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PHYSICO-CHEMICAL PROPERTIES OF PROPOLIS AND ITS APPLICATION AS A FOOD PRESERVATIVE IN FISH KOFTA

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ABSTRACT

In the current study, the local Egyptian and imported propolis were characterized. The effect of addition of local propolis at different levels (0.5, 1.0, and 1.5%) on the fish (*Oreochromis niloticus*) kofta shelf life and its quality during storage at 4±2°C for twenty days was estimated. The dominant components found in all propolis samples were guaia-1(10)-en-11-ol (Bulnesol) ranging between 7.32 to 19.5%, guaiol 8.67 to 12.7%, pinostrobin chalcone, 6.66 to 12.2%, α-curcumene 7.31 to 12.0% and β-curcumene 4.07 to 8.73%. The highest element content was potassium followed by calcium, sodium and barium for local and imported propolis, respectively. The addition of local propolis to fish kofta had no negative effect regarding the sensory properties at the beginning of storage. It was clearly noticed that the addition of propolis to fish kofta decreased the malondialdehyde production, total counts of bacterial development, and increased the DPPH inhibition in the cold stored fish kofta. And also, the lowest color changes were observed in 1.5% sample followed by 1.0%, 0.5% and finally control. It could be noted that the addition of local propolis to fish kofta prolonged its shelf life.

Keywords: Propolis, Nile tilapia, Kofta, shelf life, sensory properties

1. INTRODUCTION

Propolis is an important product of the bees that means the "city's guardian". In reviews it has been called as Russian Penicillin (Ahangari *et al.*, 2018). In the hive, honey bees prepare it to seal the cracks, smooth walls as well as keeping stability of moisture and temperature. Naturally, propolis is a gummy matter which is gathered by the honey bees from resin of leaves and flowers of some plants and it is obtained after mixing with their saliva (Gupta *et al.*, 2007). Colors of Iraqi propolis had a broad range of varieties between brownish yellow and dark brown (Ali *et al.*, 2012). Propolis is an viscus, obscure yellow to brown colored material resin odor (Seven *et al.*, 2010). Propolis is a very complex mixture and, in general, it is composed of 50% balsams and resins, 30% wax, 10% essential oils, 5% pollen and 5% of various other substances like sugars, vitamins, etc. (Bankova *et al*., 2000).

Propolis extracts have an antimicrobial activity against *S. mutans* existing in the mouth and it can be used for treatment of the mouth and teeth diseases (Duailibe *et al.*, 2007). Propolis dosages in poultry feeding markedly reduced the numbers of Enterobacteriaceae identified from chicken's crops (Kročko *et al*., 2012). Propolis contains organic acids, flavonoids, phenolics, and their derivatives. These biological components are active and responsible for its various activities such as antioxidant, antibacterial, antiviral, antifungal effects (Talas & Gulhan, 2009; Tosi *et al.*, 2007). Moreover, it can protect lipid in various systems from the peroxidation and formation of the free radicals (Seven *et al*., 2009).

Propolis extracts are commonly prepared by continuous soaking in different solvents, like water, methanol, ethanol, chloroform, dichloromethane, ether, hexane and acetone, also the ultrasonic and microwave methods was used for that purpose (Cvek *et al.*, 2008; Ahangari *et al*., 2018). Propolis collected from both temperate zone or tropical zones are slightly different in chemical composition (Anjum *et al*., 2018; Sforcin *et al.*, 2000). Several researches were conducted to identify propolis components. More than five hundred components were found in propolis, belong to flavonoids, terpenoids, phenylpropanoids, stilbenes, lignans, coumarins and their prenylated derivatives (Huang *et al.*, 2014; Sforcin, 2016). Propolis biological activities are related to a number of basic chemical compounds like phenolic acids and its esters, flavonoids, and terpenoids, such as caffeic acid phenethyl ester (CAPE), artepillin C, caffeic acid, chrysin and galangin quercetin, apigenin, kaempferol, pinobanksin 5-methyl ether, pinobanksin, pinocembrin, and pinobanksin 3-acetate (Huang *et al*., 2014).

