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## THE EFFECT OF PROPOLIS AND FROZEN STORAGE ON THE QUALITY OF SILVER CARP (*HYPOPHTHALMICHTHYS MOLITRIX*) BURGER El-Said A. El-Daly<sup>1</sup> and Samya I. A. Hassanin<sup>2</sup>

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#### Abstract

This investigation was done to determine the quality changes in fish burger, which were prepared from fresh fillets of silver carp (*Hypophthalmichthys molitrix*). Natural honey bees product (propolis) was added in different levels: 0.2%, 0.3% and 0.4% in addition to control burger without adding propolis ethanol extract (PEE) to study the effect of adding propolis on quality attributes of fish burger.

The chemical compositions and some physico-chemical properties as well as microbiological and sensory changes were evaluated during frozen storage at  $-20\pm1^{\circ}$ C for 3 month.

The results showed that addition of propolis ethanol extract (PEE) as a natural food additive at level 0.4% had a good treatment to improve the quality and accordingly increase the shelf-life of fish burger.

Keywords: propolis, silver carp burger, quality changes.

#### INTRODUCTION

In recent years, the preference of the consumers was directed towards the fast food consumption since there has been a rapid urbanization and an increase in working women population. It is well known that fish flesh has very special and important characteristics, such as  $w_3$  series fatty acids. For this reason, consumption of fish and fishery products are increasing (Tokur *et al.*, 2004). Among cultivated fresh water fish, silver carp (*Hypophthalmichthys molitrix*) has acquired great attention because of its increasing production in countries (FAO, 2007).

So, demand has come, from the fish producer to develop alternative products to increase the silver carp consumption. Many reports have been focused on products from fish mince such as fish burger (Del Nobile *et al.*, 2009). Fish burger is acceptable fast food products by the consumers in the world. In Egypt the burger prepared from beef and poultry but fish burger is not produced commercially in a large scale. Freezing and frozen storage of these burger are commonly used because of the consistent, reliable quality and ease of transportation. However, some compounds occur as a result of lipid oxidation and protein deterioration during frozen storage. These compounds cause undesirable flavor and odor changes, which affect the sensory quality (Siddaiah *et al.*, 2001). In order to increase shelf life of fresh fish and fishery products, low levels of salt and/or natural preservatives (antimicrobials and antioxidants) have been also used (Del Nobile *et al.*, 2009).

Propolis is the natural honeybee product collected by the honeybee workers from various plant buds, barks and exudates. The chemical compositions of propolis may vary according to different plant sources that bees could visit. Moreover, chemical analysis revealing that the main components of propolis are phenolic compounds including (flavonoids, aromatic acids and benzopyranes) as well as cinnamic acids derivatives, amino acids, fatty acids, terpenes, caffeic acid, vitamins, minerals and enzymes like (adinosine triphosphatase, succinic dehydrogenase,glucose 6-phosphatase). The antimicrobial and antioxidant effects of propolis are related to the synergistic effect of its compounds (Kumar *et al.*, 2008). Furthermore, most propolis components are natural constituents of food safe substances and valid alternative to synthetic preservatives (Enzo *et al.*, 2007). From the previous study propolis was tested as food preserver in beef burger at level of 0.3% to improve quality of product (Moghazy and El-Shaarawy, 2001).

The aim of this study was to processing fish burger from silver carp with addition of natural additives (propolis) and to investigate its chemical composition as well as some physico-chemical microbiological and sensory changes during frozen storage at -  $20\pm1^{\circ}$ C for 3 months.

#### **MATERIALS AND METHODS**

**Materials:** Silver carp (*Hypophthalmichthys molitrix*) fish was obtained from fish ponds at Central Laboratory for Aquaculture Research in Sharkia Governorate, Egypt. Each samples weighted were 10 Kg, while the mean of individual weight of silver carp fish was about 2.5-3 Kg. The head, skin and all fins of the fish were handily removed using a sharp knife. The whole fish was eviscerate then skinned off with special pliers and filleted. Sodium chloride: Table salt was produced by the United Industries, 6<sup>th</sup> October city, Egypt. The ingredients that used in processing fish burger formula were obtained from local market of Zagazig.

