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Research Article

Technological Attempts for Production of Low Sodium Smoked Herring Fish (Renga)

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Abstract: In this study three technological attempts were applied to produce low sodium smoked herring fish with high quality. The first technological attempt was reducing time of salting from 48 to 24 h. The second attempt was using wet salting method with different concentration of brine solution (8, 15 and 26% NaCl) instead of dry salting method. The third attempt was replacement of sodium chloride with some salt replacers such as KCl, K-Lactate and mixture of them at different levels 20, 40 and 60%. Immediately after processing all treatments were packed in polyethylene bags under vacuum and then stored at 4ºC for 3 months. Treatments were evaluated chemically (Moisture content, salt, Na, K, pH value, TVBA, TMNA, TBA and PV), physically (WHC and plasticity), microbiologically (Total bacterial count, Psychrophilic, Halophilic, coliform, *Staphylococcus aureus, Salmonella spp*, *Clostridium botulinum* and yeast and mold counts) and organoleptically. Results suggested that salt replacers (KCl, K-Lactate and mixture of them) should be used until a level of 40 % and brine solution should be used at 15% NaCl and dry salting method for 24 h instead of 48 h to obtain low sodium smoked herring fish with high eating quality.

Keywords: Dry and wet salting, low sodium, salt replacers, Smoked herring fish

INTRODUCTION

Smoked herring fish is one of the most important processed fish products in Egypt, this product also known as "renga" is commercially produced by using three main steps, the first step dry salting with NaCl, the second step is partially air drying and the final step is smoking whether by using cold or hot smoking. The main problem in commercial product it is high salt or sodium content which lead to many health problems.

Sodium chloride is the most used food additive in the fish processing industry, mainly for preserving but also for improving the taste of the product. In fact, the current demand for salted fish is driven more by the aroma and flavour of the product than for preservation purposes (Mujaffar and Sankat, 2006). Sodium intake exceeds the nutritional recommendations in several industrialized countries. Excessive intake of sodium has been linked to hypertension (Law *et al*., 1991). High blood pressure may in turn increase the risk of stroke and premature death from cardiovascular diseases. Tuomilehto *et al*. (2001) found that high sodium intake correlated with mortality and risk of coronary heart disease, independent of other cardiovascular risk factors, including blood pressure. Because of the positive correlation between sodium intake and the incidence of hypertension in old people and those with hypertension and diabetes (Hermansen, 2000), there is a tendency to reduce sodium content by substituting part of NaCl by other substances.

Potassium chloride is probably the most common salt substitute used in low or reduced salt/sodium foods. However at blends over 50:50 sodium chloride/ potassium chloride in solution a significant increase in bitterness and loss of saltiness is observed. The partial substitution of NaCl by KCl appears to be the best alternative to reduce sodium content. Indeed both salts have similar properties and potassium intake has not been linked to the development of hypertension and cardiovascular diseases (Kimura *et al*., 2004; Geleijnse *et al*., 2007).

Potassium lactate is one of the possible substitutes for NaCl. K lactate improves colour, juiciness and tenderness, enhances flavor and extends shelf-life in meat products (Choi and Chin, 2003). However, small fermented sausages with different levels of NaCl substituted by K lactate had poorer consumer acceptability when substitution levels were above 30%.This could be related to the off-flavour produced by K-lactate, which was detected by trained panelists (Guàrdia *et al*., 2006).

Sodium chloride in smoked fish not only contributes to increasing its shelf-life, but also influences its Water Holding Capacity (WHC), fat binding, colour, flavour and texture; so total or partial substitution of NaCl might lead to changes in these attributes. For this reason, it is important to know how

Table 1: Description of smoked herring fish prepared in this study

Samples	Description	Technological attempts
Control	Dry salting method with NaCl for 48 h (as a commercial method)	-
T1	Dry salting method with NaCl for 24 h	Reducing salting time to 24h instead of 48 h
8 %	Herring fish were brined in solutions of 8%, 15% and 26% NaCl. The	Wet salting method with different
15%	herring fish were submerged in brine and kept at 4±1ºC for 48 h with	concentrations of brine solution instead
26%	the fish to brine 1: 3.	of dry salting method.
20% KCl	Dry salting with (80% NaCl+20% KCl) for 48 h.	Replacement of NaCl with some salt
40%KCl	Dry salting with (60% NaCl+40% KCl) for 48 h.	replacers such as (KCl, K-Lactate and
60% KCl	Dry salting with (40% NaCl+60% KCl) for 48 h	mixture of them) at levels of 20, 40 and 60%
20% K-Lactate	Dry salting with (80% NaCl+20% K-Lactate) for 48 h.	
40% K-Lactate	Dry salting with (80% NaCl+40% K-Lactate) for 48 h.	
60%K-Lactate	Dry salting with (80% NaCl+60% K-Lactate) for 48 h.	
20% (KCL+K-Lactate)	Dry salting with (80% NaCl+10% KCl+10% K-Lactate) for 48 h.	
40%(KCl+ K-Lactate)	Dry salting with (60% NaCl + 20% KCl +20% K-Lactate) for 48 h.	
60%(KCl+K-Lactate)	Dry salting with (40% NaCl + 30% KCl + 30% K-Lactate) for 48 h.	

Na replacement affects product quality, in order to establish the feasibility of NaCl reduction. The total or partial substitution of Na in smoked fish is not an easy task and implies the study and adaptation of the smoking process to the new product. The first stage in the smoking process which has to be adapted in the case of using new salt mixtures is salting. This stage must be tailored to ensure the proper uptake of the new salt. In accordance with safety aspects, it seems reasonable that the smoked product salted with the new salt mixture should have the same a_w as the traditional one. Another criterion that could be considered is that moisture in the smoked product should be close to the corresponding commercial product (Fuentes *et al*., 2010).

The aim of this study to produce reduced or low sodium smoked herring fish by using some technological attempts:

- Reducing time of sating from 48 to 24 h
- Using wet salting method with different concentrations of brine solution (8, 15 and 26% NaCl) instead of dry salting method
- Replacement of NaCl with KCl, K-Lactate and mixture of them at levels of 20, 40 and 60 %.

MATERIALS AND METHODS

Materials: Fish: Imported frozen herring fish (*Clupea harengus*) production of Ireland .It was obtained from a local fish and seafood wholesaler at Obor market, Cairo, Egypt.

Sodium chloride: Sodium chloride is a product of El-Nasr Company for Salinas. It was purchased from the private sector shop in the local market at Giza, Egypt.

Salt replacers: Potassium chloride (KCl): Food grade potassium chloride (Purity: 98.9%) was obtained from El-Gomhouria Co. for Trading pharmaceutica, Chemicals and Medical Equipments, Cairo, Egypt.

