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CHEMICAL COMPOSITION AND ACTIVE COMPONENTS OF EGYPTIAN HONEYBEE PROPOLIS AS A NATURAL SUBSTANCES FOR PRESERVATION OF BASTARAMI .

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ABSTRACT

Propolis is a natural product collected by honeybee workers; it has been reported to have antimicrobial and antioxidant effect. Samples of bee propolis were obtained from the Dept. of Economic Entomology & Pestisicides, Fac. of Agric., Cairo Univ.,Egypt.Chemical composition and active components were evaluated. The effect of adding water extract of propolis (WEP) on the physico-chemical, microbiological quality and organoleptic properties of prepared bastarami samples with different (WEP) levels were studied.Longissimus dorsi(LD)muscles of fresh beef meat was used for preparation bastarami samples, which were treated with different concentration of (WEP) 2,4,6 and 8 % during processing, then stored at 6 ± 1°C for 0, 2, 4, 8 and 12 weeks storage, immediately after maturation. The moisture, ash, waxes, resins, balsams and essential oils contents of propolis sample were, 2.46, 21.52, 2.45, 71.66 and 1.91 % respectively, while the total phenols compounds and total flavonoids (active components) were 2.36 and 6.78 %. The ash content of all fresh treated bastarami samples with different (WEP) levels was significantly higher while, the other constituents as moisture, protein, fat and NaCl were similar as compared with control samples. During refrigerated storage, the total volatile basic nitrogen (TVBN), thiobarbituric acid (TBA) and pH values, the microbial count of aerobic, Psychrotrops and yeast and moulds of control samples were significantly ($P < 0.05$) higher than the treated samples throughout storage period. Within, the treated samples with different (WEP) levels, the addition of either 6 % or 8 % (WEP), proved to be effective in increasing shelf-life of the treated bastarami by decreasing microbial count early during storage as well as minimizing fungal and yeast count due to effect of active components. Treatments also increased antioxidant effect by reducing lipid oxidation (TBA values), and decreasing negative flavor notes, besides the lean colour also was improved. Hence, (WEP) treatments can serve as good chemical preservatives of bastarami meat products and can contribute to promote human health because they are naturally produced. In addition, it is recommended to add 6% (WEP) to prolong the shelf-life and improve the safety of bastarami production .

Keywords: Honeybee propolis, Chemical composition, Flavonoids, Antibacterial, Antifungal, Meat products.

INTRODUCTION

Propolis is one of nature's miracle. rich in vitamins A, B, C and E, amino acids, minerals such as copper, iron, manganese, calcium and Zinc (Marcucci, 1995; Burdock, 1998).

Propolis is a natural resinans substance, with a colour varying from greenish yellow to dark brown, collected by bees from tree exudates and secretions (Park and Ikegaki, 1998). The exact composition of raw propolis varies according to the source. Usually, it is composed of 50% (w/v) resins

and vegetable balsam, 30% (w/v) essential and aromatic oils, 5% (w/v) pollen and various other substance, including organic debris 5% (w/v) (Ricardo and Antonio, 1998 and Banskota ,*et al.*, 2000). When they analysed propolis contained major flavonoids, Pinocombrin, Chrysin, galangin, and Pinobanskin (Bonvehi, *et al.*, 1994 and Hegazi, *et al.*, 2000) and there were remarkable quantitative and qualitative differences in the flavonoids in propolis (Green-away, *et al.*, 1990, Koo and Park, 1997 Ricardo, and Antonio, 1998) which have antifungal, antimicrobial and antioxidant qualities (Hayashi, *et al.*, 1999). The antagonistic properties of propolis against bacteria, yeast, fungi and viruses were reported by many investigators (Dobrowolski, *et al.*, 1991 and Park, *et al.*, 1998). They also showed that, the antimicrobial properties of propolis are mainly attributed to the flavonone pinocembrin, the flavonol galangin and coffeic acid phenethyl esters, with a mechanism of action probably based on the inhibition of bacterial RNA-polymerase. Besides, Mirazoeva, *et al.*, (1997) reported that the propolis extracts have a bactericidal and synergistic effect against gram positive and some gram negative bacteria, fungi and yeast and it was effective against all pathogens. On the other hand, the water and ethanol extracts of propolis were found to retard development of rancidity and the increase of thiobarbituric acid values in foods, and this was due to the antioxidant properties of propolis (Bonvehi, *et al.*, 1994).

Propolis is a stable product, keep its antibiotic activity, even when stored for one year or longer, where addition of propolis or its extracts to manufacturad products increased bacteriostatic activity and improved pharmacological properties (Molan, 1992). Because of its biological properties, and the efficiency of crude propolis or its extracts as a natural antioxidant and antimicrobial activities, propolis is used as a preservative in food products and thus may actually prolong the shelf life of some food products, instead of the synthetic ones i.e., frozen fish (Altovieva and Ushkalova, 1971), rape seed oil (Kaczmarek and Snela, 1982).Besids Han and Park, (1996) and Han *et al.*,(2001), stated that, ethanol and water extracts of propolis were effective in preserving meat products.

In recent years attention has been focused on the use of propolis as a health back-up food in developed countries. This due to the fact that it is recognized around the world as natural healthy and beneficial compound for the body (Han, *et al.*, 2001).