Many techniques were conducted to measure the chemical compounds of propolis including HPLC, GC, TLC, GC-MS chromatographic systems as well as nuclear spectroscopic methods. These techniques led to the identification of several

compounds in propolis, such as flavonoids, terpenoids, polyphenols, esters, minerals, hydrocarbons and sugars (Ahangari *et al*., 2018).

Propolis has several applications in food products. It had a remarkable antimicrobial effect against the yeast present in deteriorated juices (Koc *et al*., 2007). Propolis was effective additive in fresh oriental sausage preservation (Ali *et al*., 2010). Silici & Karaman (2014) studied the inhibition action of propolis on patulin yielded from *Penicillium expansum* in the juice of apple and reported that propolis could be applied as natural food preservative instead of synthetic. The quality of fish products was improved by adding propolis with the level up to 0.6% as well as the shelf-life of frozen stored of such products (Hassanin & El-Daly, 2013). Propolis extract was used as edible coating for papaya fruits and exhibited a huge microbial effects without adverse effects on the physicochemical properties of the fruits (Barrera *et al*., 2015). The propolis hydroalcoholic extract was effective coating and reduce the weight loss in orange fruits up to eighteen days storage (Passos *et al*., 2016).

Many pharmaceutical actions including anti-inflammatory (Dobrowolski *et al*., 1991), immune-stimulating (Dimov *et al*., 1992), cytostatic (Frenkel *et al*., 1993) antiviral (Amoros *et al*., 1994), hepato-protective (Gonzalez *et al*., 1995), antifungal (Kujumgiev *et al*., 1999), antioxidant (Isla *et al*., 2001), antibacterial (Lu *et al*., 2003), antitumor (Orsolic *et al*., 2003), and antiprotozoal (Falcão *et al*., 2014) effects have been attributed to propolis.

The main goals of this study were to determine the physical characteristics, chemical composition of extractable part and minerals of local and impor--ted propolis. In addition, TBA (The thiobarbituric acid) number, total bacterial count, antioxidant activity, color values and sensory properties of fish kofta enriched with different levels of local Egyptian propolis during cold storage at 4°C were determined.

2. MATERIALS AND METHODS

2.1. ***Materials:*** The Nile tilapia (*Oreochromis niloticus*) fish samples were purchased from a local farm for fish production at San El-Hagar, Sharkia Governorate (Egypt), weighed 500 ±30g. Fish kept cold using icebox partially filled with ice (2 h) till delivery to Food Sci. Dept. Lab, Fac. Agric., Zagazig Univ. The local propolis was obtained from Plant Protec. Dept., Fac. Agric., Zagazig Univ. The imported propolis (Chinese product), fresh onion, fresh garlic, green coriander, edible salt, rusk and spices were obtained from local supermarkets, Zagazig City, Sharkia, Egypt.

2.2. ***Samples preparation:*** Because of the complex structure of propolis, it cannot be used directly and needs to be extracted with the help of a suitable solvent. The ethanol extract of local propolis (EELP) and imported propolis (EEIP) were prepared by mixing three grams of each type with 10.0 mL of ethanolic alcohol (95 %). The resultant solutions were shaken for 24h at the dark then filtered and frozen to separate the wax (Chang *et al*., 2008).

The *n*-hexane extract of local propolis (HELP) and imported propolis (HEIP) were obtained using another 3.0 g of each sample by cold percolation method. The samples were poured into a percolator and 10 mL of hexane was added. The blend was stirred with a glass rod at intervals for 48 h, after that the percolator was opened and the solution filtered using filter paper (Whatman, No. 1) (Talla *et al*., 2014).

2.3. Propolis characterization

2.3.1. ***Physical characteristics determination of propolis:*** Physical characteristics (appearance, form, color and odor) of local and imported propolis were determined by direct observations according to (López *et al*., 2003).