Potassium lactate (K-lactate): Food grade potassium lactate (98.7% purity) purchased from Morgan for Chemicals, Cairo, Egypt.

Methods:

Preparation of smoked herring fish: Fourteen smoked herring fish treatments were prepared in this study as shown in Table 1. Frozen herring fish were thawed overnight at 4±1°C and then washed with running water. Salting of thawed herring fish were carried out by both dry and wet salting methods. In wet salting method, herring fish were immersed in separate plastic containers containing 8, 15 and 26% (w/v) NaCl for 48 hr at 4±1°C with a fish: brine ratio of 1:3 (w/v). Also, dry salting was run at 4±1ºC for 48 h by inserting a salt layer with a fish layer. A layer of salt was placed at the bottom salting container then exchanged layers of fish and salt were placed and finally a layer of salt covered the fish. At the end of salting stage, desalting process was run by dipping of salted fish in tap water with a ratio of 1:1 (fish : water) for 30 min to remove the excessive salt on the surface of salted fish. Partial drying of desalted herring fish was achieved via the sun drying for 2 h, after that, smoking stage was applied by cold smoking method. It was carried out in a traditional smoke house. Hard wood sawdust (zan wood) was used to generate smoke. Smoking temperature was 30 -40ºC and smoking time was 10 h to obtain golden yellow color of smoke herring fish. All treatments were packaged in separately polyethylene bags under vacuum and stored at 4°C for three months. Samples were taken for analysis every month periodically.

Chemical analysis:

Moisture content: Moisture content was determined by drying of 5 g of minced smoked fish in a convection oven at 105 °C until constant weight (AOAC, 1995) Official method 985.14).

Salt content: Sodium chloride content in smoked fish samples was determined by volumetric method of Volhard (AOAC, 1995) Official method 937.09.

Sodium and potassium content: Na and K were determined in smoked fish samples by using Perkin El-Mer atomic absorption spectrophotometer (Modle 3300, USA) and this after the ashing of the sample according to the methods described by Hack (2000).

Determination of pH: pH value was estimated according to Goulas and Kontominas (2005) as follows. Ten gram of sample was homogenized in 100 ml of distilled water and the mixture was filtered. The pH of filtrate was measured using a pH meter (Jenway, 3510, UK) at ambient temperature.

Determination of total volatile basic nitrogen (TVB-N): Total Volatile Base Nitrogen (TVB-N) value was estimated by the semi-microdistillation procedure (AMC, 1979; Kirk and Sawyer, 1991). The bases are steam distilled into standard acid and back-titration with standard alkali.

Determination of trimethylamine nitrogen: Trimethylamine Nitrogen (TMAN) was determined using the above mentioned TVBN method after appropriate modification: formaldehyde was used to block the primary and secondary amines (AMC, 1979).

Determination of 2-Thiobarbituric Acid (TBA): 2-Thiobarbituric acid (TBA) value of smoked fish samples was determined color imetrically by using the method published by Kirk and Sawyer (1991).

Extraction of lipids and peroxide value: Lipid was extracted from the mixed smoked fish samples with a mixture of chloroform / methanol (2: 1 v/v) according to the method described by Folch *et al.* (1957). Peroxide value (PV) was expressed in units meq / kg lipid was determined by the titration method (Kirk and Sawyer, 1991).

Physical analysis: Water Holding Capacity (WHC) and plasticity of smoked herring were measured according to the filter - press method of Soloviev (1966).

MICROBIOLOGICAL ANALYSIS

Sample preparation: Twenty five gram of a representative and homogenized sample were mixed with 225 mL of sterile buffered 0.1% peptone water in a sterile blender, under sterile conditions, to give 1/10 dilution. Serial dilutions were prepared to be used for counting several types of bacteria and yeast and mold counts.

Bacteriological methods: Total Bacterial Count (TBC), *Staphylococcus aureus*, Coliform bacteria, Halophilic bacteria, Psychrophilic and yeast and mold counts of smoked fish were determined by using Nutrient agar, Baird-parker agar, MacConkey agar, halophilic agar, Nutrient agar and Potato Dextrose agar media, respectively according to the procedures described by APHA (1976). Incubations were carried out at 37ºC/48 h for TBC; at 37°C /24h for *Staphylococcus aureus* , Coliform and halophilic bacteria; at 7ºC/10 day for Psychrophilic and 25°C/5 day for yeasts and molds count.

Detection of salmonella: The presence or absence of salmonella was determined according to the methods described by FAO (1979) using buffered peptone as a pre-enrichment, while tetrathionate broth was used as a selective enrichment broth and S-S agar was used as a selective plating media.

Detection of Clostridium botulinum: This method is based on the detection of typical Gram positive *Bacilli* with subterminal oval spores grow on cooked meat medium and producing turbidity, gas production and digestion of the meat particles (FAO, 1979).

Organoleptic evaluation of smoked herring fish: Sensory characteristics of smoked herring fish were assessed by a panel of ten experienced panelists on the basis of a 10-point scale of each sample. The panelists were asked to evaluate several parameters (taste, appearance, odor and texture) of smoked fish treatments on a scale from 10 to 0 (Gelman *et al*., 1990). The scores were given in the decreasing order scale with 10-9 for excellent, 8-7 for good, 6-5 for fair and acceptable, 4-3 for poor and 2-1 for very poor. The mean of the scores given by the panel represented the overall sensory quality. A score less than 5 indicate that the smoked herring fish is rejected.

Statistical analysis: Data were subjected to Analysis Of Variance (ANOVA). The Least Significant Difference (LSD) procedure was used to test for difference between means (significance was defined at ($p<0.05$) as reported by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Organoleptic evaluation: The sensory properties of smoked herring fish as affected not only by salting time

Table 2: Sensory properties of smoked herring fish as affected by salting time, different concentrations of brine solution, and salt replacers