Crude propolis (honey bees glue) sample was obtained from Faculty of Agriculture, Zagazig University. Propolis samples were manually purified from impurities (wood, straw and fragments), then blended to fine particles in a Waring blender. Then propolis ethanol extract (PEE) was prepared as follow: Each 100 grams of crude propolis was dissolved in 200 ml ethanol (96%). It was shaken for half an hour and left in the laboratory over 24 hours. This procedure was repeated five times. After five days, the extract was filtered by using filter paper Watman No. 41. The propolis ethanol extract (PEE) was evaporated on water bath (40 °C) and cooling to give propolis (Kumar *et al.*, 2008). PEE natural food preservative was added in preparing silver carp burger.

**Methods:** Silver carp fillets were minced using Malounix mincer, (HV6 France) to obtain a homogenous mixture. Silver carp burger consists of the following: 82.6% minced silver carp flesh with ingredients (6% corn flour, 4% wheat flour, 2% rice flour, 0.2% garlic, 1.3% sodium chloride, 0.6% sugar, 0.1% black pepper, 0.2% onion, 0.1% cumin and 2.9% egg

white) (Tokur *et al.*, 2004). All spices were fine powder. Then all the burger ingredients were mixed well. Silver carp burger was divided into 4 groups, the first without added propolis ethanol extract (PEE, 0.0%) act as control. While the second, third and forth propolis ethanol extract (PEE) was added at ratio of 0.2%, 0.3% and 0.4% of silver carp burger respectively. The formulations were kneaded by hand until homogenous dough was obtained. Portions of 50 gm were shaped in a circular mould (9 cm diameter and 1cm thickness). Thereafter, six burger were placed on plates. In total, 24 plates were prepared, wrapped with stretch film then frozen in deep freezer (Deep freezer, model B65099A, USA) at  $-20\pm1^{\circ}$ C for 3 months. Then samples were withdrawn at random every month.

**Physico-chemical analyses:** Moisture content, total protein, lipids and ash were determined according to the methods described in AOAC (2000). Carbohydrates were calculated by difference according to Egan *et al.* (1981) as follow: Carbohydrates % = 100 - (% moisture + % crude protein + % total lipids + % ash). pH values were estimated according to the method mentioned by Özogul *et al.* (2005), using pH-meter (Orion Research Digital Ion analyzer, Model 420 a). Total volatile bases nitrogen (TVBN, mg/100g.) and trimethylamine nitrogen (TMAN, mg/100g.) values were determined according to AMC (1979). Thiobarbyturic acid (TBA, mg malonaldehyde/kg) values were determined as described by Kirk and Sawyer (1991). Peroxide values (PV) was expressed as milliequivalents of oxygen/kilogram of lipid, PV was determined according to the methods described in AOAC (2000). All the analyses were made in three replicates.

**Microbiological evaluation:** Total bacterial count (TBC), proteolytic and lipolytic bacterial count were detected according to the method described by Harrigan and Margaret (1976). Psychrophilic bacterial count (PsBC) was detected according to Swanson *et al.* (1992).

**Organoleptic evaluation:** The silver carp burger was evaluated for odor, texture, appearance and general acceptability every month during storage period. A group of 10 judges were always called upon for scoring beginning grads ranging from zero to 10 as ascribed by Teeny and Miyauchi (1972) according to the following scheme:

Score	Description	Score	Description
10	Ideal	4	Fair
9	Excellent	3	Poorly fair
8	Very good	2	Poor
7	Good	1	Very poor
6	Fairly good	0	Repulsive
5	Acceptable		-

**Statistical analysis:** Three replications of each trial were performed for the chemical composition and for TVBN, TMAN, TBA, PV, TBC, PsBC, proteolytic and lipolytic bacterial count, and sensory data were analyzed using analysis of variance (ANOVA) and the means were separated by Duncan' test (1955) at a probability level of P<0.05 SAS (2000).

### **RESULTS AND DISCUSSION**

**Chemical composition:** The chemical composition of the silver carp (*Hypophthalmichthys molitrix*) burger processed with different levels of propolis ethanol extract (PEE) is presented in Tables (1, 2 and 3). From the results, it could be noticed that silver carp burger with PEE had slight increase in moisture and ash as well as slight decrease in protein, fat and carbohydrate as compared to control burger without adding PEE. Also, the same direction was observed with increasing the level of addition PEE in treated burger. The increment in moisture could be attributed to the presence of PEE and other ingredients in the burger during frozen storage period which led to water retention as well as the effect of salt which leads to accelerating the rate of diffused moisture in fish burger. These results are in agreement with those reported by Arannilewa *et al.* (2005). From the other side the slight decrease in total protein content of frozen burger was occurred in all the samples during 3 months. The changes in

total protein may be due to protein denaturation and hydrolysis which resulted from the effect of frozen and/or microorganisms activity. These results are in agreement with those reported by Ihm *et al.* (1992a) and Asgharzadeh *et al.* (2010).

