INFLUENCE OF SMOKING METHODS AND COLD STORAGE ON QUALITY OF SILVER CARP (*Hypophthalmichthys molitrix*) FILLETS

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ABSTRACT

Effect of smoking methods and cold storage on quality of silver carp (*Hypophthalmichthys molitrix*) fillets was investigated in the present study. Fish fillets were cold and hot smoked in presence potassium sorbate (PS) and spices mixtures and or without it throughout salting process. All batches were polyethylene packaged and stored at 4±1 °C for 60 days. Results showed that although values of total volatile bases nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), and total bacterial count (TBC) were higher in cold smoked products than ones but thiobarbituric acid (TBA) and pH values were higher for hot smoked samples. Sensory evaluation indicated that cold smoked products were got high scores than hot smoked products. During cold storage, quality criteria of all treatments were gradually increased. However, all smoked silver carp products had long shelf life and still acceptable until the end of storage period. In addition, cold smoked fillets treated with spices mixtures had a superior color, odor, taste, texture, and overall acceptability scores. Therefore, essential amino acids index (EAAI) total essential amino acids (TEAA), essential amino acids (EAA), amino acid score (AS), biological value (BV) and percent satisfaction (PS/150%) were increased while grams consumed to cover the daily requirements (GDR) were decreased in this product at the initial zero time of storage and the end of storage period (60 days). In conclusion, cold smoked products were sensory more accepted than hot smoked products especially treated with spices. Also, both spices mixtures and PS in salting process improved sensory characteristics and controlled in changes rate of final products. Cold storage increased the shelf life and keeping the quality of silver carp fillets.

Keywords: Carp fish, smoking, storage, keeping quality.

INTRODUCTION

Silver carp (*Hypophthalmichthys molitrix*) is one of the main freshwater fish species, due to its fast growth rate, easy cultivation, high feed efficiency ratio, as well as high nutritional value **(Luo *et al.*, 2008).** However, freshwater fish, including silver carp, often have a strong earthy/musty taste, odour, due to significant concentrations of geosmin or 2-methyl-isobomeol (MIB) in fish flesh **(Howgate, 2004).** Furthermore, silver carp contains many intramuscular small bones. Therefore, the consumption of silver carp has been limited and the price of the fish is low. Silver carp (*Hypophthalmichthys molitrix*) may be the cheapest and most abundant freshwater fish due to its quick growth and resistance to stress, disease and rough handling. **(Aryanta *et al.*, 1991).** Smoking is one of the oldest methods that used in fish preservation; which inhibited fat oxidation, bacterial growth and may extend the shelf life of the finished product **(Shalaby, 2000 and Leroi *et al.*, 2001).** Synthetic or natural preservatives can control the development of lipid oxidation or microbial growth in fish during storage. However, consumers are always concerned about the use of artificial preservatives in food, which may

have potentially undesirable effects on human health **(Tassou *et al.*, 1995).** Potassium sorbate extended the shelf life by inhibiting the bacteria **(Beuchat, 1980),** and preventing the growth of yeasts and molds in fish **(Lueck, 1980).** Therefore, the main objective of this study is to investigate the effect of traditionally smoking methods (cold and hot) in presence some antioxidants and antimicrobial (synthetic and natural) during cold storage at 4 ± 1^0C for 60 days on the keeping quality of silver carp (*Hypophthalmichthys molitrix*) fillets.

MATERIALS AND METHODS

Materials

Fresh silver carp fish (*Hypophthalmichthys molitrix*) were obtained from Manzala Farm, Dakahlia Governorate, Egypt. Fish samples (about 2.0 kg weights) were transported in ice box to the Laboratory and immediately beheaded, eviscerated, scaled and manually filleted. Salt: Sodium chloride, fine iodized table salt (BONO) produced from Egyptian Salts & Minerals Company (EMISAL). Potassium sorbate (PS) granular was obtained from Egyptian Promoting Center (EPC). Spices; black pepper, cumin, coriander and cardamom at ratio of 1:1:1:1 were well mixed. Sawdust from beech wood as smoke source was used.