							Salt replacers								
				Brine solution %			KCl %			K-Lactate %			Mixture (Kcl+K-lactate)		
Sensory properties	Storage period	Control	T1	8	15	26	20	40	60	20	40	60	20	40	60
Taste	Zero time	8.50^{Acd}	9.20^{Aab}	8.0^{de}	9.50^{Aa}	8.80^{Abc}	8.90^{Aabc}	7.80^{Ae}	3.50^{fg}	8.70^{Abc}	7.50^{Ae}	3.0^{g}	9.0^{Aabc}	7.90^{Ade}	3.80^{f}
	3 month	6.0^{Bcd}	6.90^{Bb}	NE	7.60^{Ba}	6.30^{Bc}	7.30^{Bab}	5.50^{Bde}	NE	7.0^{Bb}	5.0^{Be}	NE	7.40^{Bab}	5.20^{Be}	NE
Odor	Zero time	8.30^{Aa}	8.50^{Aa}	8.20^{a}	8.60^{Aa}	8.30^{Aa}	8.50^{Aa}	8.40^{Aa}	8.0^{a}	8.40^{Aa}	8.20^{Aa}	8.10^{a}	8.60^{Aa}	8.40^{Aa}	8.10^{a}
	3 month	6.20^{Babc}	6.80^{Ba}	NE	6.70^{Bab}	6.10^{Bbc}	6.4^{Babc}	6.10^{Bbc}	NE	6.60^{Bab}	5.90^{Bc}	NE	6.50^{Babc}	6.10^{Bbc}	NE
Appearance	Zero time	8.0^{Aa}	8.30^{Aa}	8.10^{a}	8.50^{Aa}	8.10^{Aa}	8.20^{Aa}	8.10^{Aa}	8.10^{a}	8.20^{Aa}	8.10^{Aa}	8.0^{a}	8.30^{Aa}	8.20^{Aa}	8.0^{a}
	3 month	5.90^{Bd}	6.70^{Bab}	NE	7.0^{Ba}	6.00^{Bd}	6.80^{Ba}	6.20^{Bcd}	NE	6.6^{Babc}	6.10^{Bd}	NE	6.80^{Ba}	6.3^{Bbcd}	NE
Texture	Zero time	7.60^{Abc}	9.0^{Aa}	9.30^{a}	9.20^{Aa}	7.80^{Ab}	7.80^{Ab}	7.70^{Abc}	7.00^{cd}	7.70^{Abc}	7.60^{Abc}	6.90^{d}	7.70^{Abc}	7.5^{Abcd}	7.30^{bcd}
	3 month	5.10^{Bf}	7.20^{Bab}	NE	7.70^{Ba}	5.60^{Bef}	7.10^{Bab}	6.20^{Bde}	NE	7.0^{Bbc}	6.0^{Bde}	NE	7.30^{Bab}	6.40^{Bcd}	NE
Overall acceptability	Zero time	8.10^{Acd}	8.75^{Aab}	8.40^{bc}	8.95^{Aa}	8.25^{Acd}	8.35^{Abc}	8.0^{Acd}	6.65^{e}	8.25^{Acd}	7.85^{Ad}	6.50^{e}	8.40^{Abc}	8.0^{Acd}	6.80^{e}
	3 month	5.80^{Bb}	6.90^{Ba}	NE	7.25^{Ba}	6.0^{Bb}	6.90^{Ba}	6.0^{Bb}	NE	6.80^{Ba}	5.75^{Bb}	NE	7.0^{Ba}	6.0^{Bb}	NE

Where: Mean values in the same row (as a small letter) or column (as a capital letter) with the same letter are not significant different at 0.05 level; Control: prepared with dry salting method for 48 h; T1: prepared with dry salting method for 24 hs; NE: Not evaluated.

Table 3: Chemical and physical properties of smoked herring fish as affected by salting time, different concentrations of brine solution and salt replacers

							Salt replacers					
				Brine solution %			KCl %		K-Lactate %		KCl +K-lactate %	
Items	Storage period	Control	T1	8	15	26	20	40	20	40	20	40
Moisture	Zero time	53.67^{Ae}	57.63^{Ac}	60.91^{Aa}	59.0^{Ab}	56.46^{Ad}	54.31^{Aef}	53.11^{Aef}	53.17^{Aef}	52.98^{Aef}	53.0^{Aef}	52.71^{Af}
	3 month	50.23^{Bgh}	54.91^{Bc}	57.64^{Ba}	56.34^{Bb}	53.29^{Bd}	51.67^{Be}	51.03^{Bef}	50.76^{Bfg}	50.02^{Bh}	50.94^{Bfg}	50.21^{Bgh}
Nacl %	Zero time	7.84^{Aa}	4.25^{Ae}	3.63^{Af}	4.72^{Ae}	6.73^{Abc}	7.25^{Ab}	6.93^{Abc}	6.06^{Ad}	4.54^{Ae}	6.65^{Ac}	5.73^{Ad}
	3 month	8.35^{Aa}	4.54^{Ag}	3.97^{Ah}	5.15^{Af}	7.15^{Ac}	7.64^{Ab}	7.30^{Abc}	6.32^{Ad}	4.71^{Ag}	6.97^{Ac}	5.90^{Ae}
Na %	Zero time	3.28^{Aa}	1.83^{Ade}	1.57^{Ae}	2.02^{Ad}	2.91^{Aab}	2.54^{Abc}	1.91^{Ade}	2.49^{Ac}	1.87^{Ade}	2.45^{Ac}	1.96^{Ade}
	3 month	3.46^{Aa}	1.98^{Ad}	1.70^{Ae}	2.20^{Ad}	3.06^{Ab}	2.79^{Abc}	2.24^{Ad}	2.73^{Ac}	2.17^{Ad}	2.81^{Abc}	2.20^{Ad}
K %	Zero time	0.464^{Ae}	0.443^{Ae}	0.484^{Ae}	0.453^{Ae}	0.481^{Ae}	1.29^{Ac}	2.07^{Aa}	0.94^{Ad}	1.38^{Ac}	1.13^{Acd}	1.77^{Ab}
	3 month	0.745^{Af}	0.681^{Af}	0.72^{Af}	0.621^{Af}	0.711^{Af}	1.65^{Acd}	2.61^{Aa}	1.38^{Ae}	1.82^{Ac}	1.52^{Ade}	2.26^{Ab}
PH value	Zero time	6.09^{Aa}	6.02^{Aa}	6.14^{Aa}	6.10^{Aa}	6.06^{Aa}	5.97^{Aa}	5.90^{Aa}	5.82^{Aa}	5.79^{Aa}	6.03^{Aa}	6.00^{Aa}
	3 month	6.28^{Aa}	6.21^{Aa}	6.37^{Aa}	6.41^{Aa}	6.27^{Aa}	6.15^{Aa}	6.23^{Aa}	6.10^{Aa}	6.17^{Aa}	6.29^{Aa}	6.23^{Aa}
WHC*	Zero time	4.45^{Ba}	3.20^{Bef}	3.00^{Bf}	3.40^{Bde}	4.30^{Ba}	3.70^{Bbc}	3.95^{Bb}	3.80^{Bbc}	4.00^{Bb}	3.60^{Bcd}	3.90^{Bb}
	3 month	4.85^{Aa}	3.60^{Agh}	3.40^{Ah}	3.70^{Afg}	4.65^{Aab}	4.10^{Ade}	4.30^{Acd}	4.20^{Acd}	4.45^{Abc}	3.90^{Aef}	4.25^{Acd}
Plasticity	Zero time	1.90^{Af}	3.30^{Aab}	3.50^{Aa}	3.20^{Abc}	2.10^{Af}	3.00^{Acd}	2.90^{Ade}	2.80^{Ade}	2.70^{Ae}	3.00^{Acd}	2.80^{Ade}
	3 month	1.10^{Be}	3.00^{Ba}	3.0^{Ba}	2.80^{Bab}	1.30^{Be}	2.70^{Bbc}	2.60^{Bcd}	2.60^{Bbcd}	2.40^{Bd}	2.50^{Bcd}	2.50^{Bcd}