Until now, propolis and propolis based products have been taken mainly for health reasons and have been used only slightly in meat processing and preservation. This paper presents the chemical composition and active components of Egyptian bee propolis and evaluates the potential use of the different concentrations of propolis water extracts as a natural substances in controlling the degeneration of some cured meat products (Bastarami), its effects on the quality attributes, and also to extend the shelf-life of such product during refrigerated storage from the viewpoints of food chemistry, nutrition and safety of the consumer.

MATERIALS AND METHODS

Materials:

1. Propolis samples:

Crude propolis (Honeybees glue) samples, were obtained from the Department of Economic Entomology & Pestisicides, Fac. of Agric., Cairo Univ. Giza –Egypt. The samples were collected during summer season (July-August, 2002), to obtain a good quality of propolis. Samples were firstly purified from impurities (wood, straw, fragments and insects), then blended to fine particles in a warring blender, and stored in the dark at room temperature until use (Bonvehi, *et al.*, 1994).

2. Preparation of propolis extracts:

A stock solution of 10% (w/v) water extract of propolis (WEP) was prepared, by soaking the blended propolis in distilled water for 7 days with periodical shaking, for three hours daily at room temperature (25±1°C). The (WEP) was filtered seven times through a Whatman number one filter paper, and the undissolved particles were dried and weighted to calculate the solubility of propolis samples. A working solutions of 2%, 4%, 6% and 8 % (WEP) were prepared from the stock solution according to its solubility, then stored at 4 °C and used within 2 weeks of preparation.

3. Preparation of treated bastarami samples with WEP:

Longissimus dorsi (LD) muscles of chilled beef carcasses 24 hrs after slaughter were obtained from Cairo slaughter house (Egypt) at age 2-3 years . The meat muscles were trimmed from the external fat and tendons, then divided into five equal parts (1.5 kg weight and 30 cm length), and used for preparing bastarami samples following the usually followed standard commercial practices, as follows:

(A) Curing: Each meat sample was fissured surfacely to facilitate the penetration of the curing mixture through the muscular tissue. The curing mixture consists of NaCl as 10% from the initial meat weight, 125 ppm. sodium nitrite ($NaNO_2$) and 500 ppm. ascorbic acid, was spread uniformity on the surfaces of each meat muscle, then were left in a stainless steel racks for 12 hrs at 4°C. After curing, the drip was drained off and the muscles were washed with tap water to remove the excess of salts, then pressed with a suitable mass. (B): The (WEP) treatments: The previous cured samples were immersed for 2 min. in 0 % (WEP) (control), 2%, 4%, 6% or 8% WEP, and then left to dry in the air away from the sunlight for 2 days to reach about 45% moisture content. (C) Coating and maturation: The previous dried samples were coated with 20% of its weight with coating paste mixture (contained fenugreek powder, minced garlic, sweet pepper and water in the ratio of 15:5:3:3 (w/w), respectively, and then were left hanged at room temperature (25±1°C) for maturation up to 2- 3 days. After maturation, bastarami samples of each treatment were stored at 6±1°C for 0, 2, 4, 8 and 12 weeks. After each storage time was completed, each bastarami sample was divided into three parts for physico-chemical analysis, microbiological

quality and sensory evaluation. All the determinations were made on finely ground bastarami after removal the coating paste and were performed in triplicate.

Methods:

Chemical evaluation of propolis samples:

1. Moisture and ash contents :

It were determined according to (A.O.A.C. 1995) as follows : moisture content was determined by drying 2 gm of propolis sample to constant weight in a conventional kiln at 105°C. Ash content was determined by incineration one gm of propolis sample at 550°C to constant weight.

2. Wax, resins and total balsams :

The wax content was determined by extracting with petroleum ether (40-60°C) in a soxhelt extractor for 3 hrs. Resins and total balsams were extracted for 30 min.with methanol at room temperature, according to the methods described by Bonvehi, *et al.* (1994).

3. Essential oils :

It were determined according to the method described by Ricardo and Antonio, (1998)by distillation (50gm) of propolis sample with 220 ml distilled water in evenger extractor for 12 hrs. The oil was separated from water by cooling to 5 °C in a separately funnel, then dried over anhydrous sodium sulfate.

4. Total phenols:

It were determined by using the colorimetric methods of Folin -Denis as described by Swain and Hillis (1959) . The constituents of phenolic compounds were separated and identified according to method described by Harborne (1983) by using Thin layer chromatography technique.

5. Total flavonoids :

The determination of flavonoids were carried out as described by Zhuang *et al.* (1992) as follows : It were extracted by using 5 gm of propolis sample with water, filtrated and was completed with distilled water to known volume (50 ml) . The aqueoue solution was successively extracted with chloroform to remove the pigments and fatty materials, followed by ethyl acetate (E.A)extraction to obtain flavonoids. The (E.A)extract was concentrated to known volume. Then 1ml from extract was placed in a 10ml volumetric flask , 0.3 ml $NaNo_2$ (1:20) and 3 ml $Alcl_3$ (1:10) were added . After 6 min. 2ml (1mol -liter $^{-1}$) NaOH was added and the total was made up to 10 ml with distilled water . The solution was mixed well again and the absorbance was measured against a blank at 510 nm using a Jenway 6300 spectrophotometer since the Quercetin was used as the standard for a calibration curve . The flavonoid content was calculated using calibration curve .