Smoking process

The smoking process (fig. 1) was carried out in smokehouse in Shakshouk Fish Research Station, National Institute of Oceanography and Fisheries. The smokehouse had inside dimensions of 1.20×1.0×3.25 m. and fish samples were fixed above the smoke source by about 300 cm in cold and 150 cm in hot smoking **(Abd El-Mageed, 1994).** The investigated smoked products were periodically analyzed at intervals of two weeks.

Analytical methods

pH value was measured by checker pocket-sized pH meter with replaceable electrode (HANNA Instruments, USA), thiobarbituric acid (TBA) value and total volatile basic nitrogen (TVB-N) and Trimethylamine nitrogen (TMAN) were determined using **(AOAC, 2000)** methods. Individual amino acids were determined using reverse phase HPLC; hydrolysis, derivatization and analysis were performed according to the Pico-Tag method **(Millipore Co-operative, 1987).** Tryptophan was not determined. Indispensable amino acids (EAA) and amino acid score (A.S.) were calculated as FAO/WHO/UNU, 1985. Essential amino acid index (EAAI) and biological value (BV) were calculated **(Oser, 1959).** Microbial analysis including Total viable bacteria count (TVBC) per one-gram fish fillet samples was enumerated on plate count agar medium, the plates incubated at 30°C for 3 days as the method described by **Oxoid (1982).** Mold and yeast counts were estimated using potato dextrose agar medium; the plates were incubated at 25°C for 5 days according to the method described by **Merck (1986).** The results were expressed as $\log_{10}$ cfu/g sample. Sensory characteristics; appearance, texture, flavor, taste and overall acceptability were evaluated by ten staff members of El-Qanater El-Khairia Station for fish Research, National Institute of Oceanography and Fisheries. A ten point scale was used where 10 = excellent and 1 = extremely poor.