Where: Mean values in the same row (as a small letter) or column (as a capital letter) with the same letter are not significant different at 0.05 level; *: WHC: Water holding capacity (cm^2/0.3g) Control: prepared with dry salting method for 48 h; T1: prepared with dry salting method for 24 h

(48 and 24 h) and salting methods either dry or wet with different concentrations of brine solutions (8, 15 and 26%) but also by replacement of NaCl with KCl, K- Lactate and mixture of them at different levels (20, 40 and 60%) were presented in Table 2. From statistical analysis of these data, it could be noticed that, there were significant differences ($p<0.05$) in taste, texture and overall acceptability scores either at a zero time or after 3 months of cold storage. On the other hand, non significant differences ($p>0.05$) were recorded in odor and appearance scores between all treatments at only zero time.

The highest taste score (9.5) was recorded for smoked fish which salted with 15% brine solution followed by T1 prepared by dry salting method for 24 h; treatment which substituted NaCl with 20% mixture of KCl+K-Lactate (9.0) and finally 20% KCl (8.9) with non significant differences ($p>0.05$) between them. Moreover, taste score was significantly increased with increasing brine concentration from 8 to 15% but it was significantly decreased with increasing brine concentration from 15 to 26%. This may be due to higher salt content (strong salty taste) in the latter treatment compared with that prepared with 15% brine solution (Table 3) which led to decrease taste scores. Taste score was also affected by salting time, control sample prepared with dry salting method for 48 h had significantly lower taste score (8.50) compared with T1 prepared with dry salting for 24 h (9.20).

Also, from the same table, it could be observed that, taste score was significantly affected by types of salt replacers and their percentages. Partial replacement of NaCl with different salt replacers at level of 20% slightly improved the taste score of smoked herring fish in comparison to that of control sample. Taste scores of smoked herring fish decreased significantly by increasing substitution ratio from 20 to 60%. This may be due to the increase of bitterness as a result substitution with KCl and K- Lactate. The best substitution percent was 20% for all salt replacers followed by 40%. On the contrary, 60% substitution was refused by panelists for all salt replacers either KCl (3.5), K-Lactate (3.0) or mixture of KCl+K- Lactate (3.8) being poor products, consequently these treatments were rejected and neglected for other measurements.

Table 4: Total volatile nitrogen (mg N/100g) of smoked herring fish as affected by salting time, different concentrations of brine solution and salt replacers during cold storage

						Salt replacers					
			Brine solution %			KCl %		K-Lactate %		Kcl +K-lactate %	
Storage period	Control	T1	8	15	26	20	40	20	40	20	40
Zero time	13.65 ^{Dc}	15.06^{Db}	16.87^{Da}	15.72^{Db}	13.82^{Dc}	13.89 ^{Dc}	13.27 ^{Dc}	13.57^{Dc}	13.21 ^{Dc}	13.67 ^{Dc}	13.12 ^{Dc}
1	17.62^{Cd}	18.61^{Cc}	21.28^{Ca}	19.62^{Cb}	16.07^{Ce}	17.86^{Ccd}	16.05^{Ce}	16.25^{Ce}	15.87^{Ce}	16.57^{Ce}	16.43^{Ce}
2	21.67^{Bc}	24.28^{Bb}	27.56^{Ba}	24.10^{Bb}	20.36^{Bde}	21.18^{Bcd}	20.17^{Bde}	20.87^{Bcd}	19.62^{Be}	21.21^{Bcd}	20.27^{Bde}
3	24.71^{Ac}	29.15^{Ab}	40.63^{Aa}	28.56^{Ab}	24.37^{Ac}	24.07^{Ac}	23.68^{Acd}	23.89^{Ac}	22.78^{Ad}	24.38^{Ac}	22.62^{Ad}

Where: Mean values in the same row (as a small letter) or column (as a capital letter) with the same letter are not significant different at 0.05 level; Control: prepared with dry salting method for 48 h; T1: prepared with dry salting method for 24 h

Table 5: Trimethylamine (mg N/100g) of smoked herring fish as affected by salting time, different concentrations of brine solution and salt replacers during cold storage

						Salt replacers					
			Brine solution %			KCl %		K-Lactate %		Kcl +K-lactate %	
Storage period	Control	T1	8	15	26	20	40	20	40	20	40
Zero time	1.32^{Da}	1.68^{Da}	2.01^{Da}	1.77^{Da}	1.47^{Da}	1.57^{Da}	1.48^{Da}	1.31^{Da}	1.29^{Da}	1.61^{Da}	1.42^{Da}
1	3.79^{Ccd}	4.28^{Cb}	5.51^{Ca}	4.31^{Cb}	3.84^{Cc}	3.52^{Ccde}	3.56^{Ccde}	3.35^{Cde}	3.27^{Ce}	3.87^{Cc}	3.71^{Ccde}
2	6.41^{Bcd}	7.51^{Bb}	9.65^{Ba}	7.36^{Bb}	6.27^{Bcd}	6.46^{Bc}	6.17^{Bcd}	5.99^{Bcd}	5.83^{Bd}	6.31^{Bcd}	6.10^{Bcd}
3	8.96^{Ac}	9.82^{Ab}	14.67^{Aa}	9.73^{Ab}	8.92^{Ac}	8.68^{Acd}	8.53^{Acd}	8.10^{Ade}	7.88^{Ae}	8.45^{Acde}	8.26^{Ade}

Where: Mean values in the same row (as a small letter) or column (as a capital letter) with the same letter are not significant different at 0.05 level; Control: prepared with dry salting method for 48 h; T1: prepared with dry salting method for 24 h

Furthermore, salting time (24 and 48 h) and salting methods (dry or wet salting with different brine concentrations) as well as salt replacers did not affect the odor and appearance of smoked herring fish immediately after processing. The highest odor (8.6) and appearance (8.5) scores were recorded for treatment which salted with 15% brine solution. On the other hand, the lowest scores of these parameters were given by panelists for treatments which replaced NaCl with all salt replacers at a level of 60%. Generally, no significant differences ($p>0.05$) were recorded in both odor and appearance scores between all treatments immediately after processing.