**Table 1.** Effect of propolis ethanol extract (PEE) on moisture and protein<br/>contents (%) of silver carp burger during storage for 3 month at<br/> $-20\pm1^{\circ}C$  (\* On dry weight basis).

Paramete	r		Moist	ure %		* Protein %				
PEE %		Control	0.2	0.3	0.4	Control	0.2	0.3	0.4	
Storage period (months)	0	$71.20\pm$	$71.20\pm$	$71.20\pm$	71.20±	$65.47 \pm$	$65.47 \pm$	$65.47 \pm$	$65.47 \pm$	
		0.11	0.12	0.12	0.11	0.12	0.12	0.11	0.11	
	1	71.70± 0.12 <sup>a</sup>	71.56± 0.13 <sup>ab</sup>	$71.44 \pm 0.12^{ab}$	71.31± 0.12 <sup>b</sup>	$65.07 \pm 0.11^{ab}$	$65.22 \pm 0.12^{a}$	65.29± 0.11 <sup>a</sup>	$65.36 \pm 0.12^{a}$	
	2	$72.22\pm$ 0.12 <sup>a</sup>	$71.94 \pm 0.13^{ab}$	71.70± 0.13 <sup>b</sup>	$71.44 \pm 0.13^{bc}$	$64.65 \pm 0.13^{ab}$	$64.93 \pm 0.12^{a}$	65.10± 0.12 <sup>a</sup>	$65.22\pm 0.13^{a}$	
	3	$72.75 \pm 0.13^{a}$	72.31± 0.13 <sup>b</sup>	$71.95 \pm 0.12^{\circ}$	$71.57 \pm 0.12^{d}$	$64.24 \pm 0.12^{\circ}$	$64.66 \pm 0.12^{ab}$	$64.90 \pm 0.12^{b}$	$65.13\pm 0.13^{a}$	

<sup>a-d</sup> Means within a raw with the same superscript significantly different (P<0.05). Values are expressed as Mean  $\pm$  SE.

The data indicated significantly decrease (P<0.05) in fat in control burger compared with burger samples containing PEE (Table 2). The decrement in fat during storage may be due to enzymatic activity in addition to the mechanical mincing of fish meat accelerates oxidation due to the incorporation of oxygen in the tissue. The results agree with Siddaiah *et al.* (2001) and Tokur *et al.* (2004).

In the presence study among the different formulations there was slight decrease in carbohydrate content in groups replaced with PEE. In general, fish are known to have low amounts of carbohydrate in their muscles. The decrement in carbohydrate content may be due to decomposition in frozen fish mince during storage. However, the pronounced amount of carbohydrate observed in our study might be derived from the ingredients used in fish burger formulation such as wheat flour. The data indicated significantly a gradual (P<0.05) increase in ash contents in all samples up to the end of storage period. The increase

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in ash may be derived from the ingredients and decrease in protein, fat and carbohydrate. These results are in harmony with those obtained by Taşkaya *et al.* (2003) and Tokur *et al.* (2006).

**Table 2.** Effect of propolis ethanol extract (PEE) on fat and ash contents (%) of silver carp burger during storage for 3 month at  $-20\pm1^{\circ}$ C. (On dry weight basis).