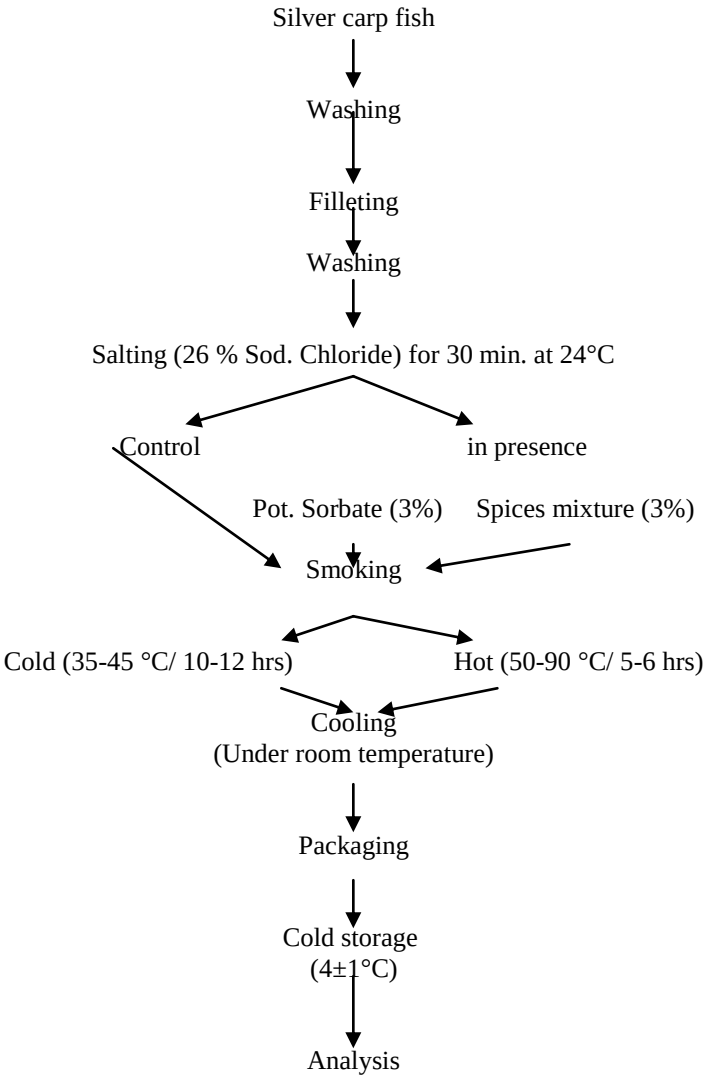

Fig. (1): Flowchart shows main steps of carp fillets smoking.

RESULTS AND DISCUSSION

TVB-N content

Table (1) shows changes in quality criteria for cold and hot smoked carp fillets during cold storage periods. TVB-N values were 15.56, 14.27 and 12.03 mg/100g sample (on wet weight basis) of cold smoked control, treated with PS and with spices samples, respectively, while they were 13.81, 11.14 and 10.02 mg /100g sample in case of hot smoked fillets at zero time. This increase in cold smoked products could be attributed to the handling prior to processing, heat and time during smoking process which caused evaporate of some TVB-N content on hot smoking or the action of protein hydrolysis caused by enzyme during the long time and lowering heat used in cold smoking. In addition, TVB-N was higher of control samples than other treatments by antimicrobial of potassium sorbate and spices. TVB-N value was continuously increased with increasing storage period

and increasing rate was higher in cold smoked than ones. At the end of storage period TVB-N of control, samples treated with PS and with spices of cold smoked samples increased to 29.21, 26.96 and 24.53 mg/100g sample respectively, while of hot smoked fillets it were increased to 27.84, 25.99 and 22.20 mg/100g sample. This increase may be attributed to the effect of microorganisms as well as autolysis processes. However, these values of TVB-N for smoked silver carp products were considered accepted until the end of storage period. In this respect **Greene and Cumuze (1982)** reported that the maximum acceptable levels of (TVB-N) for smoked fish was 30 mg/100g sample flesh. These results are nearly in agreement with **Hegazy (1998).**

Table (1): The mean valus in quality properties of smoked silver carp fillets as affected by storage at 4 ± 1 ^{0}C (as wet weight basis).

Storage Period (days)	Smoking methods;					
	Cold smoking			Hot smoking		
	Control	*PS	Spices	Control	*PS	Spices
Total volatile bases nitrogen (TVB-N mg/100gm flesh)						
0	15.56	14.27	12.03	13.81	11.14	10.02
15	16.71	14.73	13.46	14.07	11.41	11.08
30	18.60	16.27	15.58	16.88	13.79	13.71
45	23.37	20.23	19.74	20.21	17.88	16.91
60	29.21	26.96	24.53	27.84	25.99	22.20
Trimethylamine nitrogen (TMA-Nmg/100gm flesh)						
0	0.87	0.72	0.50	0.69	0.47	0.42
15	1.19	1.05	0.85	1.17	0.95	0.76
30	1.86	1.58	1.38	1.81	1.31	1.14
45	2.65	2.35	2.18	2.67	2.38	1.95
60	4.38	3.89	3.63	4.17	3.69	3.16
Thiobarbituric acid (TBA mg Malonaldhyde/Kgm flesh)						
0	0.60	0.52	0.48	0.63	0.59	0.56
15	0.66	0.61	0.60	0.75	0.71	0.68
30	0.90	0.82	0.83	1.02	0.95	0.91
45	1.24	1.16	1.17	1.44	1.34	1.28
60	1.92	1.66	1.62	2.12	1.82	1.73
pH value						
0	5.27	5.02	5.12	5.98	5.83	5.87
15	5.35	5.15	5.25	5.98	5.8	5.90
30	5.65	5.48	5.60	6.02	5.90	5.95
45	6.35	6.10	6.20	6.27	6.08	6.12
60	6.85	6.35	6.48	6.55	6.23	6.35
Total viable bacterial count (TVBC Log_{10} cfu/gm flesh.)						
0	3.75	3.63	3.55	3.61	3.52	3.50
15	3.76	3.65	3.56	3.61	3.54	3.50
30	3.78	3.70	3.58	3.63	3.55	3.51
45	4.25	3.75	3.62	3.71	3.61	3.55
60	4.90	3.81	4.68	3.78	3.65	3.61
Yeasts and molds (Log_{10} cfu/gm flesh.)						
0	-	-	-	-	-	-
15	-	-	-	-	-	-
30	-	-	-	-	-	-
45	0.80	0.50	0.65	-	-	-
60	1.12	0.68	0.90	0.92	0.34	0.75