6. Extraction and Identification of flavonoids :

Flavonoids extracted and identificated according to Markham (1982) as follows: A small amount of sample (2gm) was immersed in 2 M HCl and heated in a test tube for 30-40 min. At 100°C. the extract was cooled and filtrated.The hydrolyzate was extracted with ethyl acetate,concentrated to dryness , dissolved in 1 ml ethanol . Aliquots were used for spoting on PC and developed by using solvent system (acetic acid conc:HCl:water 30:3:10).

Chemical evaluation of fresh bastarami samples :

Fresh samples of beef bastarami of the different treatments were chemically analyzed for their moisture, fat, crude protein, ash and sodium chloride (NaCl) contents, according to the standard procedures of the A.O.A.C. (1995). The minerals content was determined,by using the ash obtained from one gram of each sample, which was dissolved in 100 ml HCl (1N). Zinc (Zn), Iron (Fe), Calcium (Ca), Potassium (K), Phosphorus (P) and Magnesium (Mg) were determined using a pye unicum Sp/900, atomic absorption spectroscopy technique as described by A.O.A.C. (1995).Sodium chloride content was determined in different treatments by titration with standard solution of silver nitrate (Ag No_3).

Determination of pH values:

pH value of the bastarami samples were determined according to the A.O.A.C. (1995) procedures. Ground samples (30-g) were blended with 90-ml double distilled water in a Waring Blender to provide uniform slurries. The slurrie were poured into beakers and fluted filter papers were placed over the slurries. After equilibration, pH values (±0.01) were recorded using (Model 601 A Digital).

Determination of the total volatile basic nitrogen (TVBN).

The (TVBN) of the bastarami samples was determined according to the method described by Malle and Tao, (1987), using the steam distillation. The (TVBN) was expressed as mg. nitrogen / 100 g flesh.

Thiobarbituric acid (TBA)

The extent of lipid oxidation was determined by the (TBA) method as reported by Tarladgis, et al., (1960). The(TBA)value was expressed as milligrams of malonaldehyde (MA) per kilogram of bastarami sample, using a conversion factor of 7.8. In all cases, three determinations were carried out and the average of the three results recorded.

Colour evaluation:

Hunter L (Lightness), a (redness) and b (yellowness) values were determined on a minimum of 4 locations per each sample after each storage periods using the procedures of AMSA (1991) with a Hunter Lab. Colorimeter (Model D25-2). The mean values for L, a and b measurements were determined, and these were used to calculate chroma and the values, where, Chroma = (a2 + b2)1/2 and Hue =Tan^{-1} (b/a).

Microbiological analysis:

Duplicate 25 g each of ground bastarami sample were collected aseptically, immediately after each storage period, and analysed for aerobic plate count (APC), Psychrotrophs plate count (Pspc), total yeast&moulds and Salmonella, according to the microbiological methods appropriate for meat as recommended by MIRINZ, (1991). Results were expressed as colony forming units per gram (Cfug-1) or presence/absence (Salmonella only).

Sensory evaluation:

All the bastarami samples were subjected to a panel testing at each examination period, using 10 trained members from the Faculty staff of different age, sex groups. Small slices were prepared from the bastarami samples and were coded with random numbers, then panelists evaluated the samples for colour, flavour and texture as recommended by the AMSA, (1995). (10 = most desirable and zero = least acceptable).

Statistical analysis :

This entire experiment was conducted as a randomized complete block design with three replications. The data were analyzed by the General Linear Models procedure (SAS , 1994). Treatments were compared using the multiple range test (Duncan, 1955), at the 0.05 level of significance.

RESULTS AND DISCUSSION

Chemical evaluation of propolis sample:

The results in table (1) represent the chemical compostion and active components of propolis sample. From the results , it could be noticed that the moisture and ash contents were 2.46% and 21.52%, respectively, while the moisture and ash contents of different propolis samples from various botanic and geographic origins reported by Bonvehi, *et al.*, (1994), ranged from 1.8 to 3.2% and from2.6 to 21.5%, respectively.They also reported that the water content of propolis depends on the extraction technique applied.

The phenolic components are the most representative part of the resin fraction and have the antioxidant and antimicrobial effect (Hayashi , *et al.* , 1999 , and Banskota , *et al.* , 2000).From the results of total phenols content as given in Table (1) , it could be noticed that the phenolic compounds of Egyptain propolis sample was slightly lower (2.36%) , compared with values (2.68 and 2.89 %) reported for propolis samples from Brazil and China respectively by Bonvehi , *et al.*, (1994) , and Marcucci, (1995). Besides, TLC identification indicated the percentage of Gallic acid (0.07%), cinnamic acid (0.20%), vanillin (1.18%), caffeic acid (0.036%), m-cumaric acid (0.24%), 4-hydroxy benzoic acid (0.46%) and not identifed compounds (0.174%).Spectrophometric determination of flavonoids content of propolis was (6.78%) and PC indicated the precentage of rutin (3.01%) , quercetin (1.26%), apigenin (1.86%) and not identifed (0.65%) .Such these results indicated flavonoids are predominant in the phenolic fraction , and the

rutin followed by apigenin are the most constituents of flavonoids components , while , the gallic and caffeic acid consituents appeared in smaller percentages in the phenolic fraction .