Parameter Fat %			Ash	n %					
PEE %		Control	0.2	0.3	0.4	Control	0.2	0.3	0.4
Storage period (months)	0	17.38± 0.05 <sup>a</sup>	17.38± 0.04 <sup>a</sup>	17.38± 0.05 <sup>a</sup>	17.38± 0.05 <sup>a</sup>	$9.27 \pm 0.03^{a}$	$9.27 \pm 0.03^{a}$	$9.27 \pm 0.02^{a}$	$9.27 \pm 0.03^{a}$
	1	$17.04 \pm 0.06^{ab}$	17.10± 0.06 <sup>b</sup>	$17.18 \pm 0.05^{ab}$	17.27± 0.05 <sup>a</sup>	$10.41 \pm 0.04^{a}$	$10.03 \pm 0.03^{b}$	9.80± 0.03 <sup>c</sup>	$9.59 \pm 0.05^{\rm d}$
	2	$16.65 \pm 0.07^{ab}$	$16.84 \pm 0.07^{b}$	$16.97 \pm 0.06^{ab}$	17.14± 0.07 <sup>a</sup>	$11.14 \pm 0.05^{a}$	$10.87 \pm 0.04^{b}$	$10.41 \pm 0.05^{\circ}$	$9.93 \pm 0.05^{d}$
	3	$\begin{array}{c} 16.20 \pm \\ 0.09^{d} \end{array}$	$16.51 \pm 0.08^{\circ}$	16.76± 0.07 <sup>b</sup>	$17.02 \pm 0.08^{a}$	13.06± 0.06 <sup>a</sup>	$12.72 \pm 0.06^{b}$	11.00± 0.07 <sup>c</sup>	$\begin{array}{c} 10.22 \pm \\ 0.06^{d} \end{array}$

 $^{a\text{-d}}$  Means within a raw with the same superscript significantly different (P<0.05). Values are expressed as Mean  $\pm$  SE.

**Table 3.** Effect of propolis ethanol extract (PEE) on carbohydrate contents (%) (On dry weight basis) and pH of silver carp burger during storage for 3 month at  $-20\pm1^{\circ}$ C.

Parameter	•		Carbohy	drate %		рН			
PEE %		Control	0.2	0.3	0.4	Control	0.2	0.3	0.4
	•	7.88±	7.88±	7.88±	7.88±	6.65±	6.64±	6.63±	6.60±
	U	$0.017^{a}$	$0.016^{a}$	$0.018^{a}$	$0.015^{a}$	0.015 <sup>a</sup>	0.014 <sup>a</sup>	$0.014^{a}$	0.013 <sup>a</sup>
<i>a</i> .	1	7.48±	7.65±	7.73±	7.78±	6.75±	6.68±	6.66±	6.62±
Storage		$0.020^{c}$	0.021 <sup>b</sup>	$0.022^{ab}$	0.019 <sup>a</sup>	0.016 <sup>a</sup>	0.013 <sup>ab</sup>	0.017 <sup>b</sup>	0.015 <sup>c</sup>
period	•	6.96±	7.36±	7.52±	7.71±	6.86±	6.70±	6.69±	6.65±
(months)	2	0.018 <sup>d</sup>	0.018 <sup>c</sup>	0.016 <sup>b</sup>	0.019 <sup>a</sup>	$0.017^{a}$	0.017 <sup>b</sup>	0.015 <sup>bc</sup>	0.016 <sup>c</sup>
		6.50±	7.11±	7.34±	7.69±	6.99±	6.79±	6.71±	6.67±
	3	$0.020^{d}$	0.017 <sup>c</sup>	0.021 <sup>b</sup>	0.019 <sup>a</sup>	0.019 <sup>a</sup>	$0.018^{b}$	0.017 <sup>bc</sup>	0.016 <sup>c</sup>

<sup>a-bc</sup> Means within a raw with the same superscript significantly different (P<0.05).

Values are expressed as Mean  $\pm$  SE.

**Physico-chemical changes:** pH-values are presented in Table (3). The initial pH values were 6.65, 6.64, 6,63 and 6.60 for control and samples with propolis (0.2, 0.3 and 0.4%) respectively. During freezing storage, the pH values of control samples were significantly a gradual (P<0.05) increased reaching to 6.99 at the 3<sup>rd</sup> month. A slight increase in pH values was obtained during storage periods in samples containing propolis. The

increase of pH may be attributed to the enzymatic degradation of fish muscles and production of volatile basic components (Ruiz-Capillas and Moral, 2001). Whereas by increasing the addition level of propolis extract the pH value slightly decreases. These may be attributed to the chemical composition of propolis (Kumar *et al.*, 2008).

The changes in TVBN and TMAN during frozen storage at  $-20\pm1^{\circ}$ C for 3 months are shown in Table (4): In our studies the TVB-N and TMAN values of silver carp burger in all groups increased with advancing of storage periods. The increasing rate was the highest in control samples as compared with samples containing propolis ethanol extract (PEE). However, samples containing 0.4% (PEE) revealed the lowest amount of TVB-N (11.94 mg/100g.) and TMAN (4.43 mg/100g.) at the end of storage period.