2.3.2. ***Gas chromatography coupled to mass spectrometry (GC-MS):*** GC-MS was conducted as described by Chang *et al*., (2008). The test was carried out in a gaseous chromatograph (model GC-17A, USA) supplied with a capillary column (DB-5 30 meters), 0.25 mm of I.D., 0.25 µm of thin layer, in addition to a mass spectrometer of the same type, model GCMS-QP5000, supplied with database of 330.000 mass spectra. The experiment were conducted under the following conditions: initial temperature of column at 60°C, injector at 220°C and mediator at 240°C; program column 60-240°C at 3°C/min, 20 min at 240°C. One microliter of the specimen was provided under helium as transporter gas. The apparatus run with energy of 70 eV and the mass indicator included molecules between 40 to 450 Da.

2.3.3. ***Determination of minerals content:*** Potassium (K), calcium (Ca), sodium (Na), magnesium (Mg), barium (Ba), chrome (Cr), manganese (Mn), iron (Fe), nickel (Ni) and zinc (Zn) concentrations were determined as described by Maat (2015) using atomic absorption spectrophotometer (iCE 3000 series, England).

2.4. ***Preparation of fish kofta and treatments:*** When fish samples arrived at the Lab., the fish were directly prepared (by cutting the heads, fins e.t.c.), gutted, dressed and carefully washed under tap water, then filleted and skinned by hand. The yield of flesh was 38%. The prepared fillets were cut and minced by a kitchen mincer (Kenwood MG510,

England) using a 3 mm diameter holes plate. Minced fish were mixed with the ingredients to produce Nile tilapia fish kofta mince (Hassanin & El-Daly, 2013). The fish kofta mince [87% Nile tilapia fillet mince, 1.7% fresh onion, 1.3% fresh garlic, 6.5% green coriander, 1.7% salt and 1.3% spices mixture (25% black pepper, 25% cardamom, 20% Chinese cubeb, 10% cinnamon, 10% red pepper, 10% laurel leaf)] were divided into four batches. 5 mL distilled water was added to the first batch and served as control sample of fish kofta (coded FKControl). The second, third and fourth batches were mixed with 0.5, 1.0 and 1.5 g/100g of local Egyptian propolis (each propolis quantity were dissolved in 5mL of distilled water) to produce treated fish kofta and coded FK0.5%, FK1.0% and FK1.5%, respectively. Thereafter, the control and treated fish kofta samples were manually formed in spherical shapes (in 15±1 cm^3) and coated with rusk. Polyethylene packages were used for packaging then the samples were cold stored at 4±2°C for 20 days.

2.4.1. ***Thiobarbituric acid determination:*** The thiobarbituric acid (TBA) was measured according to Kirk & Sawyer (1991) and recorded as mg malondialdehyde/kg tested sample.

2.4.2. ***DPPH· radical scavenging measurement:*** The free-radical scavenging activities of the fish kofta enriched with various concentrations of local propolis (0.5, 1.0 and 1.5%) as well as control samples were tested as bleaching of the stable 2,2-diphenyl-1-picrylhydrazil (DPPH·) according to Matsushige *et al.*, (1996). Ten grams of each examined sample were extracted with 10 mL of methanol with shaking for 30 min, and then the resulted blend was filtered using Whatman filter paper no. 1. Four milliliters of the tested sample was added to methanol and then (1 mL) of DPPH was added to final concentration of DPPH 2 mM. After mixing for 10 sec, it was left to stay out of light for 30 min at room temperature (27±2°C). After the incubation the absorbance was detected at 520 nm using a 6405 UV/vis spectrophotometer. The percent (I%) of scavenging activity was measured using the absorbance of the tested samples and the blank control sample as following:

$$I\,\% = \frac{A_{520}\ \text{blank} - A_{520}\ \text{sample}}{A_{520}\ \text{blank}} \times 100$$

2.4.3. ***Bacteriological evaluation:*** Total bacterial count (TBC) was determined as described by Harrigan & McCance (1976).

