٤ ± ٠.١ و ٣.١٢ ± ٠.١٢ و ٤.١٦ ± ٠.١٤ و ٣.٣١ ± ٠.١١ وكان الأس الهيدروجينى ٦.٨١ ± ٠.٢١ و ٦.٦٥ ± ٠.٢٣ و ٦.٥٩ ± ٠.٢٥ و ٦.٥٣ ± ٠.٢٤ وكانت متوسط قيم المثيل أمين الثلاثى ١٤.٤١ ± ٠.٥٤ و ١١.٢٤ ± ٠.٤٢ و ١٠.٢٤ ± ٠.٣٩ و ٩.٨٥ ± ٠.٣٨ مجم / ١٠٠ جم وكانت متوسط قيم حمض الثيوباربيتريك ٤.٩٨ ± ٠.٣١ و ٣.٨٥ ± ٠.٣٢، ٣.١٢ ± ٠.٢٤ و ٢.٥٤ ± ٠.٢٣ مجم مالون ألدهايد /كجم وبلغت نسبة الهستامين فى نهاية الأسبوع الثامن ٢٢.١ ± ١.٥٦ و ١٧.٣٢ ± ١.٤٩ و ١٦.٤٢ ± ١.٤٥ و ١٣.٩٥ ± ١.٣٥ مجم/ كجم لكل من المجموعة الضابطة، المجموعة المجوفة، المجموعة المعالجة بالكركم و المجموعة المجوفة مع المعالجة بالكركم علالتوالي. وبالفحص البكتيرى وجد أن عدد كان العدد الكلى للبكتريا المعوية ١٠ × ٢.١ ± ١٠ × ٤.٥، ١٠ × ١.٦ ± ١٠ × ٣.٦، ١٠ × ٠.٤٥ ± ١٠ × ١.٢١ و ١٠ × ٠.٠٩ ± ١٠ × ٠.٢٤، ١٠ × ٢.٣ ± ١٠ × ٠.٣٥ و ١٠ × ٢.٨ ± ١٠ × ٠.٢٤ خلية بكتيرية /جرام بعد نهاية الأسبوع الثامن لكل من المجموعة الضابطة، المجموعة المجوفة، المجموعة المعالجة بالكركم و المجموعة المجوفة مع المعالجة بالكركم على التوالي.