**Table 4.** Effect of propolis ethanol extract (PEE) on total volatile bases nitrogen (TVBN) and trimethylamine nitrogen (TMAN) (mg/100g) contents of silver carp burger during storage for 3 month at  $-20\pm1^{\circ}$ C.

Doromoto			TVDN (n	a/100a)		TMAN(mg/100g)			
PEE %		Control 0.2 0.3 0.4			Control	0.2	0.3	0.4	
	0	$8.97 \pm 0.022^{a}$	$8.96 \pm 0.022^{a}$	8.96± 0.021 <sup>a</sup>	8.96± 0.021 <sup>a</sup>	1.89± 0.011 <sup>a</sup>	1.88± 0.011 <sup>a</sup>	$1.85 \pm 0.11^{a}$	$1.83 \pm 0.011^{a}$
Storage period (months)	1	$11.57 \pm 0.033^{a}$	$10.96 \pm 0.031^{b}$	$9.66 \pm 0.032^{\circ}$	$9.26 \pm 0.025^{cd}$	$4.63 \pm 0.012^{a}$	$3.83 \pm 0.012^{b}$	$2.94 \pm 0.013^{c}$	$2.68 \pm 0.013^{cd}$
	2	$14.75 \pm 0.042^{a}$	$\frac{13.34\pm}{0.037^{b}}$	$10.75 \pm 0.030^{\circ}$	$9.64 \pm 0.027^{d}$	$7.42\pm 0.015^{a}$	$6.24 \pm 0.015^{b}$	$4.45 \pm 0.014^{c}$	$3.58\pm 0.013^{d}$
	3	$18.22 \pm 0.051^{a}$	$15.81 \pm 0.043^{b}$	$11.94 \pm 0.032^{c}$	$10.11 \pm 0.028^{d}$	$10.50 \pm 0.018^{a}$	8.70± 0.017 <sup>b</sup>	$6.30 \pm 0.015^{\circ}$	$\begin{array}{c} 4.42 \pm \\ 0.014^{d} \end{array}$

<sup>a-d</sup> Means within a raw with the different superscript are significantly different (p<0.05). Values are expressed as Mean ± SE.

The increment in TVB-N and TMAN during frozen storage may be due to the breakdown of protein by bacterial enzymes into non-protein N-compounds and due to the conversion of TMAO to TMA (Kelleher *et al.*, 1981). In addition, increase of pH values is referring to formation and accumulation of TVBN and TMAN. However the breakdown occurred in burger protein in the presence of propolis extract was of low rate might be due to antimicrobial agent of propolis extract. An identical behavior was described by Asgharzadeh *et al.* (2010).

In the present study Table (5) showed a continuous increase in TBA and PV in all groups during storage period. The developments of TBA and PV values were very slow in burger containing PEE significantly (p<0.05) compared to control burger. The highest TBA value was obtained at the 3<sup>rd</sup> month; at 1.83 mg malonaldehyde/kg for control burger but the samples did not reach detectable levels of rancidity. Moreover, samples containing 0.4% PEE revealed the lowest amount of TBA and PV at the end of storage period. This could be explained as a result of ice crystals formation which could injure the cell and cause the release of pro-oxidant molecules (hemoproteins and metal ions) for lipid oxidation (Asgharzadeh *et al.*, 2010).

**Table 5.** Effect of propolis ethanol extract (PEE) on TBA (mg<br/>malonaldehyde/kg.) and PV (milliquivalent peroxide/ kg.)<br/>contents of silver carp burger during storage for 3 month<br/>at  $-20\pm1^{\circ}$ C.