*PS: Potassium sorbate

TMA-N content

Hot smoking of silver carp fillets caused a reduction of TMA-N content than cold smoking; this reduction might be due to the effect of heat used during smoking, which led to volatilization of TMA-N. In addition, TMA-N was increased during the cold storage period in all smoked products. TMA-N were increased from 0.89, 0.72 and 0.50 mg/100g sample (on wet weight basis) after smoking of cold smoked control, treated with PS and treated with spices fillets, respectively, to 4.38, 3.89 and 3.63 mg/100g sample at the end of storage period. While, these values of hot smoked fillets were 0.69, 0.47 and 0.42 mg/100g sample after smoking process and increased to 4.17, 3.69 and 3.16 mg/100g sample respectively. This increase of TMA-N value of smoked fish during storage may be due to cleavage of TMA-N oxide naturally present in the fish muscles and/ or the formation of TMA-N itself from betain and cholin, which present in the fish muscles **(Ibrahim and El-Zonfuly, 1980).** These values of TMA-N for smoked silver carp products were considered accepted until the end of storage period according to **Egyptian standards (1996)** which reported that smoked fish should not be more than 10.0 mg/100g flesh sample of TMA-N.

TBA value

TBA values increased after smoking, as well as these increased regularly and gradually as the time of cold storage increased. The increasing rate of TBA value was higher in hot smoked fish products than others during storage period. In addition, TBA value was higher of control samples than other treated by antimicrobial of PS and spices table (1). This increase in the TBA value during hot smoking could be due to the influence of heat for long time and much reduction in moisture that promoted lipid oxidation, and absorption of some TBA-reacting substances from the smoke itself **(El-Akeel, 2003). Greene and Cumuze (1982)** reported that 10 mg /kg fish as a higher level for acceptance. This TBA value rise was mainly because of both auto oxidations of fish fat and bacteriological with the formation of some TBA- reacting substances during storage **(Hammad, 1985 and El-Akeel, 1988).**

pH value

The obtained results of table (1) revealed that a decrease trend of pH value immediately after smoking process of cold smoked than hot smoked samples. This decrease of pH value may be attributed to absorb high amount of some organic acids from smoke during the long time and lowering heat of cold smoking **(Dessouki, 1971).** Also, pH values of smoked fish showed a gradual but slight increase during storage, might be mainly due to the activity of some bacteria and the fish tissues enzymes on fish protein decomposition causing the formation of total volatile nitrogen bases, which increased the pH value of smoked samples **(Etman, 1985).**

TVBC

Total viable bacteria count (TVBC) of hot smoked silver carp fillets was decreased as compared with that of cold smoked samples at zero time (table, 1). This redaction could be attributing to the effect of smoke constituents, which had an antimicrobial effect, in addition to heating and dehydration through hot smoking process. These data indicated that TVBC of all smoked fish samples were raised during cold storage and until the end of storage period and was lower in cold and hot smoked fillets treated by antimicrobial spices and PS than control samples. Therefore, TVBC of all investigated smoked samples did not reach to the maximum level even at the end of storage period, this indicates to high quality microbiologically fillets. These results support the finding of **Schulze and**

Zimmermann (1983) who reported that the maximum accepted level of total microbial count for smoked fish is 10^6 cells / gm flesh. Our results are in agreement with reported by **Schulze and Zimmermann (1983); Hammad (1985); El-Akeel (1988) and Abd El-Mageed (1994).**

Table (2): The mean values of smoked silver carp fillets as affected by storage at 4±1°C.

