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THE EFFECT OF USING ANTIOXIDANTS, ANTIFUNGAL AGENTS AND PACKAGING METHODS ON THE QUALITY OF SMOKED MACKEREL FISH.

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ABSTRACT

Mackerel fish are considered one of the main raw fish to manufacture smoked fish products. Mackerel fish were prepared as butterfield shape, dry salting was carried out, potassium sorbate (PS), and natamax (NM) were used as antifungal agents, while sodium erythobate (SE) was added as an antioxidant. Processing of cold smoking, both normal and vacuumed package were achieved and then cold storage at 4°C up to 60 days was done.

Vacuumed package samples showed low moisture loss, low salt concentration (CS) and relatively high water activity values (a_w) within all intervals time of storage. Use of vacuumed package, sodium erythorbate (SE) in addition to natamax (NM) or potassium sorbate (PS) led to delay the proleins breakdown depending on decreasing rate observed development of values of free amino nitrogen (FAN), total volatile nitrogen (TVN) and non-prolein nitrogen (NPN), which were detected during cold storage. Also, antioxidant and antifungal agents reduced the increment rate of thiolsarbituric acid (TBA) and peroxide value (PV). The treatment comprised both sodium erythorbate and natamax was more efficient in this field.

Use of the aforementioned antioxidant and antifungal agents with participation the vacuumed package improved quality characteristics and keeping quality of smoked mackerel fish throughout the cold storage period by inhibition the bacterial development and prevention mould and yeast growth.

INTRODUCTION

Smoking is one of the oldest fish processing, it's less expensive than some other methods and suitable for developed countries. The world production from smoked fish increased from 808704 metric tons in 1981 to 918394 metric tons in 1990 (FAO, 1990). Actually, the world production could be estimated that foregoing one million meyric tones.

Dry salting was carried out with salt crystals alone (Zaitsev *et al.*, 1969) or with brine solutions with salt and glucon-d-lactone (Neroal, 1972) or by using sodium ascorbate (Cuppett *et al.*, 1989).

The smoked mackerel lost some of its moisture during the storage at 4°C with or without packing. However, the storage in the polyethylene bags markedly increased the water losses rather than the parchement paper (Dessouki, 1971). On the other hand, the shelf-life of vacuum packed smoked trout fillets and whole smoked trout were 10-12 and 16-18 days at 4-6°C, respectively (Schneider and Hildebrandt, 1985).

Sorbic acid and its salts increased the shelf-life of smoked fish by halting the growth of mould (Debever and Voets, 1972). Potassium sorbate extended the shelf-life by inhibition of bacteria (Beuchat, 1980), and the final product remained safety because of the ability of potassium sorbate to inhibit

pathogens as *St. aureus* and *Cl. botulimum* (Gray, 1980). Sodium bicarbonate solution reduced markedly the microbial growth in treated smoked fish (Curran *et al.*, 1990).

The addition of 0.1% sodium erythorbate was conduced to inhibit the development the oxidative rancidity and to contribute in maintenance a higher flavor score (Iredale and York, 1977). Deeping of halk fillets in a 3% sodium erythorbate solution for 3 seconds extended the shelf-life because of its ability to inhibit the formation of peroxides and off-flavor (Licciardello *et al.*, 1980). Pfrzer (1986) reported that sodium erythorbate is strong reducing agents acting as oxygen scavengers and reducing molecular oxygen. Erythorbates are more stable under acidic conditions than they are under neutral conditions.

The thiobarbituric acid value (TBA), acid value (AV), and peroxide value (PV), increased were gradually after salting and smoking processes and within cold storage period especially in the presence of oxygen (Bhuiyan *et al.*, 1986 and El-Akeel, 1988).

The main aim of the present work is possibility to produce a smoked mackerel fish product with low sodium chloride (3% approx.) by cold smoking process and to study the effect of using sodium erythurbate (SE) as an antioxidant agent and both potassium sorbate (PS) and natamax as antifungal agents during cold storage at 4°C for two months using normal and vacuumed packaging.