2.4.4. ***Color determination:*** Color characteristics (lightness (L), redness (a) and yellowness (b)) of treated and untreated fish kofta were measured as described by Modi *et al.*, (2009) using Hunter Lab color analyzer (Hunter Lab Color Flex EZ, USA). The rusk layer of the tested fish kofta was removed before measuring its color. After calibration of the apparatus, the means of three replicates were recorded. The overall color changes were determined by the delta-E relation as follows:

$$\Delta E = \sqrt{(L-L_0)^2 + (a-a_0)^2 + (b-b_0)^2}$$

2.4.5. ***Sensory evaluation:*** Fish kofta samples were assessed for sensory properties at the beginning of the storage period as described by Alasalvar *et al.*, (2001). After frying at 160°C for 5 min using corn oil, the fillets were gently drained for 5 minutes after frying to remove the excess oil (Jayasena *et al.*, 2018). Panelists were asked to evaluate fish kofta samples for color, taste, texture, appearance, odor and overall acceptability. The samples served warm to the panelists after coding using letters and randomly presented to the panelists. A group of 10 judges (members of Food Sci. Dept., Fac. Agric., Zagaizg Univ.) were always invited upon for scoring from zero to ten.

2.5. ***Statistical analysis:*** The obtained data were analyzed using MSTAT-C program (MSTAT-C, 1986) for analysis of variance. Means were recorded and analyzed using Fisher's protected least significant differences (LSD) test at 5% probability level (Steel *et al.*, 1997). Tests were conducted in triplicates and recorded as mean ± standard deviation (SD). Means having the same letters are not significantly different.

3. RESULTS AND DISCUSSION

3.1. ***Physical characteristics of propolis:*** Data in Table 1 present the physical characteristics of local and imported propolis. Data of physical characteristics (appearance, form, color, and odor) of local and imported propolis reflected wide differences between propolis samples. Such variations may be due to geographical origin and flora vegetation of each propolis sample (Ali *et al.*, 2012).

Table 1: Physical characteristics of local and imported propolis.

Propolis sample	Appearance and form	Color	Odor
Local	Rigid waxy	Reddish brown	Very aromatic resinous
Imported	Rigid, powder	Light brown	Aromatic resinous

3.2. *Chemical composition of propolis extracts:* Data in Table 2 present the chemical composition of ethanol extract of local propolis (EELP), ethanol extract of imported propolis (EEIP), hexane extract

of local propolis (HELP), and hexane extract of imported propolis (HEIP). Thirteen components were identified in EELP and HEIP, while twenty nine components were identified in EEIP and HELP. The most dominant components found in all propolis samples were guaia-1(10)-en-11-ol (bulnesol) ranging between 7.32 to 19.5%, guaiol 8.67 to 12.7%, pinostrobin chalcone, 6.66 to 12.2%, α-curcumene 7.31 to 12.0% and β-curcumene 4.07 to 8.73%. On the other hand, 2-methoxy-4-vinyl-phe-nol and lanceol were found only in the case of EELP, while cinnamyl alcohol (2-propen-1-ol, 3-phe-nyl); spiro [4.5] dec-6-en-8-one, 1,7-dimethyl-4-(1-methylethyl); 10-12-pentacosadiynoic acid and iso-aromadendrene epoxide were found only in the case of EEIP. EELP contains the highest total identified components recording 99.4%. The propolis composition markedly related to place and time of collection (Bankova *et al.*, 1998). Wiryowidagdo *et al.*, (2009) identified 37 components from ethanolic extract of propolis collected from tropical zone (Java, Indonesia).

Results in Table 3 show the mineral content of local and imported propolis samples. The data revealed that propolis is considered a rich source for minerals. The highest element content was potassium followed by calcium, sodium and barium recording 5271.0, 11007.5; 2433.8, 1817.0; 1013.3, 673.3 and 162.7, 169.9 ppm for local and imported propolis, respectively. Ca, K, Mg, Na, Al, B, Ba, Cr, Fe, Mn, Ni, Sr and Zn were discovered by atomic emission/absorption spectrometry in propolis samples collected from different regions (Cvek *et al*., 2008).