Period (day)	Smoking methods;					
	Cold smoking			Hot smoking		
	Control	*PS	Spices	Control	*PS	Spices
Appearance						
0	9.0	9.0	9.0	8.5	8.0	8.0
15	8.8	8.2	9.0	7.5	7.3	7.5
30	7.2	7.5	8.5	7.2	7.0	7.2
45	6.5	7.0	7.2	6.4	6.8	7.0
60	5.8	6.2	6.4	6.0	6.3	6.8
Odor						
0	8.5	7.5	9.0	7.6	7.0	8.0
15	7.8	7.2	8.5	7.0	6.8	7.2
30	7.0	6.8	7.5	6.5	6.5	7.0
45	5.5	6.2	6.5	5.5	6.0	6.5
60	5.0	5.8	6.0	5.0	5.5	6.2
Taste						
0	8.5	7.0	9.0	8.0	7.5	8.5
15	8.0	6.7	8.8	7.5	7.0	8.3
30	6.5	6.5	7.5	7.0	6.6	8.0
45	5.6	6.0	7.0	6.0	6.0	7.5
60	5.0	5.2	6.0	5.2	5.5	6.6
Texture						
0	8.0	7.5	8.5	7.5	7.3	7.5
15	7.6	7.2	8.2	7.2	7.0	7.2
30	6.6	7.0	8.0	6.5	6.8	7.0
45	5.2	6.6	7.0	5.8	6.5	6.6
60	4.8	5.0	5.5	5.0	5.3	6.0
Overall acceptability						
0	8.5	7.7	9.0	8.0	7.3	8.0
15	8.0	7.3	8.6	7.3	7.0	7.5
30	6.8	7.0	8.0	6.8	6.7	7.3
45	5.7	6.5	7.0	6.0	6.3	6.9
60	5.2	5.6	6.0	5.3	5.6	6.4

Score limits: Excellent: 8.6 -10; Very good: 7.6 - 8.5; good: 6.6 – 7.5 and Accepted: 5- 6.5.

*PS: Potassium sorbate

Mould and yeast

The same table (1) showed that the mould and yeast were not detected in all smoked products. Mould and yeast were appeared in cold smoked products after 45 days of storage and remained up to the end of storage period, while, in hot smoked samples it detected after 60 days. In addition, it could be concluded that mould and yeast were decreased in products treated by spices and PS as antioxidant and antifungal agents showed a strong action in prevention of fungal

growth in treated samples. These results coincided with that found by **Rabie, *et al.* (2002).**

Sensory evaluation

Cold smoked fillets were more sensory accepted than the hot smoked fillets especially treated with spices (table, 2). Also, cold smoked product treated with spices was considered the best treatment. It had got highest scores of color, odor, taste and texture and overall acceptability. On the other hand, quality changes in sensory characteristics were affected by extending storage period. However, all smoked silver carp products had long shelf life and still acceptable and in good conditions until 60 days. The smoked fish fillets had developed spoilage characteristics, which were disliked by the panel taste and became inedible. Thus, the unacceptability of these products after its mentioned keeping quality period was due to textural softening, off flavor, development of rancidity and the growth of microorganisms. These results are in agreement with found by **Abd El-Mageed (1994).**