MATERIALS AND METHODS

A. Materials:

1. Raw fish:

Frozen mackerel fish (*Scomber scombrus*) imported by the Food Star Company for Import and Export, Alexandria, Egypt.

2. Antioxidant:

Sodium erythorbate (SE) was produced by Pfizer Company (USA). It was purchased from the local market by Egyptian Promoting Center (EPC), Cairo, Egypt. Each polyethylene bag contains 1/2 kg.

3. Antifungal agents:

Two kinds of antifungal agents used in present study were produced by Pfizer Company (USA), it were obtained by Egyptian Promoting Center (EPC), Cairo, Egypt, namely:

- Natamax (NM), which is combined from natamicin and lactose at ratio of 1:1. A small plastic container with 100g was purchased.
- Potassium sorbate (PS) granular, produced by Ueno Fine Chemicals Industry, Ltd, Japan.

4. Salt (NaCl):

For salting process, the salt (NaCl) is a grade NO_2. It is a product for El-Nasr Salines Company, Egypt.

5. Smoke source:

Sawdust and shaves of beech wood were used as a source of smoke at ratio of 1:1 (w/w).

6. Packaging materials:

- Cartone boxes lined with a parchment paper were used for normal packaging. Each box contains 6.8 unites of smoked fish.
- Polpropylene sacks for packaging the smoked fish under vacuum were used. Each sack contains two unites of smoked fish.

B. Methods:

Preparation of fish samples:

Frozen mackerel fish were thawed at room temperature (24°C) within 4 hrs, and then were dressed as a butterfly shape. The thawed fish were immersed for 5 min into sodium erythorbate (SE) solution containing 30 gm/liter. Dry salting was carried out by covering fish with dry salt and spices (1 salting mixture : 3.3 fish) for 15 min at room temperature (24°C) and then samples were rinsed with tap water in order to remove to excess the dry salt. Salting mixture was composed of 96% salt, 2% sugar, 2% spices. The spices and sugar mixture were added to salt at ratio of 2%. The mixture of spices composed of hot paprika, sweet paprika, black pepper, common and thimath. The butterfly fish samples were treated with antifungal agents by mixing potassium sorbate (PS) as 3% of the added salt on 4 gm natamax (NM) / kg fish.

Salted treated samples were kept on a net shelves and allowed to drain and semi-dried for overnight (16 hrs) inside a ventilated room. Cold smoking was achieved at 27°C for 24 hours, at the Sea Food's Misr Company for Fish Trading and Processing, Kafr El-Sheikh, Egypt.

The final smoked samples were devided into two parts, the first was packaged into polypropylene sacks under vacuum, while the second was packaged into carton boxes lined with parchment paper. Both two mentioned packaged samples were stored at 4°C for two months into domestic home refrigerator "Electrostar".

Analytical methods:

Moisture content, crude protein content, crude fat, ash content, sodium chloride content, total soluble nitrogen (TSN), free amino nitrogen (FAN) and pH value were determined as described in A.O.A.C. (1990).

Non-protein nitrogen (NPN) was determined by the method of Jacobs (1962). Total volatile nitrogen (TVN) was estimated according to the method described by Mwansyemela (1973). The peroxide value (PV) was estimated as described by A.O.C.S. (1981). Thiobarbituric acid value (TBA) was measured colorimetrically at 538 nm using Bouch & Lomb colorimeter spectronic 20 according to the method described by Pearson (1968).

Water activity (a_w) was calculated by the equation mentioned by Demeyer (1979).

Microbiological examinations:

Total bacterial counts (TC) per one gram fish fillet samples were enumerated on plate count agar medium, the plates incubated at 30°C for 3 days as the method described by Sharf (1966).

Mould and yeast counts were estimated using potato dextrose agar medium, the plates were uncubated at 25°C for 5 days according to the method described by Merck (1986).