3.3. *Fish kofta characteristics:* Data in Table 4 illustrate the sensory properties of fresh fish kofta containing different concentrations of local propolis (0.5, 1.0, and 1.5%) as well as control sample. It was clearly noticed that all studied samples recorded high score (> 8) for all sensory properties. Statistically, no significant ($p \leq 0.05$) differences were founded between all samples for color, taste, texture, appearance, odor and overall acceptability. The addition of propolis to fish kofta has no negative effect regarding to the sensory properties. Ali *et al*., (2010) reported that fresh sausage treated with 0.6% of propolis extract was fully accepted by judges.

It is known that fish and their products have short shelf life due to the lipid oxidation (Özogul *et al.*, 2005). Thiobarbyturic acid (TBA) value has been greatly used to determine the degree of the secondary oxidation of lipids (Nishimoto, 1985). TBA reactive substances are responsible for the second stage of auto oxidation during which the aldehyde and ketone formed (Lindsay, 1994). Data in Table 5 summarize the TBA values of fish kofta containing different concentrations of propolis (0.5, 1.0 and 1.5%) and control samples during cold storage at 4°C for 20 days. The TBA values of all treatments increased gradually during the storage period. However, the increase of TBA values was very slow in the samples treated with high propolis concentration. At the 20th day of storage, the maximum TBA value was detected in the FKControl sample reaching 5.83±0.40 mg malondialdehyde/kg, while the minimum was in the FK1.5% sample reaching 1.15±0.07 mg malondialdehyde/kg. Accordingly, it was clearly

Table 2: Chemical composition (%) of EELP, EEIP, HELP and HEIP

Retention times (min)	Component (%)	EELP	EEIP	HELP	HEIP
19.73	Phenyl ethyl Alcohol= Benzene ethanol	4.24	6.65	1.83	1.10
26.38	Acetic acid, 2-phenylethyl ester= Phenyl ethyl acetate	*	2.29	1.61	1.27
29.32	2-Methoxy-4-vinylphenol	2.50	*	*	*
29.56	Cinnamyl alcohol = 2-Propen-1-ol, 3-phenyl	*	4.82	*	*
30.92	Propionic acid, phenethyl ester	*	*	*	0.70
34.04	α-Guaiene	1.09	1.00	1.75	1.76
35.22	α-Bergamotene	2.04	2.88	2.68	2.83
35.88	β-curcumene	4.06	4.07	8.23	8.73
36.14	α-Curcumene	7.59	7.31	11.65	12.09
36.71	*Trans*-α-Bergamotene	1.08	1.19	1.82	1.75
37.27	β-Bisabolene	3.46	3.81	5.42	5.53
37.56	Sesquicineole	*	*	1.06	0.95
38.07	Farnesol	1.04	1.11	1.38	1.23
38.54	*cis*-α-Bisabolene	*	*	2.07	2.27
38.70	Methyl 10,12-pentacosadiynoate	1.96	1.90	*	*
41.39	Guaiol	10.65	8.67	11.65	12.73
43.00	γ-Eudesmol	4.75	3.55	4.70	5.04
44.06	β-Eudesmol	4.17	*	5.08	4.09
44.31	Guaia-1(10)-en-11-ol= Bulnesol	7.32	19.59	10.90	10.77