In addition , the essential oils content of propolis sample (1.91%) was slightly higher than was found in the different propolis samples by Bonvehi, *et al.*, (1994).

Table (1): Chemical composition and active components of propolis samples (g/100 g sample)

Components	Percentage	Active components of balsams			
Moisture	2.46	Gallic acid	0.07	Rutin	3.01
Ash	21.52	Cinnamic acid	0.20	Quercetin	1.26
Wax	2.45	Vanillin	1.18	Apigenin	1.86
Essential oils	1.91	Caffeic acid	0.036	Not identified	0.65
Resins and balsams	71.66	*m*-Cumaric acid	0.24	Total flavonoids	6.78
		hydroxy benzoic acid	0.46		
		Not identified	0.174		
		Total phenols	2.36		

In crude propolis, with its high wax percentages (2.45%), the contents of its active substances (phenolic components) were rather low (Marcucci, 1995). Therefore, it could be concluded that, the active components of Egyptian propolis used in this study, was greater than other propolis samples from different geographic origins, indicating that it can be used as a natural substances for preservation of bastarami , due to its known antioxidant and antimicrobial effect(Green-away , *et al.*, 1990, and Han , *et al.*, 2001) .

Chemical evaluation of fresh bastarami samples (after processing):

Data presented in Table (2) revealed that high ash percentage in all treated bastarami samples with different concentrations of (WEP), as compared with control, that is due to the addition of (WEP), which contained high ash content (21.52%).However, the other constituents of different treatments of bastarami samples were similar to control. The bastarami samples treated with either 6% or 8% (WEP) have higher mineral content than other samples . This could be explained by the rapid diffusion of (WEP) during curing process into the muscular tissues. Besides, the addition of (WEP) resulted in a remarkable increase of Ca. Fe and Mg contents in treated samples with (WEP) as compared with the control samples. These results confirmed the findings of Marcucci, (1995)and Hegazi,*et al.*,(2000).

Table (2): Chemical evaluation of fresh bastarami samples , as affected by using different concentrations of water extract of propolis (WEP)

Bastrami ssmples (B.s)	Moisture %	Chemical constituents (on dry wt. basis)					Mineral content (mg/100 g sample)				
		Protein	Fat	Ash	NaCl	Ca	K	P	Fe	Zn	Mg
Untreated bastrami sample (control0	46.18	65.73	10.92	15.88	13.94	65.89	76.01	88.91	3.66	2.10	17.02
B.S with 2% WEP	45.70	66.07	10.60	16.41	13.74	68.46	76.19	89.18	4.18	2.65	17.73
B.S with 4% WEP	45.18	66.11	10.61	16.92	13.70	70.12	76.48	90.05	4.65	2.86	18.35
B.S with 6% WEP	45.11	66.18	10.60	17.11	13.63	72.32	76.82	91.36	5.18	2.96	18.98
B.S with 8% WEP	45.01	66.32	10.63	17.18	13.58	72.55	76.80	91.65	5.26	2.96	19.12

Total volatile basic nitrogen (TVBN):

(TVBN) is considered an important factor to measure the extent of protein degradation to amino acids and putrefaction of the proteinaceous constituents (Han, *et al.*, 2001). The (TVBN) values of all untreated and treated bastarami samples, were found to be increased significantly ($P < 0.05$), as the storage time increased up to 12 weeks (Fig. 1). As expected, more protein was converted to amino acids at a longer storage period, since the degradation of bastarami was enhanced by long storage period. For example, after 8 and 12 weeks of storage, the (TVBN) values for the control sample were 30.6 and 46.2% mg, respectively. However, it could be noticed that, the highest (TVBN) values were obtained from the control samples, during refrigerated storage after 2, 4, 8 and 12 weeks of storage, while all the treated bastarami samples yielded (TVBN) values lower than those of the corresponding control samples (Fig. 1). This was due to the preservative action of (WEP) in the treatment groups, where, 6% (WEP) followed by 8%(WEP) recorded the lowest (TVBN) values at all five storage periods. This showed that 6% (WEP) reduced protein degradation to the greatest extent compared with other treatments of different concentrations of(WEP). For instance, after 12 weeks of storage, the bastarami treated with 2%, 4%, 6% and 8% (WEP) yielded (TVBN) values which were 29.45, 28.3, 21.18 and 21.16% mg lower, respectively, than the controls as shown in (Fig. 1). These reduced (TVBN) values indicated a corresponding reduction in protein degradation. Therefore, it could be concluded that, the shelf-life of bastarami supplemented with either 6% or 8%(WEP) was longer than other treatments including control sample. As a result of this study, natural propolis may be used for preservation of bastarami which stored at 6±1°C.