RESULTS AND DISCUSSION

Data presented in Table (1) showed the chemical composition and some physical properties of raw and smoked mackerel fish. Smoking process conduced to decrease the moisture content because of the evaporation and separation of some fluids as drip. The crude protein and fat contents were also decreased due to the leaching out of juice and loss of nitrogenous compounds during salting stage and releasing fatty matters during smoking process. These results confirmed the data obtained by Bhuiyan *et al.* (1986).

Table 1: Chemical properties of raw and butterflied smoked mackerel fish at zero time (on wet weight basis).

Parameters	Raw fish	Butterfly smoked fish	Parameters	Raw fish	Butterfly smoked fish
Moisture (%)	70.50	53.98	NPN* (mg/100g)	152	143
Protein (%)	18.15	16.42	SPN* (g/100g)	5.74	4.46
Fat (%)	10.53	20.11	Salt* (%)	0.13	6.60
Ash (%)	0.82	9.49	TBA (mg/kg)	2.27	4.03
FAN* (mg/100g)	135	150	PV (meq/kg fat	1.59	3.86
TVN* (mg/100g)	49	64	pH value	6.20	5.55
TSN* (g/100g)	5.89	4.60	a_w	0.998	0.967

* On dry weight basis.

Also, from Table (1), it is obvious that free amino nitrogen (FAN) and total volatile nitrogen (TVN) markedly increased because of protein constituents breakdown, while total soluble nitrogen (TSN) and non-protein nitrogen (NPN) decreased because of their loss during salting and smoking process. These data are in agreement with those obtained by Shiau and Chai (1985) and Hassab Allah (1997).

The obtained results revealed that the thiobarbituric acid (TBA) and peroxide values (PV) increased after salting and smoking process due to auto-oxidation of fish lipids and formation of hydroperoxides and malonaldehyde (El-Akeel, 1988).

The pH value and water activity (a_w) showed obvious decrease due to absorption of organic acids vapor produced during smoking process and drying of moisture, respectively as obtained results by Hammed (1985).

Data given in Table (2) showed the moisture percentage, salt concentration and water activity values in case of using normal and vacuumed packaging during cold storage at 4°C of butterflied-smoked mackerel fish.

Results showed a continuous and gradual decline in moisture contents throughout the storage period at 4°C of all normal and vacuumed packaged samples, but with different rates, where the percentages of moisture loss were in between 22 to 25% and 6 to 9% in case of normal and vacuumed package, respectively. This decrease of moisture contents during storage might be due to the separation of fluids from fish tissues.

Regarding to the development of salt concentrations (SC) during cold storage at 4°C, it could be observed that the vacuumed packaging led to decrease the salt concentrations during cold storage comparing to the normal packaging. This could be attributed to the kind of bags, which might have caused greater loss in moisture content in case of normal package compared to polypropylene bags, which used in vacuumed package, and to loss of a part of dry matter as volatile substances during storage period. This observation manifested that vacuumed package samples gave smoked products with low salt content at consumption time, which reflects the needing response of some consumers. These results are in agreement with findings by Gunnar *et al.* (1980).

The decrease in water activity (a_w) was more pronounced in normal packaged samples because they were more water loss as found by Hirn and Hirvela (1994).

Neither antioxidant (SE) nor antifungal agents (NM and PS) showed any effect on moisture, salt concentration and water activity during storage period.

Table 2: Effect of cold storage at 4°C and packaging methods on moisture content, salt concentration and water activity (a_w) of smoked mackerel fish.