44.99	α-Bisabolol	4.94	3.78	5.54	5.76
45.76	α-Tumerone	1.48	1.73	1.97	1.85
48.00	Spiro[4.5]dec-6-en-8-one,1,7-dimethyl-4-(1-methylethyl)	*	0.78	*	*
49.16	10-12-Pentacosadiynoic acid	*	0.75	*	*
50.35	Lanceol, cis	2.27	*	*	*
50.81	β-Citrylideneethanol	*	*	1.26	1.47
51.03	Isoaromadendrene epoxide	*	1.85	*	*
55.40	Palmitic acid, ethyl ester= Hexadecanoic acid, ethyl ester	1.29	0.90	0.70	0.65
56.63	12-Methyl-E,E-2,13-octadecadien-1-ol	2.55	1.60	*	*
58.50	10-Heneicosene	3.39	1.57	*	*
58.95	9,12-Octadecadienoyl chloride, (Z,Z)- = Linoleic acid chloride	2.18	1.51	1.50	1.28
59.41	Octadecanoic acid, methyl ester = Methyl stearate	*	*	0.67	0.73
60.54	Linoleic acid ethyl ester	1.14	*	0.92	0.98
61.73	Hi-oleic safflower oil	*	1.54	*	*
60.87	Oleic acid=9-Octadecenoic acid (Z)	2.24	0.88	1.72	1.09
61.61	Heptadecanoic acid, ethyl ester	1.61	*	0.96	0.92
62.23	Nonadecanol	*	*	0.94	0.84
63.11	1-Hexadecanol, 2-methyl	1.27	*	0.95	0.78
65.06	1-Heptatriacotanol	2.52	*	*	*
66.40	Ethyl iso-allocholate	1.12	1.53	*	*
67.58	*cis*-10-Nonadecenoic acid	1.10	0.85	0.93	0.89
71.81	Pinostrobin chalcone= 2-Propen-1-one, 1-(2,6-dihydroxy-4-methoxyphenyl)-3-phenyl-, (E)-	12.27	9.72	6.71	6.66
76.47	7-Heptadecyne, 17-chloro	2.08	1.35	2.73	2.57
Total identified		99.40	99.18	99.33	99.31

* Not Detected.

Table 3: Minerals content (ppm) of local and imported propolis.

Elements (ppm)	Local propolis	Imported propolis
Calcium (Ca)	2433.8	1817.0
Potassium (K)	5271.0	11007.5
Magnesium (Mg)	14.7	33.5
Sodium (Na)	1013.3	673.3
Barium (Ba)	162.7	169.9
Chrome (Cr)	8.1	7.7
Iron (Fe)	124.1	153.2
Manganese (Mn)	15.7	12.8
Nickel (Ni)	2.9	3.2
Zinc (Zn)	13.4	16.3

Table 4: Sensory evaluation of fish kofta supplemented with propolis at different concentrations compared to control at the beginning of cold storage at 4°C

Treatments	Color	Taste	Texture	Appearance	Odor	Overall acceptability
FKControl	8.58±0.38	8.06±0.48	8.11±0.50	8.37±0.61	8.29±0.67	9.20±0.40
FK0.5%	8.34±0.59	8.50±0.50	8.50±0.47	8.40±0.68	8.40±0.75	9.33±0.40
FK1.0%	8.90±0.60	8.81±0.58	8.15±0.50	8.48±0.50	8.60±0.73	9.32±0.52
FK1.5%	8.70±0.70	8.83±0.65	8.00±0.60	8.17±0.38	8.37±0.55	9.11±0.55
LSD at 0.05 %	NS	NS	NS	NS	NS	NS

LSD= least significant difference; NS= Not Significant.

Table 5: Effect of propolis addition compared to control on TBA concentration (mg malonaldehyde/kg fish kofta) during 20 day cold storage at 4°C

Treatments	Storage time (day)					
	0	4	8	12	16	20
	TBA concentration (mg malondialdehyde/kg fish kofta)					
FKControl	0.20±0.10	1.90±0.20^{a}	2.73±0.35^{a}	4.78±0.45^{a}	5.04±0.41^{a}	5.83±0.40^{a}
FK0.5%	0.21±0.20	0.47±0.20^{b}	0.98±0.14^{b}	1.73±0.39^{b}	1.93±0.22^{b}	2.60±0.73^{b}
FK1.0%	0.19±0.08	0.40±0.6^{b}	0.80±0.10^{b}	0.99±0.20^{c}	1.54±0.33^{bc}	1.72±0.19^{c}
FK1.5%	0.18±0.12	0.38±0.05^{b}	0.75±0.21^{b}	0.97±0.23^{c}	1.22±0.21^{c}	1.15±0.07^{d}
LSD at 0.05 %	NS	0.27	0.42	0.63	0.57	0.55

LSD= least significant difference; NS= Not Significant.

noticed that the addition of propolis to fish kofta processing decrease the malondialdehyde production for fish kofta during cold storage. Such results were reviewed by Hassanin & El-Daly (2013) who studied the effect of propolis and garlic on *Oreochromis niloticus* fillets during frozen storage. On the other hand, Asgharzadeh *et al.*, (2010) showed that the increase of TBA during cold storage may be due to lipid hydrolysis and also chemical prooxidant molecules (hemoproteins and metal ions) which caused lipid oxidation.