Thiobarbituric acid (TBA) values:-

(TBA) values were determined, as an index for lipids oxidation taking place in the processed bastarami samples during their refrigerated storage. The results illustrated in Fig. (2) indicated that (TBA) values in the bastarmi samples without added (WEP) (control), were the highest, as compared with the treated samples which had the lowest values, at any time of refrigerated storage period. This indicates that, the (WEP) significantly retarded the oxidative changes in treated samples compared with untreated ones. However, the effect on the retardation of (TBA) construction differed in the

various (WEP) levels, where the samples treated with either 6% or 8%(WEP) showed significantly ($P < 0.05$) the lowest (TBA) value, at any time of refrigerated storage period. Therefore, the preservation period of bastarami product supplemental with 6% or 8% (WEP) was longer than those supplemental with 2% or 4% WEP. The effect of propolis activity, which varied according to the(WEP) concentration, may be attributed to their contents of flavonoids and phenolic compounds (Marcucci, 1995 and Hayashi, *et al.*, 1999). Moreover, it could be concluded that propolis, as a natural bee product has an antioxidant activity and may be used as suitable alternative for chemical preservatives used in bastarami production .

pH values:

Data presented in Table (3), showed that, the pH values of all treated and untreated samples was not significantly ($P < 0.05$) different at zero or after two weeks storage. But when storage time was extended to 4 weeks up to 12 weeks storage, the control samples had significantly higher pH values than treated samples with different (WEP) levels. Within samples treaded with (WEP), the samples with 6% (WEP), had significantly ($P < 0.05$) lower pH values during storage period, as compared with other treatments. This could be explained by the fact that even during curing process, protein denaturation and breakdown took place, as was indicated by the increase in the (TVBN) values (Fig. 1). However, the increase in pH value of control samples may result in the development of "off flavors" (Table 3), so it is an important factor to consider with regard to bastarami quality. pH, also is an important factor for the colour of meat, as changing pH can cause myoglobin to be more readily oxidized to metmyoglobin, with lower colour intensity (Cross, *et al.*, 1986).

From the results obtained in Figs. (1 and 2), and Table 3, it could be concluded that the (TVBN), (TBA) and pH values of bastarami samples treated with 6% (WEP), were significantly ($P < 0.05$)become the limits permitted by the Egyptian standard for bastarami (Kramlich, *et al.*, 1975and E.S. 1991).

Colour evaluation:

Colour is a primary factor in Judging meat and meat products quality (Eckert, *et al.*, 1997). Means for main effects for colour evaluation of bastarami samples (Table 3), showed that, the redness (a* values) and yellowness (b* values) were slightly decreased, as the storage time increased during refrigerated storage for 12 weeks, indicating that, the colour of bastarami samples becomes darkr. Besides, the control samples and samples treated with 4% (WEP) had more b* values than other treatments, at any time of storage period. However, the fact that hue value decreased as storage time increased, indicating that, the samples were becoming more reddish purple. On the other hand, the chroma value (which measure the saturation of colour in sample), was significantly ($P < 0.05$) higher after two weeks storage than after storage for 4, 8 and 12 weeks, this indicated increased graying in bastarami samples over storage weeks. Besides, as the storage time increased, the bastarami samples changed in colour from

Table (3): Effect of different WEP levels on the pH , Hunter color values, and sensory attributes of bastarami samples, during refrigerated storage at 6+1°C for 12 weeks.