Nutritional value

Cold smoked fillets treated with spices product was considered the best treatment organolepticaly. Thus, it was analyzed for its content of different amino acids (g/16g N), amino acid score (A.S), essential amino acids index (EAAI) and biological value (B.V.) at the initial zero time and the end of storage period (60 days). Results in table (3) showed that tryptophan was not determined and the most of amino acids decreased at the end of storage period. amino acid score (A.S.) of the product before storage was higher than 100, this indicated that there is no deficiency in any essential amino acids score compared with FAO references protein and high quality of the product. While, A.S. of the same product after storage period was less than 100 of histidine (81) which considered the restricting amino acid (R.A) (having the lowest A.S). Results also showed total amino acids (TAA) and total essential amino acids (TEAA) were 94.76 and 49.37 of the product immediately after smoking and decreased to 84.15 and 44.11 at the end of storage period. In addition, (EAAI) and (B.V.) were 89.75and 86.10 which decreased to 67.28 and 61.61 at the end of storage period of the same product. This indicating the high quality protein in the product at zero time of storage period compared at the end of storage. Thus, this decrease of total amino acids and other parameters during storage may be attributed to the reaction between amino groups and aldehydes, which present in fish tissues as a result of smoking and / or formed during lipid oxidation **(El-Akeel, 1988 and 2003).**

Results presented in table (4) showed that grams consumed to cover the daily requirements (G.D.R) of adult man and percent satisfaction of these requirements(P.S./150 %) when consuming 150g of the product in relation to **USRDA (1989)** (as g/100g sample). It was noticed that the restricting (R.A) was histidine amino acid (highest GDR "193"g and lowest PS/150 % "78") of the product before storage, while it was (highest GDR "258"g lowest PS/150% "58") after storage period. From these results, it could be concluded that the cold smoked fillets treated with spices was with high quality at zero time of storage. This confirmed by the increase of TEAA, EAA, A.S., EAAI, B.V.%, P.S.150% and decrease GDR values than the same parameters in the same product at the end of storage period (60 days).These changes in protein quality are depending mainly on the increase of storage period. These results are in agreement with reported by **El-Akeel (2003).**

Table (3): Amino acids composition of cold smoked silver carp fillets treated with spices as affected by storage at 4±1°C.

Amino acid	FAO/WHO/ UNU (1985)	Cold smoked fillets treated with spices samples			
		At zero time of storage		After 60 days of storage	
	g/16gm N	g/16gm N	A.A.S	g/16gm N	A.A.S
Aliphatic:					
Isoleucine	1.3	5.75	442	5.17	398
Leucine	1.9	9.45	497	8.55	450
Threonine	0.9	4.85	539	4.26	473
Valine	1.3	3.95	304	3.44	265
Serine		14.96		13.62	
Glycine		8.10		7.12	
Alanine		4.52		4.11	
Aromatic:					
Phenyalanine		5.12		5.02	
Tyrosine		2.10		1.95	
Phenyl.+Tyr	1.9	7.22	380	6.97	367
Sulpher:					
Methionine		7.95		7.30	
Cystine		2.20		1.95	
Met. + Cyst	1.7	10.15	597	9.25	544
Heterocyclic:					
Tryptophan	0.5	ND	ND	ND	ND
Proline		2.28		2.12	
Acidic:					
Aspartic		6.54		5.60	
Glutamic		5.07		4.22	
Basic:					
Histidine *	1.6	1.85	116	1.30	81*
Lysine	1.6	6.15	384	5.17	323
Arganine		3.92		3.25	
TAA		94.76		84.15	
TEAA		49.37		44.11	
EAAI		89.75		67.28	
B.V		86.10		61.61	

ND: not determined. TAA: Total amino acids. TEAA: Total essential amino acids.
EAAI: Essential amino acids index. B.V.: Biological value. AAS: Amino acid score.
* R.A.: Restricted essential amino acid.

Table (4): Nutritional value of cold smoked silver carp fillets treated by spices as affected by storage at 4 ± 1 °C.