Texture scores of smoked herring fish were significantly affected by salting time and brine concentration but not affected by types of replacers and their percentages up to 40% level. The highest texture score (9.3) was given for treatment which salted with 8% brine solution followed by 15% (9.20) and finally treatment prepared by dry salting for 24 h with non significant differences ($p>0.05$). The above mentioned treatments had significantly higher texture score (more tender) when compared with control sample, due to lower moisture content in control sample (Table 3) which made smoked fish texture firmer. Texture of fish muscle has been related to water content and lipid content. Dunajski (1979) and Jittinandana *et al*. (2002) stated that fish higher in lipid or water content were softer in texture. Also, from the same table, it could be observed that, no significant differences ($p>0.05$) were recorded in texture score between control sample and salt replacers treatments until NaCl had been replaced to a level of 40% by K-Lactate and 60% by KCl, or mixture of them. Texture scores of salt replacers treatments were slightly decreased with increasing replacement ratio from 20 to 60%.

Overall acceptability scores of smoked herring fish were increased significantly with decreasing salting time from 48 to 24 h and brine concentration from 26 to 15%. On the other hand, overall acceptability did not affected by replacement of NaCl with any type of salt replacers up to 40% level but it was significant decreased by increasing replacement ratio to 60%. Generally, the highest value of overall acceptability was recorded for smoked herring fish prepared with 15% brine solution (8.95) followed by treatment prepared by dry salting method for 24 h (8.75) with non significant differences between them. Meanwhile, the lowest values of overall acceptability were recorded for treatments which replaced NaCl with all salt replacers at a level of 60%. This results partially agreement with those obtained by Fuentes *et al*. (2011) reported that partial sodium replacement (50% NaCl: 50% KCl) did not affect sensory evaluation of smoked sea bass.

All sensory properties were significantly decreased with advancement of cold storage period for all treatments. Concerning, the evaluation of smoked herring fish prepared 8% brine solution, sensory properties did not carried out at the end of cold storage (3 months), because this treatment might be unsafe for human consumption and rejected due to the deterioration in their chemical parameters such as TVN and TMN (Table 4 and 5) which showed higher than the permissible limits as reported by EEC (1995) and Egyptian standards (2005).

Results of organoleptic evaluation suggest that salt replacers (KCl, K-Lactate and mixture of them) should

be used until a level of 40% and brine solution should be used at 15% NaCl and dry salting method for 24 hr instead of 48 h to obtain low sodium smoked herring fish with high eating quality.

Chemical and physical properties of smoked herring fish: Data presented in Table 3, illustrate chemical and physical properties of smoked herring fish as affected by salting time, different concentrations of brine solution as well as salt replacers and their percentages. From these results it could be noticed that, there were significant differences ($p<0.05$) in moisture content between treatments at either a zero time or after 3 months of cold storage. Moisture content of smoked herring fish ranged from 52.71 to 60.91% immediately after processing. Industrial specifications for smoked finished products generally recommended water content in the fish flesh of less than 65% (Cardinal *et al*., 2001). This is in agreement with our values.

Salting time and wet salting method with different concentrations of brine had significant effects on the moisture content of smoked herring fish. The moisture content significantly decreased from 57.63 to 53.67% when the time of salting increased from 24 to 48 h. This is in accordance with the results obtained by Birkeland and Bjerkeng (2005) found that dry matter of cold smoked fillets was increased significantly by increasing the time of salting. Also, moisture content of smoked herring fish significantly decreased with increasing brine concentration from 8 to 26%. Similar results were recorded by Jittinandana *et al*. (2002) reported that, increasing the brine concentration decreased ($p<0.05$) moisture content of rainbow trout fillets. Moisture content was significantly higher in smoked fish prepared with 8% brine solution (60.91%) than those in other treatments. This may be due to higher salts concentration (26%) and dry salting leads to more protein denaturation, resulting in rapidly loss water due to the salting out process and reduced water holding capacity.

Moisture content did not affected by the type of salt replaces and their percentages. Smoked herring fish treatments replaced NaCl with KCl, K-Lactate and mixed of them at a level of 20 and 40% showed slightly lower moisture content than that of control sample without significant differences between them. Moreover, moisture content of all treatments was decreased significantly with advancement of cold storage period. This decrease may be due to the drip loss and partially the evaporation of moisture through the polyethylene bags which were used for smoked fish packing.

Also, from the same table, it could be notice that, salt content of smoked herring fish significantly decreased from 6.73 to 3.63% with decreasing brine concentration from 26 to 8% and also significantly decreased from 7.84 to 4.25% by decreasing salting time from 48 to 24 h. Birkeland and Bjerkeng (2005) reported that salt content of cold smoked fillets was increased significantly by increasing the time of salting. Moreover, salt replacers KCl, K-Lactate or mixture of them had significantly effects on salt content of smoked herring fish. Partial replacement of NaCl with K-Lactate at a level of 20 and 40% had higher effect on reduction of NaCl content when compared with KCl or mixture of KCl+K-Lactate at the same levels, may be explained on the basis that the used method for determination of NaCl depends on assessment of Cl (and not the Na); at the same time Cl is present in both NaCl and the substitute salt e.g., the KCl and mixture of KCl+K-Lactate, leading thereby, to the apparent increase (not true) of determined NaCl in low sodium treatments. Generally, lower percentage of NaCl was recorded in treatment prepared with 8% brine solution (3.63%). On the other hand, control sample and treatment prepared with 26% had significantly higher salt content compared with other treatments. Jittinandana *et al*. (2002) found that salt content of products soaked in higher brine concentration was greater than of those from the lower brine concentration for the same brining time.

Sodium and potassium contents of smoked herring fish ranged from 1.57 to 3.28% and 0.443 to 2.07% respectively immediately after processing. The highest Na content (3.28%) was recorded for control sample followed by treatment salted with 26% brine solution (2.91%) with non significant between them. Sodium content was decreased significantly with decreasing not only sating time and brine concentration but also by partial replacement of NaCl with salt replacers. The highest decrement was recorded for treatment prepared with 8% brine solution (52.13%) followed by T1 treatment prepared with dry salting for 24 h (44.20%) and treatments which replaced NaCl with both K-Lactate (42.99%) ; mixture of KCl+K-Lactate (41.77%) and KCl (40.24%) at a level of 40% when compared with control sample. Moreover, sodium content was also decreased significantly with increasing replacement ratio from 20 to 40% in all salt replacers treatments.

Potassium content was significantly affected by type of salt replacers and their percentages but not affected by either salting time or brine concentrations. Partial replacement of NaCl with KCl resulted higher potassium content than replacement of NaCl with K-Lactate. This may be due to higher potassium level in KCl (52.7%) than in K-Lactate (30.40%). Moreover, by advancement of cold storage time, the levels of NaCl, Na and K of all treatments slightly increased which is possibly due to the loss in moisture content and according by increases in dry matter including the salt.