Para-Meters	Treat-ments	Package method	Storage period in days						Changes %
			0	4	8	16	30	60	
Moisture (%)	C	NP	53.98	51.50	50.82	49.11	42.99	40.30	-25
		VP		53.08	52.41	52.11	51.82	50.02	-7
	SE+NM	NP		50.46	49.15	48.00	42.50	41.73	-23
		VP		53.51	52.18	51.98	50.63	49.15	-9
	SE+PS	NP		51.30	50.23	48.39	43.51	41.91	-22
		VP		53.61	52.84	52.20	51.94	50.86	-6
Salt Conc. (%)	C	NP	5.33	5.94	6.39	7.25	9.44	11.00	+106
		VP		5.60	5.82	6.12	6.31	6.92	+30
	SE+NM	NP		6.03	6.63	7.65	9.44	10.58	+99
		VP		5.44	5.74	6.22	6.52	7.70	+45
	SE+PS	NP		5.96	6.35	7.08	8.78	10.10	+90
		VP		5.44	5.78	6.01	6.23	6.27	+31
Water activity a_w	C	NP	0.967	0.963	0.960	0.954	0.938	0.926	-4.20
		VP		0.965	0.964	0.962	0.960	0.956	-1.23
	SE+NM	NP		0.962	0.958	0.951	0.938	0.929	-3.87
		VP		0.966	0.964	0.961	0.959	0.951	-1.68
	SE+PS	NP		0.963	0.959	0.956	0.943	0.933	-3.49
		VP		0.966	0.964	0.962	0.961	0.956	-1.15

C = Control sample NM = Natamax SE = Sodium erythorbate
NP = Normal package PS = Potassium sorbate VP = Vacuumed package.

Data presented in Table (3) showed a continuous hydrolysis of protein content which took place during the storage of smoked fish. This degradation was indicated by the increase of FAN, TVN and NPN resulted from the effect of proteolytic enzymes found in tissues and also to microbial

contamination. Also, it could be noticed that using of such antioxidant, and antifungal agents delayed the proteinaceous constituents breakdown compared to the control samples. Participation of vacuumed packaged with antioxidant and antifungal agents inhibited the acceleration of proteinaceous constituents breakdown more than the normal package. No preference between NM and SP as antifungal agents to delay proteinaceous constituents breakdown.

Data in Table (3) summarized that the increase of FAN, TVN and NPN in all samples with different levels due to temperature, kind of package, duration of storage and rates of protein breakdown. These findings are in concordance with mentioned data by Magnusson and Martinsdoter (1995).

Also, from data presented in Table (3), a slight decrease in protein solubility was noticed of vacuumed-packaged samples compared to normal-packaged samples. The effects of antioxidant and antifungal agents on the protein solubility are not evident.

Table 3: Effect of packaging methods and efficiency of antioxidant and antifungal agents on protein decomposition and solubility of smoked fish during cold storage.

Para-Meters	Treat-ments	Package method	Storage period in days						Changes** (%)
			0	4	8	16	30	60	
FAN (mg/ 100g)	C	NP	150	159	201	219	233	262	+75
		VP		159	223	244	269	281	+87
	SE+NM	NP		156	174	131	195	203	+35
		VP		155	168	199	210	234	+56
	SE+PS	NP		154	178	193	212	227	+51
		VP		154	205	220	255	250	+53
TVN* (mg/ 100g)	C	NP	29	48	93	96	133	161	+448
		VP		33	56	73	90	110	+274
	SE+NM	NP		38	76	88	103	118	+302
		VP		33	46	51	60	81	+174
	SE+PS	NP		45	83	90	113	125	+341
		VP		37	57	71	74	91	+212
NPN (mg/ 100g)	C	NP	143	219	232	365	464	530	+241
		VP		205	227	269	307	343	+140
	SE+NM	NP		201	247	299	336	427	+199
		VP		193	216	244	261	286	+160
	SE+PS	NP		194	219	287	327	366	+156
		VP		169	237	297	297	304	+113
TSN (g/ 100g)	C	NP	4.60	4.29	4.31	6.85	7.95	8.97	+89
		VP		4.30	4.71	6.34	7.03	7.19	+56
	SE+NM	NP		4.32	4.65	6.92	7.69	8.31	+81
		VP		4.24	4.31	5.65	6.19	7.10	+54
	SE+PS	NP		4.26	4.49	6.51	7.15	7.84	+70
		VP		4.32	4.51	6.48	7.06	7.53	+64

* On wet weight basis ** Changes (%) at the end of storage period.
C = Control sample PS = Potassium sorbate NM = Natamax
SE = Sodium erythorbate NP = Normal package VP = Vacuumed package.