Parameter		TBA	(mg malo	naldehyde	e/kg.)	PV (milliquivalent peroxide/ kg.)			
PEE %		Control	0.2	0.3	0.4	Control	0.2	0.3	0.4
Storage period (months)	0	$0.18 \pm 0.003^{a}$	$0.18 \pm 0.003^{a}$	$0.18 \pm 0.002^{a}$	$0.18 \pm 0.002^{a}$	$0.95 \pm 0.005^{a}$	$0.95 \pm 0.005^{a}$	$0.95 \pm 0.006^{a}$	$0.95 \pm 0.005^{a}$
	1	$0.69 \pm 0.004^{a}$	$0.58 \pm 0.003^{b}$	$0.48 \pm 0.003^{\circ}$	$0.38 \pm 0.002^{d}$	1.96± 0.009 <sup>a</sup>	$1.75 \pm 0.008^{ab}$	$1.38 \pm 0.007^{b}$	$1.15 \pm 0.006^{c}$
	2	$\begin{array}{c} 1.25 \pm \\ 0.008^{a} \end{array}$	$1.05 \pm 0.006^{b}$	$0.87 \pm 0.005^{\rm c}$	$\begin{array}{c} 0.60 \pm \\ 0.004^{d} \end{array}$	$3.17 \pm 0.013^{a}$	$\begin{array}{c} 2.61 \pm \\ 0.012^{b} \end{array}$	$1.91 \pm 0.011^{c}$	$\begin{array}{c} 1.41 \pm \\ 0.010^{d} \end{array}$
	3	1.83± 0.013 <sup>a</sup>	1.59± 0.012 <sup>b</sup>	$1.35 \pm 0.011^{\circ}$	$\begin{array}{c} 0.95 \pm \\ 0.008^{d} \end{array}$	$4.60 \pm 0.021^{a}$	$\begin{array}{c} 3.52 \pm \\ 0.018^{b} \end{array}$	$2.63 \pm 0.016^{\circ}$	$\begin{array}{c} 1.71 \pm \\ 0.012^{d} \end{array}$

<sup>a-d</sup> Means within a raw with the different superscript are significantly different (p<0.05). Values are expressed as Mean  $\pm$  SE.

The TBA and PV values were very slowly increased in fish burger containing PEE may be due to antioxidants properties of ingredients and propolis extracts, which retarded the development of rancidity and the decrease of thiobarbituric acid values (Hemeida and Abd-Alfattah, 1993). These results are in agreement with Siddaiah *et al.* (2001) and Al-Bulushi *et al.* (2005).

**Microbiological evaluation:** The achieved results presented in Table (6) and (7) showed the changes in bacterobiological count of fish burger processed from silver carp with different levels of propolis during storage for 3 month at  $-20\pm1^{\circ}$ C. The total bacterial count (TBC), protolytic and lipolytic bacterial count (Log 10 CFU/g) in all treatments showed significant decrease (P<0.05) between all burger processed from silver carp and containing (0.0, 0.2, 0.3 and 0.4%) of propolis extract (P<0.05). As it can be inferred, that the lowest content of total bacterial count, protolytic bacterial count were detected in fish burger containing 0.4% followed by 0.3 and 0.2% propolis respectively, compared to control samples at the end of storage period.

**Table 6.** Effect of propolis ethanol extract (PEE) on total bacterial count (TBC) and psychrophilic bacterial count (PsBC) (Log10 CFU/g) contents of silver carp burger during storage for 3 month at  $-20\pm1^{\circ}$ C.

Paramete	ameter TBC (Log10 CFU/g)					Ps BC (Log10 CFU/g)				
PEE %		Control	0.2	0.3	0.4	Control	0.2	0.3	0.4	
Storage period (months)	0	$4.57 \pm 0.017^{a}$	$4.48 \pm 0.018^{a}$	$4.45 \pm 0.016^{a}$	$4.41 \pm 0.017^{a}$	$1.33 \pm 0.012^{a}$	1.29± 0.011 <sup>a</sup>	$1.28 \pm 0.010^{a}$	$1.27 \pm 0.011^{a}$	
	1	$4.48 \pm 0.017^{a}$	$4.34 \pm 0.017^{ab}$	$4.22\pm 0.018^{b}$	$4.09 \pm 0.017^{bc}$	1.46± 0.013 <sup>a</sup>	$1.38 \pm 0.012^{b}$	$1.35 \pm 0.013^{bc}$	$1.30\pm 0.012^{c}$	
	2	$\begin{array}{c} 4.37 \pm \\ 0.018^a \end{array}$	$4.15 \pm 0.017^{b}$	$3.95 \pm 0.019^{bc}$	$3.74 \pm 0.018^{c}$	$1.61\pm 0.013^{a}$	1.49± 0.013 <sup>b</sup>	$1.44 \pm 0.013^{bc}$	$1.35 \pm 0.012^{c}$	
	3	$4.26 \pm 0.018^{a}$	$3.97 \pm 0.019^{b}$	$3.69 \pm 0.019^{c}$	$\begin{array}{c} 3.38 \pm \\ 0.018^d \end{array}$	$1.75 \pm 0.012^{a}$	1.60± 0.013 <sup>b</sup>	$1.51 \pm 0.012^{c}$	1.38± 0.013 <sup>d</sup>	

 $^{a\text{-d}}$  Means within a raw with the different superscript are significantly different (p<0.05). Values are expressed as Mean  $\pm$  SE.