The effect of local propolis addition on total bacterial counts (TBC) in fish kofta during storage under refrigeration at 4°C for 20 days is shown in Table 6. TBC were determined immediately after the preparation of fish kofta and after 4, 8, 12, 16 and 20 days of cold storage. The decrease of TBC for all treated samples comparing with control samples was in harmony with the increase of propolis levels. However, the supplementation with propolis had a significant impact on the TBC in fish kofta comparing with control samples. At the beginning of storage, the TBC in FKControl, FK0.5%, FK1.0% and FK1.5% were 2.67± 0.07×10^{3}, 2.52±0.07 ×10^{3}, 2.60±0.10×10^{3} and 2.47±0.05×10^{3} CFU/g. At the 20th day of storage, these values reached 63.00±0.39×10^{3}, 21.38± 0.20×10^{3}, 19.45±0.36×10^{3} and 12.97±0.86×10^{3} CFU/g,

Table 6: Effect of local propolis addition on total bacterial counts (TBC) in fish kofta during cold storage at 4±2C°

Sample	Total bacterial count (x10^{3} CFU g^{-1}) during storage time (day)					
	0	4	8	12	16	20
FKControl	2.67±0.07^{a}	7.31±0.31^{a}	20.82±0.80^{a}	44.95±0.51^{a}	61.53±0.53^{a}	63.00±0.39^{a}
FK0.5%	2.52±0.07^{bc}	4.55±0.05^{b}	6.66±0.12^{b}	14.77±0.19^{b}	20.04±0.20^{b}	21.38±0.20^{b}
FK1.0%	2.60±0.10^{ab}	3.06±0.18^{c}	6.21±0.10^{bc}	12.43±0.19^{c}	18.43±0.31^{c}	19.45±0.36^{c}
FK1.5%	2.47±0.05^{c}	3.01±0.11^{c}	5.56±0.09^{c}	10.54±0.21^{d}	11.88±0.13^{d}	12.97±0.86^{d}
LSD at 0.05%	140.68	353.16	772.57	575.32	615.01	536.31

LSD= least significant difference

respectively. The increase in propolis level led to a significant reduction of TBC in fish kofta during 20 days of cold storage compared to control samples. A high antimicrobial activity of propolis in various food products was reported (Ali *et al*., 2010; Koc *et al*., 2007; Silici & Karaman, 2014). Flavonoids, tanins, and steroids are the major components responsible for the bioactive properties of propolis. El Sohaimy & Masry (2014) reported that the Egyptian and Chinese propolis contains high concentrations of phenolic components those lead to their biological activity as antimicrobial agent.

DPPH radical scavenging activity is famously used to study the antioxidant capacity of differing components. Results in Table 7 show that the ability of the fish kofta samples containing different levels of local propolis (0.5, 1.0, and 1.5%) to inhibit the DPPH free radical. The DPPH inhibition was significantly increased with the increasing of propolis level for fish kofta treatments in comparison with control samples. Accordingly, the FK 1.5% treatment kept the highest DPPH inhibition during all storage period (from the beginning to the 20th day). At the 20th day of cold storage the DPPH inhibition of FKcontrol was 8.64±0.31%, while FK 1.5% recorded 40.22±0.24%. All the tested propolis extracts had high DPPH radical scavenging activity in various food systems (Talas & Gulhan, 2009; Tosi *et al*., 2007). Flavonoids and caffeic acid phenethyl ester found in propolis, are reviewed to be antioxidants against lipid peroxidation in the cells (Seven *et al*., 2010).