Table 4: Effect of packaging methods and efficiency of antioxidant and antifungal agents on thiobarbituric acid (TBA) and peroxide value (PV) of smoked fish during cold storage.

Samples	Treatments		Para-meters	Storage period in days					% of Increase
				0	8	16	30	60	
Smoked Mackerel Samples	Normal Package	Control	TBA	4.03	5.03	7.13	10.54	12.94	+221
			PV	3.86	10.04	12.33	15.39	18.96	+391
		SE+ NM	TBA	4.03	5.43	5.39	6.25	8.32	+106
			PV	3.86	7.08	8.60	10.35	12.83	+232
		SE+ PS	TBA	4.03	4.95	5.53	6.48	8.98	+148
			PV	3.86	5.98	9.31	10.49	11.80	+206
	Vacuumed package	Control	TBA	4.03	4.03	4.84	5.61	7.05	+75
			PV	3.86	6.08	7.64	10.83	11.74	+204
		SE+ NM	TBA	4.03	4.29	4.38	4.59	5.01	+24
			PV	3.86	5.64	5.75	7.53	8.10	+110
		SE+ PS	TBA	4.03	4.05	4.68	4.94	5.13	+27
			PV	3.86	4.80	6.18	7.60	9.93	+157

TBA values were expressed as mg malonaldehydr / kg sample.
PV values were expressed as meq / kg fat.
PS = Potassium sorbate NM = Natamax SE = Sodium erythorbate

Data tabulated in Table (4) indicated that both thiobarbituric acid (TBA) and peroxide (PV) values were gradually increased during prolongation of cold storage period at 4°C for 60 days. The smoked control samples showed the highest increases for TBA. The type of package affected the development of TBA and PV values, where the vacuumed package reduced the development TBA and PV values in mode more pronounced comparing to the normal package. Using of antioxidant (SE) led to delay the development of TBA and PV at the end of cold storage period. The treatment of SE + NM showed ability to inhibit the increasing of TBA and PV values more than the treatment of SE + PS, for both vacuumed and normal packaged samples, respectively. These findings are in concordance with Pfizer (1994) and Hassab Allah (1997).

Data presented in Table (5) proved that a marked increase in total bacterial count (TBC) either in vacuumed or normal packages was observed, this is may be back to the biodegradation of fish ingredients such as proteins and fats. Only vacuum process reduced the TBC from 29000 x 10^3 cell/ g to 2500x10^3 cell/g after 60 days of cold storage. It should be concluded that the vacuumed package markedly improved the shelf-life of product by suppression the development of visible bacteria as reported by Whittle *et al.* (1991).

Periodic declines in TBC through the cold storage period due to the use of mixture of antifungal and antioxidants agents were observed (Table 5). Natamax (NM) was more successful than potassium sorbate (PS) to prevent the bacterial growth during cold storage giving 230 x 10^3 and 18 x 10^3 cell/g, while PS caused final TBC ranged from 490 x 10^3 and 32 x 10^3 cell/g in normal and vacuumed package, respectively.

Results tabulated in Table (6) show that the mould and yeast were not detected during cold storage of smoked fish samples packaged under vacuum condition. In the normal packaged samples particularly the control

sample, mould and yeast development began after 30 days and remained up to the end of storage period giving 32 x 10^2 cell/g. Using of NM and PS as antifungal agents showed a strong action in prevention of fungal growth in all treated samples. These findings are similar to those reported by Whittle *et al.* (1991).

Table 5: Effect of packaging methods and efficiency of antioxidant and antifungal agents on total bacterial counts (cell/g)of smoked fish during cold storage.