On other side, changes in psychrophilic bacterial count (PsBC) (Log10 CFU/g) indicated that, a gradual increase of PsBC was observed during storage period at  $-20\pm1^{\circ}$ C in control more than samples treated with propolis. However, the results showed slight increase of PsBC in samples treated with propolis throughout storage period. The

psychrophilic load counts developed may be due to the presence of psycrophilic bacteria which activated at low temperature. The results are in agreement with those obtained by Elzbita and Marina (1987) and Suvanich *et al.* (2000a). Generally, the reduction in numbers of microorganisms as indicated previously may be due to the mechanical damage of bacterial cell caused by crystals during freezing as well as due to bactericidal and bacteriostatic properties of propolis (Hegazi *et al.*, 2000). Also, other the ingredients used in fish burger as spices, sodium chloride, garlic and onion powder, which were all effective in inhibiting the bacterial growth. These results are in harmony with those obtained by Al-Bulushi *et al.* (2005).

**Table 7.** Effect of propolis ethanol extract (PEE) on total lipolytic and proteolytic bacterial count (Log10 CFU/g) contents of silver carp burger during storage for 3 month at  $-20\pm1^{\circ}$ C.

Parameter	•	Lipolytic (Log10 CFU/g)				Protolytic (Log10 CFU/g)			
PEE %		Control	0.2	0.3	0.4	Control	0.2	0.3	0.4
	0	$1.45 \pm$	$1.39\pm$	$1.38\pm$	1.37±	$1.56\pm$	1.52±	$1.51\pm$	$1.50\pm$
		0.011	0.012	0.012	0.011	0.012	0.011	0.012	0.012
Storage period (months)	1	1.35± 0.012 <sup>a</sup>	$1.28 \pm 0.012^{b}$	1.22± 0.013 <sup>bc</sup>	1.17± 0.012 <sup>c</sup>	$1.45 \pm 0.011^{a}$	1.39± 0.012 <sup>b</sup>	$1.34 \pm 0.012^{bc}$	1.29± 0.011 <sup>c</sup>
	2	$1.23 \pm 0.013^{a}$	$1.15 \pm 0.011^{b}$	$1.03 \pm 0.012^{bc}$	$0.93 \pm 0.013^{c}$	$1.32 \pm 0.011^{a}$	$1.24 \pm 0.011^{b}$	$1.14 \pm 0.011^{c}$	$1.05 \pm 0.009^{d}$
	3	1.13± 0.012 <sup>a</sup>	$1.02 \pm 0.011^{b}$	$0.85 \pm 0.011^{\circ}$	$0.71 \pm 0.011^{d}$	1.20± 0.012 <sup>a</sup>	1.10± 0.011 <sup>b</sup>	$0.95 \pm 0.009^{\circ}$	$\begin{array}{c} 0.81 \pm \\ 0.009^d \end{array}$

<sup>a-d</sup> Means within a raw with the different superscript are significantly different (p<0.05). Values are expressed as Mean  $\pm$  SE.

**Organoleptic evaluation:** The analyses of sensory scores for silver carp burger with different levels of propolis extract are shown in Table (8). During storage period, there was gradual decrease in the scores. The scores were significantly high (P<0.05) in burger containing 0.4% propolis extract and followed in order by the burger containing 0.3, 0.2 % and control respectively. These results may be attributed to propolis extract which have a role in inhibiting microbial growth, biochemical changes and quality loss. Moreover the sensory properties of burger decreased as TVB-N and TMAN, TBA, PV and TBC values increased. The results coincide with those given by Tokur *et al.* (2006).

In conclusion, this study recommends using of 0.4% propolis ethanol extract (PEE) as an antimicrobials and antioxidants agents to keep the quality of silver carp burger during storage at  $-20\pm1^{\circ}$ C for 3 month.