Color changes assays (L, a, b and Δ E values) of fish kofta containing different concentrations (0.5, 1.0 and 1.5%) of propolis and control sample during cold storage at 4°C are presented in Figure 1. The values are from the L-scale (light vs. dark), the a-scale (red vs. green) and the b-scale (yellow vs. blue) as well as Δ E. The differences between color values during the storage period were high in the FKControl, but these values get closer with the treatment with propolis addition in FK0.5%, FK 1.0% and FK1.5%. The lowest color changes were in FK1.5% followed by FK1.0%, FK0.5% and FK control. These could be attributed to the components found in propolis which act as strong antioxidants and antimicrobial resulting in a great preservative behavior and delay the quality loss (a stable fish kofta color) comparing with control sample (Hassanin & El-Daly, 2013; Tosi *et al*., 2007).

Table 7: Effect of propolis addition on DPPH· radical inhibition (%) during 20 day cold storage

Treatments	Storage time (day)					
	0	4	8	12	16	20
	DPPH· inhibition (%)					
FKControl	15.24±0.84^{d}	14.73±0.20^{d}	12.53±0.56^{d}	10.53±0.21^{d}	9.94±0.33^{d}	8.64±0.31^{d}
FK0.5%	27.54±1.56^{c}	25.49±0.90^{c}	23.15±0.82^{c}	21.54±1.46^{c}	18.28±1.10^{c}	16.53±0.55^{c}
FK1.0%	44.99±1.60^{b}	39.41±0.99^{b}	37.86±0.95^{b}	35.68±1.33^{b}	32.69±0.82^{b}	30.45±1.23^{b}
FK1.5%	56.49±1.55^{a}	49.59±1.47^{a}	46.15±1.18^{a}	45.48±1.35^{a}	42.50±1.25^{a}	40.22±0.24^{a}
LSD at 0.05 %	2.69	1.89	1.71	2.27	1.78	1.32

LSD= least significant difference; NS= Not Significant.

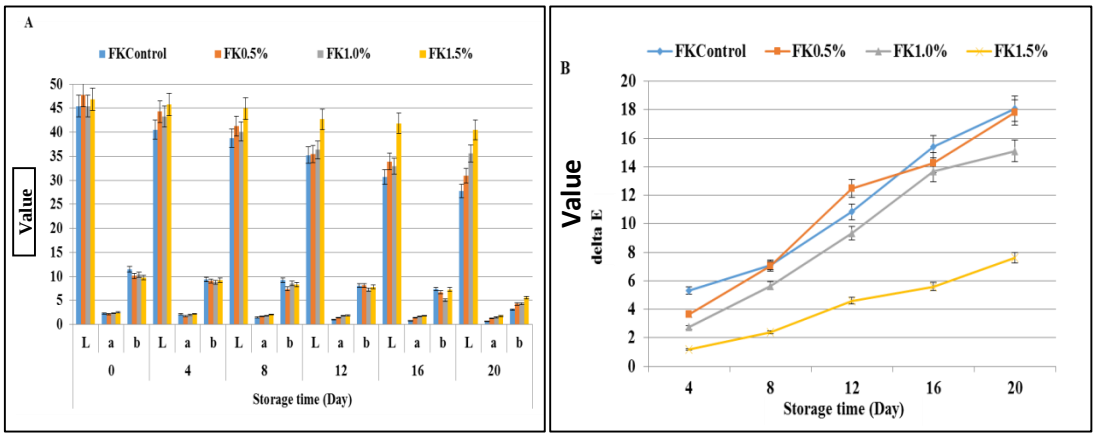

Figure 1: Color changes in Hunter coordinates L, a and b (A); Color changes L, a and b (expressed as delta E) (B) for fish kofta containing 0.5, 1.0 and 1.5% propolis (FK0.5%, FK1% and FK1.5%, respectively) and control sample (FKControl) during cold storage at 4±2°C.

Conclusion

From the results of the GC-MS analyses it could be concluded that thirteen chemical components were identified in the case of EELP and HEIP while twenty nine components were identified in the case of EEIP and HELP. Moreover, the addition of Egyptian propolis to fish kofta increased the DPPH inhibition and decreased the TBA values, the TBC and color changes of tested samples during cold storage at 4±2°C for 20 days.

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