The initial pH value of smoked herring fish was ranged from 5.79 to 6.14 indicating the freshness of all samples. These values are partially in agreement with that of Goulas and Kontominas (2005) found a pH value of 6.12 for smoked chub mackerel. No significant differences ($p>0.05$) were recorded in pH values between all treatments at either a zero time or after 3 months of cold storage. Moreover, pH values of all treatments were slightly increased with increasing storage time. The increase in pH may be attributed to production of volatile basic components such as ammonia, trimethylamine and total volatile nitrogen by fish spoilage bacteria (Ruiz-Capillas and Moral, 2005).

Water holding capacity and plasticity values of smoked herring fish ranged from 3.0 to 4.45 and 1.95 to 3.50 cm^2/0.3 gm, respectively immediately after processing (Table 3). A statistically significant ($p<0.05$) lower water holding capacity and plasticity were found in control sample and treatment prepared with 26% brine with non significant differences between them versus the smoked fish treatments prepared with 8, 15% brine solution and that prepared with dry salting method for 24 hr. These results are in agreement with those reported by Gallart-Jornet *et al*. (2007) who found that higher salts concentration (25%) and dry salting leads to more protein denaturation, (the myofibrillar protein rapidly loose water due to the salting out process) resulting in changes in texture and reduced water holding capacity.

Water holding and plasticity values were significantly decreased with increasing brine concentration from 8 to 26%. The presence of high concentrations of salt in muscle gradually increases the water holding capacity obtaining a maximum at an ionic strength of 1M (≈ 5.8% salt) as reported by Offer and Knight (1988). At high ionic strengths, water holding capacity decreases, apparently by a salting-out effect due to water binding by the salt and concurrent dehydration of the protein (Martínez-Alvarez *et al*., (2005). Replacement of NaCl with all salt replacers KCl, K-Lactate and mixture of them) at a level of 20 and 40% led to improve water holding capacity and plasticity when compared with control sample. By increasing replacement ratio from 20 to 40% WHC and plasticity slightly decreased in all salt replacers treatments. Also, WHC and plasticity in all treatments significantly decreased with increasing cold storage time.

Total Volatile Bases Nitrogen (TVBN): TVBN is widely used as an indicator of fish spoilage, its increase is related to the activity of spoilage bacteria and endogenous enzymes (Ozogul *et al*., 2004; Ruiz-Capillas and Moral, 2005). Changes in total volatile nitrogen of smoked herring fish as affected not only by time of salting and different concentrations of brine solution but also by salt replacers and their percentages during cold storage are given in Table 4. TVBN of different smoked herring fish treatments ranged from 13.12 to 16.87 mg N/100g being non significant differences ($p>0.05$) not only between control sample and treatments prepared by replacement of NaCl with all salt replacers but also between the above-mentioned treatments and smoked fish treatment prepared with 26% brine concentration immediately after processing. Also, TVBN of smoked herring fish significantly decreased with increasing both of brine concentration and salting time either at a zero time or along of cold storage period. This is associated with lower moisture content and higher salt level which reducing spoilage bacteria growth and activity of endogenous enzymes. These results are in agreement with that reported by Yanar *et al*. (2006) found that increased salt concentration had a positive effect on reduction of TVN of hot smoked tilapia during cold storage.

As time of cold storage progressed the total volatile nitrogen values of all treatments increased significantly. This increase is expected because it is related to bacterial spoilage (Connell, 1990; Goulas and Kontominas, 2005). TVBN of smoked herring fish sample prepared with 8% brine reached the value of 40.63mgN/100g after 3 months of cold storage, exceeding the upper acceptability limit set the EU (EEC, 1995) and Connell (1990) for TVN values of fish (35 mg N/100 g fish flesh). On the other hand, TVN of other smoked herring treatments remained lower than the acceptability limit at any time of cold storage.

Generally, salt replacers led to slight decrease in TVBN values for treatments when compared with control during cold storage. Also, K-Lactate was more effective on TVN reduction when compared with KCl. This may be due to lactates exhibit antimicrobial properties against non pathogenic (Chen and Shelef, 1992) and pathogenic (Miller and Acuff, 1994) microflora as well as delay growth of meat spoilage microorganisms (Brewer *et al*., 1995).

Trimethylamine Nitrogen (TMAN): TMAN is produced from Trimethylamine Oxide (TMAO) possible partly by action of intrinsic enzymes but certainly through bacterial action, is the main component responsible for a pleasant "fishy" odour (Rodriguez *et al*., 1999; Shakila *et al*., 2003). Changes in TMAN of different smoked herring fish treatments during cold storage are shown in Table 5. From statistical analysis of these data it could be noticed that, no significant differences ($p>0.05$) were recorded in TMAN values (ranged from 1.29 to 2.01 mg N/100 g) between all treatments immediately after processing.

After 1 month of cold storage TMAN value of smoked herring fish treatment prepared with low salt concentration (8% brine) was significantly ($p<0.05$)

Table 6: Thiobarbituric acid (mg malonaldhyde / kg) of smoked herring fish as affected by salting time, different concentrations of brine solution and salt replacers during cold storage

						Salt replacers					
			Brine solution %			KCl %		K-Lactate %		(Kcl+K-lactate) %	
Storage period	Control	T1	8	15	26	20	40	20	40	20	40
Zero time	1.85^{Ca}	0.93^{Db}	0.51^{Cc}	0.86^{Cb}	1.62^{Da}	1.77^{Ca}	1.69^{Ca}	1.61^{Da}	1.58^{Ca}	1.65^{Ca}	1.51^{Da}
1	2.98^{Ba}	1.37^{Cd}	0.76^{Ce}	1.18^{Cd}	2.87^{Ca}	2.81^{Ca}	2.67^{Bab}	2.42^{Cbc}	2.29^{BCc}	2.65^{Cabc}	2.61^{Cabc}
2	3.88^{Aa}	1.97^{Bc}	1.36^{Bd}	1.89^{Bc}	3.75^{Ba}	3.80^{Ba}	3.72^{ABa}	3.16^{Bb}	3.11^{Bb}	3.37^{Bb}	3.30^{Bb}
3	4.32^{Aa}	2.62^{Ad}	1.89^{Ae}	2.46^{Ad}	4.07^{Aab}	4.26^{Aa}	4.19^{Aab}	3.62^{Ac}	3.61^{Ac}	3.87^{Aabc}	3.76^{Abc}

Where: Mean values in the same row (as a small letter) or column (as a capital letter) with the same letter are not significant different at 0.05 level; Control: prepared with dry salting method for 48 h; T1: prepared with dry salting method for 24 h

Table 7: Peroxide value (meq /kg) of smoked herring fish as affected by salting time, different concentrations of brine solution and salt replacers during cold storage