Storage time	Treatments	pH value	Hunter color values				Sensory attributes			
			L*	a*	B*	Hue	Chroma	Flavor	Colour	Texture
Zero Time	1- Untreated B's (control)	5.6^{a}	31.36^{a}	16.89^{c}	12.10^{a}	46.32^{d}	20.78^{b}	9.6^{a}	8.5^{ab}	7.65^{a}
	2- Treated B's with 2% WEP	5.64^{a}	32.35^{b}	16.90^{c}	10.98^{c}	47.20^{c}	20.15^{bc}	9.5^{a}	8.69^{ab}	7.66^{a}
	3- Treated B's with 4% WEP	5.64^{a}	33.47^{c}	16.67^{c}	12.01^{a}	46.65^{d}	20.55^{b}	9.5^{a}	8.70^{ab}	7.71^{a}
	4- Treated B's with 6% WEP	5.64^{a}	32.98^{b}	17.98^{b}	11.90^{b}	48.65^{b}	21.56^{a}	9.6^{a}	8.95^{a}	7.69^{a}
	5- Treated B's with 8% WEP	5.64^{a}	32.45^{c}	18.03^{a}	11.92b	49.60^{b}	21.61^{a}	9.5^{a}	8.96^{a}	7.68^{a}
2 weeks	1- Untreated B's (control)	5.66^{ab}	32.54^{b}	16.41^{c}	10.58^{c}	45.20^{e}	19.53^{cd}	9.2^{ab}	7.30^{de}	7.08^{b}
	2- Treated B's with 2% WEP	5.65^{ab}	32.66^{b}	16.60^{c}	10.54^{c}	46.85^{d}	19.66^{cd}	9.0^{ab}	8.60^{ab}	7.46^{b}
	3- Treated B's with 4% WEP	5.66^{ab}	33.60^{b}	16.74^{c}	12.05^{a}	46.90^{d}	20.63^{b}	9.3^{ab}	8.50^{ab}	7.66^{a}
	4- Treated B's with 6% WEP	5.65^{ab}	31.76^{b}	16.99^{c}	10.84^{c}	47.89^{c}	20.05^{bc}	9.4^{ab}	6.90^{ab}	7.65^{a}
	5- Treated B's with 8%WEP	5.65^{ab}	33.62^{b}	16.74^{c}	10.80^{c}	46.88^{d}	19.92^{c}	9.4^{ab}	8.89^{ab}	7.65^{a}
4 weeks	1- Untreated B's (control)	6.50^{f}	33.16^{c}	15.67^{d}	10.28^{c}	42.30^{h}	18.74^{d}	8.6^{c}	8.20^{f}	6.92^{c}
	2- Treated B's with 2%WEP	5.76^{bc}	32.66^{b}	14.66^{e}	11.25^{b}	42.50^{h}	18.48^{de}	8.6^{c}	8.02^{b}	7.12^{bc}
	3- Treated B's with 4%WEP	5.74^{bc}	31.76^{e}	15.67^{d}	12.01^{a}	45.89^{e}	19.74^{c}	8.5^{c}	8.02^{b}	7.56^{ab}
	4- Treated B's with 6%WEP	5.72^{b}	33.62^{c}	16.74^{c}	10.80^{c}	46.90^{d}	19.92^{c}	8.7^{c}	8.65^{ab}	7.60^{ab}
	5- Treated B's with 8%WEP	5.71^{b}	33.76^{c}	16.74^{c}	10.80^{c}	48.25^{d}	19.92^{c}	8.6^{c}	8.60^{ab}	7.62^{c}
8 weeks	1- Untreated B's (control)	7.6^{g}	33.21^{c}	14.62^{e}	11.28^{b}	39.65^{k}	18.47^{de}	5.20^{g}	4.38^{g}	4.6^{f}
	2- Treated B's with 2%WEP	5.79^{cd}	32.90^{b}	14.66^{e}	11.24^{b}	41.05^{d}	18.47^{de}	7.80^{d}	6.95^{e}	6.55^{cd}
	3- Treated B's with 4%WEP	5.80^{cd}	32.05^{b}	13.60^{f}	12.01^{a}	42.60^{h}	18.14^{de}	7.6^{d}	6.95^{e}	6.55^{cd}
	4- Treated B's with 6%WEP	5.75^{c}	33.62^{c}	16.74^{c}	10.80^{c}	43.18^{g}	19.92^{c}	8.05^{cd}	7.55^{c}	6.60^{cd}
	5- Treated B's with 8%WEP	5.75^{c}	33.16^{c}	14.66^{e}	11.25^{b}	44.12^{f}	8.64^{de}	8.02^{cd}	7.53^{c}	6.60^{cd}
12 weeks	1- Untreated B's (control)	8.36^{h}	32.95^{c}	13.37^{f}	10.48^{c}	32.26^{m}	16.99^{f}	2.30^{h}	3.08^{h}	3.80^{g}
	2- Treated B's with 2%WEP	5.96^{e}	32.66^{b}	13.58^{e}	10.49^{c}	37.18^{j}	17.16^{e}	6.6^{f}	6.18^{f}	5.3^{e}
	3- Treated B's with 4%WEP	5.90^{de}	32.26^{b}	15.67^{d}	12.01^{a}	39.25^{k}	16.74^{fg}	6.5^{f}	6.20^{f}	5.8^{de}
	4- Treated B's with 6%WEP	5.81^{cd}	33.42^{c}	13.89^{f}	10.56^{c}	39.88^{k}	17.92^{e}	7.30^{e}	7.55^{c}	6.12^{d}
	5- Treated B's with 8%WEP	5.82^{cd}	33.01^{c}	14.66^{f}	11.59^{a}	39.90^{k}	17.26^{e}	7.2^{e}	7.5^{c}	6.11^{d}

abc- means within column not followed by the some letters are significantly different (P < 0.05) L* = Lightness b* = yellowness a*= redness B's = Bastarami sample WEP = water extract of propils.