Paramete	r		Od	lor			Tex	ture	
PEE %		Control	0.2	0.3	0.4	Control	0.2	0.3	0.4
	0	$9.00\pm$	9.00±	9.00±	9.00±	9.00±	9.00±	9.00±	9.00±
	U	$0.082^{a}$	$0.082^{a}$	$0.083^{a}$	$0.082^{a}$	$0.071^{a}$	$0.072^{a}$	$0.007^{a}$	$0.073^{a}$
	1	8.30±	$8.50\pm$	8.70±	$8.80\pm$	8.20±	8.60±	8.70±	$8.80\pm$
Storege	1	0.063 <sup>c</sup>	$0.062^{b}$	$0.070^{ab}$	$0.074^{a}$	$0.064^{ad}$	0.065 <sup>c</sup>	0.071 <sup>b</sup>	$0.070^{a}$
	2	$7.50\pm$	7.90±	$8.20\pm$	$8.50\pm$	7.30±	7.90±	8.30±	8.70±
	2	$0.052^{c}$	$0.052^{bc}$	$0.052^{b}$	0.063 <sup>a</sup>	0.051 <sup>d</sup>	0.061 <sup>c</sup>	0.072 <sup>b</sup>	$0.075^{a}$
	3	$6.50\pm$	$7.40\pm$	7.80±	$8.20\pm$	$6.60\pm$	7.60±	7.85±	8.60±
Storage		0.051 <sup>d</sup>	0.051 <sup>c</sup>	.050 <sup>b</sup>	0.063 <sup>a</sup>	0.052 <sup>d</sup>	0.043 <sup>c</sup>	$0.065^{b}$	0.071 <sup>a</sup>
(months)			Appea	arance	Over all acceptability				
	•	$8.80\pm$	8.90±	8.90±	8.90±	89.3±	89.7±	89.7±	89.7±
	U	$0.071^{a}$	$0.072^{a}$	$0.072^{a}$	$0.073^{a}$	0.41 <sup>a</sup>	$0.60^{a}$	$0.50^{a}$	0.71 <sup>a</sup>
	1	7.80±	8.00±	8.50±	8.70±	81.0±	83.7±	86.3±	87.7±
	I	0.066 <sup>bc</sup>	$0.070^{b}$	0.073 <sup>ab</sup>	$0.074^{a}$	0.70b <sup>c</sup>	0.41 <sup>b</sup>	0.73 <sup>ab</sup>	$0.82^{a}$
	2	6.63±	7.50±	8.07±	8,37±	71.4±	77.7±	81.9±	85.2±
	2	0.054 <sup>d</sup>	0.063 <sup>c</sup>	0.071 <sup>b</sup>	$0.072^{a}$	0.60 <sup>d</sup>	0.72 <sup>c</sup>	0.71 <sup>b</sup>	0.43 <sup>a</sup>
	2	5.60±	6.90±	7.60±	8.00±	62.3±	73.0±	77.5±	82.7±
	3	0.045 <sup>d</sup>	0.056 <sup>c</sup>	0.064 <sup>b</sup>	$0.071^{a}$	0.51 <sup>d</sup>	$0.70^{c}$	0.62 <sup>b</sup>	$0.40^{a}$

**Table 8.** Effect of propolis ethanol extract (PEE) on odor, texture, appearance and over all acceptability of silver carp burger during storage for 3 month at  $-20\pm1^{\circ}$ C.

<sup>a-d</sup> Means within a raw with the different superscript are significantly different (p<0.05). Values are expressed as Mean  $\pm$  SE.

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تأثير استخدام البروبلس والتخزين بالتجميد علي جودة برجر سمك المبروك الفضى سامية ابراهيم على حسنين قسم بحوث التصنيع ومراقبة الجودة، المعمل المركزي لبحوث الثروة السمكية،

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أجريت هذه الدراسة لتقييم برجر سمك المبروك الفضى المجهز من شرائح بدون جلد مع استخدام منتج طبيعى للنحل (صمغ النحل - البروبلس) المستخلص بالايثانول propolis ethanol extract (PEE) وكانت مستويات الإضافة هى ٠.٠ ، ٠.٠ ، ٠.٠ % بالإضافة إلى تصنيع برجر سمك المبروك الفضى بدون بروبلس (كنترول) وذلك لمعرفة تأثير استخدام البروبلس على خصائص جودة البرجر خلال التخزين بالتجميد. وقد تم تقدير التركيب الكيميائى، وبعض الخواص الفيزيائية والكيميائية والميكروبيولوجية والحسية للبرجر خلال التخزين عند(-٥. ٢ °م) لمدة ثلاثة أشهر وقد أوضحت النتائج انه يمكن أضافه مستخلص البروبلس كمادة حافظة طبيعيه بنسبة ٠.٠ % وذلك للمحافظة على جودة برجر السمك وبالتالى إطالة صلاحيته.