						Salt replacers					
			Brine solution %			KCl %		K-Lactate %		(Kcl+K-lactate) %	
Storage period	Control	T1	8	15	26	20	40	20	40	20	40
Zero time	3.87^{Da}	3.26^{Dbc}	2.92^{Dc}	3.32^{Db}	3.78^{Da}	3.79^{Da}	3.68^{Dab}	3.65^{Cab}	3.59^{Dab}	3.61^{Dab}	3.56^{Cab}
1	5.41^{Ca}	4.29^{Cc}	3.57^{Cd}	4.67^{Cbc}	4.98^{Cab}	5.39^{Ca}	5.72^{Ca}	4.92^{Bab}	4.67^{Cbc}	4.86^{Cab}	4.71^{Bb}
2	7.61^{Ba}	5.86^{Bcd}	4.52^{Be}	5.43^{Bd}	6.87^{Bb}	7.43^{Ba}	7.24^{Bab}	6.30^{Bc}	6.18^{Bc}	6.02^{Bc}	5.84^{Bcd}
3	10.77^{Aa}	7.20^{Ae}	5.85^{Aj}	6.55^{Af}	9.67^{Ab}	10.50^{Aa}	10.57^{Aa}	8.68^{Acd}	8.39^{Ad}	9.10^{Ac}	8.69^{Acd}

Where: Mean values in the same row (as a small letter) or column (as a capital letter) with the same letter are not significant different at 0.05 level; Control: prepared with dry salting method for 48 h; T1: prepared with dry salting method for 24 h

higher and increased with a significantly higher than the corresponding values of all other treatments throughout cold storage period, indicating the preservative effect of higher salt concentration and salt replacers. This effect is due to the decrease water activity (a_w) and thus prevention of growth of many spoilage microorganisms (Leroi *et al*., 2000). TMAN of treatment prepared with 8% brine reached the value of 14.67 mg N/100 g after 3 months of cold storage, exceeding the upper acceptability limit set by Egyptian Standards (2005) for TMNA values of smoked fish (10 mg N/100 g). Meanwhile, TMAN values of all other treatments not exceeded this limit at any time of cold storage.

From the same table, it could be noticed that, TMNA values were significant decreased with increasing brine concentration from 8 to 26 % and salting time from 24 to 48 hr. In this concern, Hansen *et al*. (1995) reported that decreasing salt levels resulted higher concentration of TMNA in cold-smoked salmon. Moreover, at any time of cold storage, treatments which prepared with replacement of NaCl with salt replacers at a levels of 20 and 40% were slightly lower TMNA when compared with both control sample and treatment prepared with 26 brine solution being non significant differences ($p>0.05$). Similar results were obtained by Fuentes *et al*. (2011) who reported that partial sodium replacement with KCl did not affect trimethylamine of smoked sea bass. Also, lower TMNA values of K-Lactate treatments in comparison with the KCl treatments and other treatments may be attributed to antibacterial properties of K-Lactate as above reported for TVBN values.

Lipid oxidation: To evaluate the degree of lipid oxidation, the Thiobarbituric Acid (TBA) and Peroxide Value (PV) were determined. Changes in TBA and PV of smoked herring fish as affected by salting time and different concentrations of brine solution as well as salt replacers during cold storage are shown in Table 6 and 7. TBA and PV of all smoked fish treatments ranged from 0.51 to 1.85 mg malonaldhyde / kg sample and 2.92 to 3.87 meq /kg respectively immediately after processing. From statistical analysis of these data, it could be noticed that no significant differences ($p>0.05$) in both TBA and PV were observed not only between control sample and smoked herring fish prepared with 26% brine but also between the abovementioned treatments and smoked treatments prepared by replacement of NaCl with all salt replacers at a levels of 20 and 40%.

Moreover, TBA and PV of smoked herring fish were increased significantly with increment brine concentration and salting time either at a zero time or throughout storage period. These results are in agreement with that obtained by Augbourg and Ugliano (2002) and Yanar *et al*. (2006) point out that increasing salting concentration in brine accelerates the rate of peroxide and TBA values in salted horse mackerel and hot-smoked tilapia during storage. Also, TBA and peroxide values not significantly affected by replacement of NaCl with KCl either at immediately after processing or during cold storage but significantly decreased by replacement of NaCl with K-Lactate during cold storage. This may be attributed to antioxidants properties of K-Lactate (Tan and Shelef, 2002; Sallam, 2007).

TBA and peroxide values significantly increased during cold storage ($p<0.05$) in all treatments. At the end of cold storage (after 3 months) the highest values of PV (10.77 meq/kg) and TBA (4.32 mg malonaldhyde/kg) were recorded for control sample. On the other hand, the lowest values were recorded for

Table 8: Total bacterial count (*cfu/gm*) of smoked herring fish as affected by salting time, different concentrations of brine solution and salt replacers during cold storage

						Salt replacers					
			Brine solution %			KCl %		K-Lactate %		(Kcl+K-lactate) %	
Storage period	Control	T1	8	15	26	20	40	20	40	20	40
Zero time	6.24×10^{3}	1.82×10^{4}	2.70×10^{5}	4.61×10^{4}	8.27×10^{3}	7.76×10^{3}	6.17×10^{3}	4.75×10^{3}	1.17×10^{3}	4.63×10^{3}	3.95×10^{3}
1	8.27×10^{4}	3.29×10^{5}	8.71×10^{5}	5.27×10^{5}	9.27×10^{4}	9.26×10^{4}	7.96×10^{4}	1.13×10^{4}	5.62×10^{3}	2.16×10^{4}	9.39×103
2	3.78×10^{5}	9.82×10^{5}	7.68×10^{6}	1.21×10^{6}	5.32×10^{5}	4.67×10^{5}	3.21×10^{5}	7.21×10^{4}	1.36×10^{4}	6.0×10^{4}	5.87×10^{4}
3	1.52×10^{6}	3.56×10^{6}	5.17×10^{7}	4.48×10^{6}	2.21×10^{6}	1.91×10^{6}	1.36×10^{6}	2.88×10^{5}	4.68×10^{4}	4.01×10^{5}	2.1810^{5}

Control: prepared with dry salting method for 48 h; T1: prepared with dry salting method for 24 h

Table 9: Psychrophilic bacteria (*cfu/gm*) of smoked herring fish as affected by salting time, different concentrations of brine solution and salt replacers during cold storage

						Salt replacers					
			Brine solution %			KCl %		K-Lactate %		(Kcl+K-lactate) %	
Storage period	Control	T1	8	15	26	20	40	20	40	20	40
Zero time	6.0×10^{1}	7.0×10^{1}	1.0×10^{2}	8.5×10^{1}	5.5×10^{1}	6.0×10^{1}	5.5×10^{1}	4.5×10^{1}	3.0×10^{1}	6.0×10^{1}	5.0×10^{1}
1	$6.75\times x10^{2}$	9.87×10^{2}	4.26×10^{3}	1.11×10^{3}	5.32×10^{2}	6.22×10^{2}	5.86×10^{2}	3.87×10^{2}	3.13×10^{2}	4.20×10^{2}	$3.61x10^{2}$
2	5.97×10^{3}	8.21×10^{3}	1.35×10^{4}	7.56×10^{3}	4.81×10^{3}	5.64×10^{3}	4.97×10^{3}	3.25×10^{3}	1.99×10^{3}	3.73×10^{3}	$2.89x10^{3}$
3	2.67×10^{4}	3.42×10^{4}	8.12×10^{4}	5.24×10^{4}	2.78×10^{4}	1.98×10^{4}	1.02×10^{4}	9.11×10^{3}	7.34×10^{3}	9.81×10^{3}	$7.15x10^{3}$