Amino acid	** USRDA (1989) (gm /100gm sample)	Cold smoked samples treated with spices					
		At zero time of storage			After 60 days of storage		
		g/100gm sample	GDR (gm)	P.S.% /150gm	g/100gm sample	GDR (gm)	P.S.% /150gm
Aliphatic:							
Isoleucine	0.819	1.61	51	294	1.56	53	283
Leucine	1.197	2.66	45	333	2.58	46	326
Threonine	0.567	1.36	42	357	1.29	44	341
Valine	0.819	1.11	74	203	1.04	79	190
Serine		4.21			4.12		
Glycine		2.28			2.15		
Alanine		1.27			1.24		
Aromatic:							
Phenyalanine		1.44			1.52		
Tyrosine		0.59			0.59		
Phenyl.+Tyr.	1.197	2.03	59	254	2.11	57	263
Sulpher:							
Methionine		2.23			2.21		
Cystine		0.62			0.59		
let. + Cyst	1.071	2.85	38	394	2.79	38	394
Heterocyclic:							
Tryptophan	0.315	ND	ND	ND	ND	ND	ND
Proline		0.64			0.64		
Acidic:							
Aspartic		1.84			1.69		
lutamic		1.42			1.28		
Basic:							
istidine *	1.008	0.52	193	78	0.39	258	58
Lysine	1.008	1.73	58	259	1.56	65	230
Arganine		1.10			0.98		

ND: not determined. G.D.R: grams consumed to cover the daily requirements for adult man.

P.S %. / 150g: the percentage satisfaction of the grams daily requirements for adult man.

*R.A. = Restricted essential amino acid.

** USRDA: United State Recommended Dietary Allowances.

CONCLUSION

Cold smoked products were sensory more accepted than hot smoked products especially treated with spices. Also, both spices mixtures and pot. Sorbate in salting process improved sensory tests and controlled in changes rate of final products. Cold storage was increased the shelf life and keeping the quality of silver carp fillets.

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تأثير طرق التدخين وظروف التخزين المبرد على جودة شرائح سمك المبروك الفضى

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يهدف هذا البحث الى دراسة تأثير طرق التدخين بطريقتين (على البارد وعلى الساخن) وظروف التخزين المبرد (٤ ± ١ مº) على جودة شرائح سمك المبروك الفضى.حيث تم الحصول على عينات سمك المبروك الفضى من بحيرة المنزلة وتم عمل ٣ معاملات (الأولى عينة الكنترول والثانية إضافة سوربات البوتاسيوم بنسبة (٣%) والثالثة إضافة مخلوط التوابل (الفلفل الأسود، الكمون، الكزبرة والحبهان بنسبة ١:١:١:١) بنسبة (٣%) لكل من المنتجات المعاملة بالتدخين على البارد وعلى الساخن. وقد أظهرت النتائج المتحصل عليها مايلى:

ارتفعت قيم كل من القواعد النيتروجينية وثلاثى ميثايل الأمين والعدد الكلى البكتيرى فى المنتجات المدخنة على البارد، فى حين ارتفعت قيم حمض الثيوباربتيوريك والأس الهيدروجينى فى المنتجات المدخنة على الساخن. والتقييم الحسى اظهر حصول المنتجات المدخنة على البارد على أعلى القيم خاصة المعاملة بمخلوط التوابل مما ترتب علي ذلك تقدير القيمة الغذائية لهذا المنتج. وبناءا على هذه الدراسة فان المنتجات المدخنة على البارد خاصة المعاملة بمخلوط التوابل كانت أعلى قابلية حسيا مقارنة بالمنتجات الأخرى. علاوة على ذلك فقد ادت المعاملة بمخلوط التوابل وبسوربات البوتاسيوم الى التحكم فى معدل التغير فى معايير جودة المنتجات محل الدراسة مقارنة بالعينة الكنترول. وقد أدى التخزين على البارد أدى إلى أطالة فترة الصلاحية لكل عينات الأسماك المدخنة.