Samples	Treatments		Total bacterial counts x 10^3					
			Storage period in days					
			0	4	8	16	30	60
Smoked Mackerel Samples	Normal Package	Control	150	140	3600	3800	4100	29000
		SE+NM	13	12	20	43	190	230
		SE+PS	23	26	36	60	210	490
	Vacuumed package	Control	130	13	320	390	810	2500
		SE+NM	13	9	12	13	15	18
		SE+PS	23	13	13	18	21	32

SE = Sodium erythorbate PS = Potassium sorbate NM = Natamax

Table 6: Effect of packaging methods and efficiency of antioxidant and antifungal agents on mould and yeast counts (cell/g)of smoked fish during cold storage.

Samples	Treatments		Mould and yeast counts x 10^2					
			Storage period in days					
			0	4	8	16	30	60
Smoked Mackerel samples	Normal Package	Control	-	-	-	-	0.9	32
		SE+NM	-	-	-	-	-	-
		SE+PS	-	-	-	-	+	0.3
	Vacuumed package	Control	-	-	-	-	-	-
		SE+NM	-	-	-	-	-	-
		SE+PS	-	-	-	-	-	-

SE = Sodium erythorbate PS = Potassium sorbate NM = Natamax
(-) = No growth (+) = Low growth.

Finally, it could be concluded that the use of aforementioned antioxidants and fungal agents during cold storage led to retard protein hydrolysis and fat oxidation as well as mould and yeast growth. This action becomes more pronounced if the vacuum package is done, guarding the quality of smoked fish.

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تأثير إستخدام مضادات الأكسدة ومضادات الفطريات وطرق التغليف على جـــودة الأسماك المدخنة

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تعتبر أسماك الماكريل واحدة من الأسماك الخام الرئيسية لتصنيع منتجات الأسماك المدخنة. وقد تـم تجهيز أسماك الماكريل فى الصورة المفتوحة buttrflied حيث أجرى التمليح بالطريقة الجافة وعوملـت الأسماك خلالها بكل من سوربات البوتاسيوم (PS) والناتـامكس (NM) كمضـادات فطريـة وكذلـك أيريثوربات الصوديوم (SE) كمضاد أكسدة ، وتم تدخين الأسماك على البارد وأجريت تعئبتـها بالطريقـة العادية وكذلك بطريقة التفريغ وخزنت على درجة ٤ °م لمدة ٦٠ يوما.

وقد أظهرت العينات المغلفة تحت تفريغ إنخفاض معدل فقدها للرطوبة ، وتركيز ملحـى منخفـض ونشاط مائى مرتفع نسبياً خلال مراحل التخزين البارد.

كذلك إتضح أن أستخدام التغليف تحت تفريغ بالإضافة إلى إستخدام كل من ايريثوربات الصوديـوم (SE) كمضاد أكسدة وكل من الناتامكس (NM) وسوربات البوتاسيوم (PS) كمضادات فطرية أدى إلـى تأخير إنحلال البروتينات حيث أن قيم النتروجين الأمينى الحر FAN والنتروجين المتطاير الكلـى TVN وكذلك النتروجين الغير بروتينى NPN كانت معدلات زيادتها أثناء التخزين منخفضة مقارنه بالعينة القياسية ، كما كانت معدلات زيادة قيم حمض الباربتوريك (TBA) وكذلك رقم البيروكسيد (PV) منخفضة أيضـاً على إمتداد فترة التخزين. وقد أظهرت المعاملة المشتملة على كل من أيريثوربات الصوديوم مع الناتـامكس أكثر فعالية فى هذا المجال من المعاملة بكل من أيريثوربات الصوديوم مع سوربات البوتاسيوم.

كذلك ظهرت فعالية الإستخدام المشترك لطريقة التغليف تحت التفريغ مع إستخدام تلـك المضـادات الفطرية ومضادات الأكسدة فى تثبيط النمو البكتيرى ومنع النمو الفطرى مما يؤدى يقيناً إلى تحسين صفـات الجودة والحفاظ عليها طوال فترة التخزين البارد.