Control: prepared with dry salting method for 48 h; T1: prepared with dry salting method for 24 h

Table 10: Halophilic bacteria (*cfu/gm*) of smoked herring fish as affected by salting time, different concentrations of brine solution and salt replacers during cold storage

						Salt replacers					
			Brine solution %			KCl %		K-Lactate %		(Kcl+K-lactate) %	
Storage period	Control	T1	8	15	26	20	40	20	40	20	40
Zero time	5.96×10^{2}	9.51×10^{2}	4.30×10^{3}	8.62×10^{2}	6.76×10^{2}	5.62×10^{2}	4.87×10^{2}	4.61×10^{2}	4.10×10^{2}	4.5010^{2}	3.65×10^{2}
1	1.87×10^{3}	6.80×10^{3}	9.18×10^{3}	3.20×10^{3}	3.16×10^{3}	1.69×10^{3}	9.23×10^{2}	7.70×10^{2}	6.20×10^{2}	7.20×10^{2}	6.90×10^{2}
2	5.13×10^{3}	1.25×10^{4}	3.27×10^{4}	9.17×10^{3}	7.71×10^{3}	6.37×103	5.92×10^{3}	1.85×10^{3}	8.50×10^{2}	1.23×10^{3}	9.26×10^{2}
3	1.56×10^{4}	6.67×10^{4}	9.37×10^{4}	4.81×10^{4}	2.17×10^{4}	2.17×10^{4}	1.37×10^{4}	4.06×10^{3}	1.97×10^{3}	4.37×10^{3}	2.37×10^{3}

Control: prepared with dry salting method for 48 h; T1: prepared with dry salting method for 24 h

Table 11: Total yeasts and molds count (*cfu/gm*) of smoked herring fish as affected by salting time, different concentrations of brine solution and salt replacers during cold storage

						Salt replacers					
			Brine solution %			KCl %		K-Lactate %		(Kcl+K-lactate) %	
Storage period	Control	T1	8	15	26	20	40	20	40	20	40
Zero time	ND	1.0×10^{1}	4.0×10^{1}	2.0×10^{1}	ND	ND	ND	ND	ND	ND	ND
1	ND	4.0×10^{1}	9.5×10^{1}	7.0×10^{1}	ND	ND	ND	ND	ND	ND	ND
2	ND	1.15×10^{2}	$2.10\ 10^{2}$	1.68×10^{2}	ND	ND	ND	ND	ND	ND	ND
3	ND	1.95×10^{3}	2.85×10^{3}	2.30×10^{3}	ND	ND	ND	ND	ND	ND	ND

Control: prepared with dry salting method for 48 h; T1: prepared with dry salting method for 24 h; ND = Not detected

smoked herring fish prepared with low salt concentration (8% brine). Finally, TBA values of all treatments were lower than the general TBA limit for smoked fish as mentioned by Egyptian Standards (2005) which reported that TBA values not exceed 4.5 malonaldhyde/kg.

Microbiological quality of smoked herring fish: Microbial load represented in Total Bacterial Count (TBC), Psychrophilic Bacteria (PsB), Halophilic Bacteria (HB) and yeast and mold counts (*cfu/gm*) of smoked herring fish as affected not only by salting time and different concentrations of brine solution but also by type of salt replacers and their percentages during cold storage is shown in Table 8, 9, 10 and 11. Initial counts of total bacterial, psychrophilic, halophilic bacteria and yeast and mold of all smoked fish treatments ranged from 1.17×10^{3} to 2.70×10^{5}, 3.0×10^{1} to 1.0×10^{2} , 3.65×10^{2} to $4.30\times10^{3}$and 0.0 to 4.0×10^{1} *cfu/gm*, respectively. The low initial counts of abovementioned microorganisms indicating good levels of hygiene during handling and processing. The highest microbial growth (TBC, Psy B and HB) was recorded for smoked herring fish prepared with 8% brine when compared with other treatments at any time of cold storage.

Also from the same tables, it could be noticed all microorganisms (TBC, Psy B and HB) were decreased with increment of brine concentration from 8 to 26% and salting time from 24 to 48 h. This may be due to increase salt content by increasing brine concentration or salting time (Table 3) which led to more decrease in water activity (a_w) and thus prevention of growth of many spoilage microorganisms (Leroi, *et al*., 2000).

Smoked herring fish treatments prepared by replacement of NaCl with K-Lactate or mixture of (KCl+K-Lactate) were lower microbial growth (TBC, Psy B and HB) when compared with control sample. This may be due to antimicrobial properties of K-Lactate (Brewer *et al*., 1995; Tan and Shelef, 2002). Meanwhile replacement of NaCl with KCl had no effect on reduction of microbial growth.

From the same data, it could be observed that all microorganisms (TBC, Psy B and HB) counts were also affected by cold storage time. By advancement of cold storage, the TBC, PsyB and HB counts were increased in all treatments. These results are in line with those obtained by Fuentes *et al.* (2011) who reported that, mesophilic bacteria, enterobacteriaceae and H_2S-producing bacteria counts in smoked sea bass increased during cold storage. At the end of cold storage (after 3 months) it could be noticed that smoked herring fish prepared with 8% brine solution had the highest TBC (9.15×10^7 cfu/g), psychrophilic bacteria (8.12×10^4 cfu/g) and halophilic bacteria (9.37×10^4 cfu/g) in comparison with other treatments.

All smoked herring fish treatments except T1 and treatments prepared with 8 and 15% brine solution were completely free from yeast and mold (Table 11). The highest count of yeast and mold (40 cfu/g) was recorded for smoked herring fish prepared with 8% brine solution immediately after processing. Also, yeast and mold counts of smoked herring fish increased with increasing cold storage time.

Finally, all smoked herring fish treatments were completely free from colifrom bacteria, *staphylococcus aureus*, *salmonella spp* and *clostridium spp* either at a zero-time or along cold-storage periods, indicating good level of hygiene during the handling, processing and storage.

CONCLUSION

Salt replacers (KCl, K-Lactate and mixture of KC+K-Lactate) should be used until a level of 40% and brine solution should be used at 15% NaCl and dry salting method for 24 h instead of 48 h to obtain low sodium smoked herring fish with high eating quality.

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