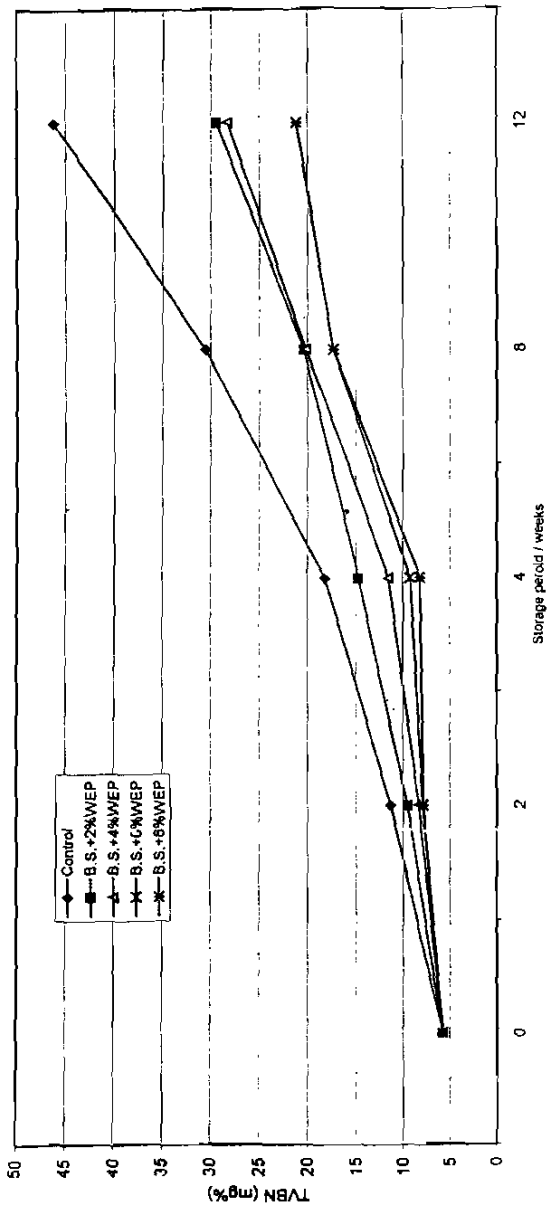
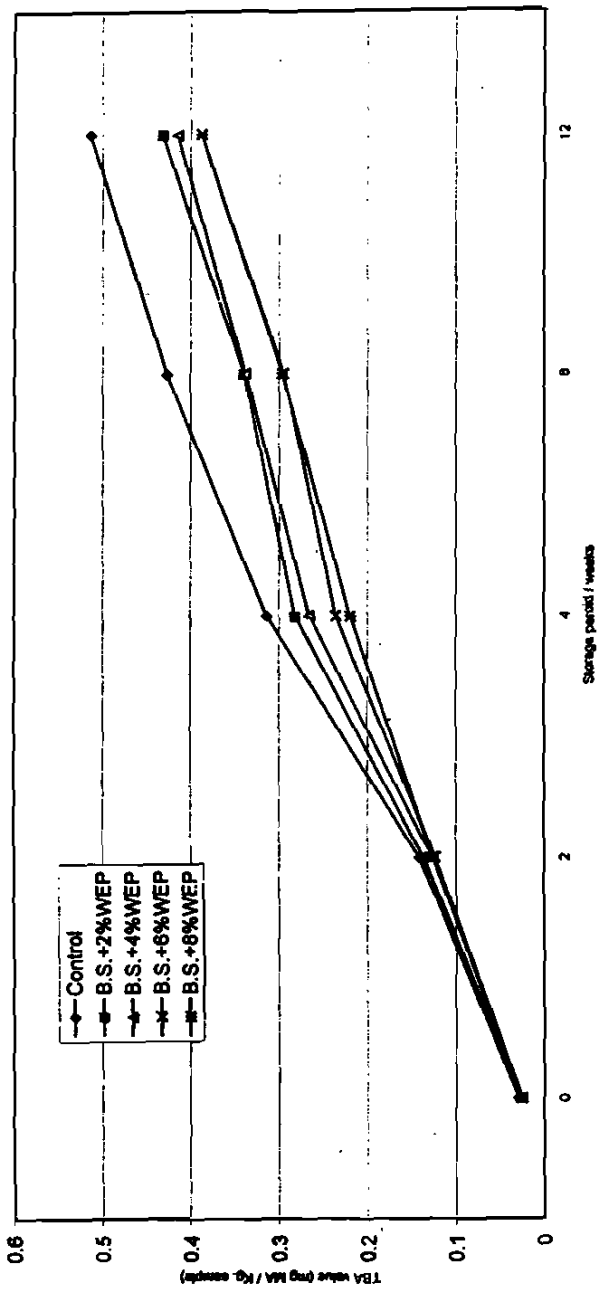


Fig.(1):Changes in the total volatile basic nitrogen (TVBN) values (mg%) of bastarami samples, as affected by using different concentration of water extract of propolis (WEP), during refrigerated storage.at 6 ± 1°C for 12 weeks.



Fig(2): Changes in thiobarbituric acid valuea (TBA) of bastarami samples, aa affected by using different concentrations of water extract of propolla (WEP), during refrigerated storage at 6 ±1c for 12 weeks.

moderately bright cherry red to slightly dark red, while the treated samples with either 6% or 8% (WEP), were significantly ($P < 0.05$) higher in lean colour, as compared with other treatments during storage period, indicating that, the antioxidant effect of (WEP), prevented the breakdown and oxidation of myoglobin into metmyoglobin during refrigerated storage period (St. Angelo, *et al.*, 1987 and Eckert, *et al.*, 1997)yielding bastarami with more acceptable colour .

Microbiological evaluation:

The results in (Table 4) showed that the type of additive, storage environment and storage time all affected ($P < 0.05$) the microbial load during refrigerated storage (Table 4). The obtained results declared that the aerobic plate counts (ApCs), Psychrotrophs plate counts (Ppcs) and total yeast and mould counts (Log10 CFu/g) of all bastarami samples were significantly ($P < 0.05$) increased during refrigerated storage period as the storage time increased up to 12 weeks. Meanwhile, the microbial count in bastarami samples without added (WEP) (control) were of an order of magnitude higher than those samples treated with different concentrations of (WEP), at any time of refrigerated storage, indicating that, the use of(WEP) treatments, resulted in a significant decrease in the microbial populations ($P < 0.05$), which indicates the presence of significant time-treatment interactions, where, the higher (WEP) concentration, the lower the microbial content . This is due to the destructive effect of (WEP) on some microbial population and inhibitory effect on the other bacteria (Dobrowolski, *et al.*, 1991 and Burdock, 1998). These results were in accordance with the findings reported by Mirazoeva, *et al.*, 1997), who found that the propolis extracts produce a toxicological model, which inhibited growth of bacteria. The effect of (WEP) treatments on fungal and the total count of yeasts and moulds was pronounced (Table 4). Reduction was dramatic in bastarami samples treated with high WEP (6% or 8%). On the other hand, it was observed that the control samples showed significantly ($P < 0.05$) continuous increase in the microbial count during the whole period of refrigerated storage with visible growth after only 2 weeks storage, hence it reached the predefined spoilage level of log 7.8 (CFu/g) for aerobic bacteria, after 4 weeks storage, while no predfined spoilage resulted in the bastarami treated with either 6% or 8% (WEP), even after 8 weeks of storage. These results proved that the use of 6% (WEP) was more efficient than other concentrations, as strong antibacterial and antifungal effect for protecting bastarami product during its storage period and prolonged the shelf-life. Commonly the antimicrobial activity of propolis may be due to the fact that propolis is a complex of organic substances, and contains several flavonic compounds which have antibacterial properties (Green-away *et al.*, 1990; Koo and Park, 1997 and Ricardo and Antonio 1998).

Table (4): Effect of different WEP levels on the microbiological quality of bastarami samples, during refrigerated storage at 6+1°C for 12 weeks.

Treatments	Storage period, weeks				
	0	2	4	8	12
	Aerobic plate count (Log_{10}/g)				
1- Untreated B's (control)	5.10^{b}	7.3^{cd}	7.8^{d}	$8.0d^{e}$	9.3^{e}
2- Treated B's with 2% WEP	4.7^{a}	5.6^{bc}	6.8^{c}	6.9^{c}	7.8^{d}
3- Treated B's with 4% WEP	4.7^{a}	5.3^{b}	6.6^{b}	6.9^{c}	7.5^{d}
4- Treated B's with 6% WEP	4.5^{a}	4.8^{a}	4.8^{a}	5.7^{bc}	5.8^{bc}
5- Treated B's with 8% WEP	4.5^{a}	4.8^{a}	5.4^{b}	5.8^{bc}	5.10^{b}
	Psychrotrops plate count (log10/g)				
1- Untreated B's (control)	3.4^{b}	4.1^{c}	4.8^{d}	6.1^{ef}	6.6^{f}
2- Treated B's with 2% WEP	3.1^{ab}	3.4^{b}	3.6^{bc}	5.5^{e}	6.2^{ef}
3- Treated B's with 4% WEP	2.8^{a}	3.0^{ab}	3.2^{ab}	4.5^{cd}	5.6^{e}
4- Treated B's with 6% WEP	2.8^{a}	2.8^{a}	3.0^{ab}	3.6^{bc}	3.6^{bc}
5- Treated B's with 8% WEP	2.9^{a}	2.8^{a}	3.1^{ab}	3.8^{bc}	3.9^{bc}
	Total yeast and mould count (log10/g)				
1- Untreated B's (control)	2.7^{d}	2.8^{d}	4.7^{f}	5.5^{g}	6.6^{h}
2- Treated B's with 2% WEP	1.8^{ab}	2.5^{c}	3.7^{ef}	3.9^{ef}	4.3^{f}
3- Treated B's with 4% WEP	1.9^{ab}	2.2^{c}	2.8^{d}	3.2^{e}	3.7^{ef}
4- Treated B's with 6% WEP	1.6^{a}	1.7^{a}	1.8^{ab}	1.9^{ab}	1.90^{ab}
5- Treated B's with 8% WEP	1.6^{a}	1.8^{ab}	1.9^{ab}	1.92^{ab}	1.98^{b}
	*Salmonella				
All samples	-	-	-	-	-

*** Salmonellae, were not detected in any sample.**
abc .... Means with different superscript in a row or column differ significantly (P<0.05).
WEP = water extract of propolis.
B.S: Bastarami sample

In comparison with other antimicrobial and antifungal agents used in different meat products, it could be concluded that, the (WEP) is advisable to be used as a natural preservative with no toxic effects even in large doses in the field of meat products (bastarami) to safeguard the consumer from the microorganisms and its toxic metabolites, besides, the effect on keeping quality, make such bastarami product preferable.

Sensory evaluation:

Results in Table 3 represent the results of the sensory attributes of the different treatments of bastarami samples with (WEP) during refrigerated storage for 12 weeks. The results revealed that means for any attributes of the all samples, were not significant ($P < 0.05$), during the first two weeks of storage, while, during storage for 4, 8 and 12 weeks, the values of sensory attributes decreased gradually, where, the control samples had significantly ($P < 0.05$) lower scores of flavour, colour and texture.

Flavour is a major criterion affecting the quality of any final meat product, and the addition of non-meat ingredients has been reported to increase positive flavour notes and decrease off flavour in meat systems (Green and Cumize, 1982). Results in Table 3, showed that, the samples

treated with different (WEP) concentrations have significantly ($P < 0.05$) higher positive flavour scores than control samples during refrigerated storage period, besides, within the treated samples,there were no significant differences in flavour during 4 weeks storage. On the other hand , the samples treated with 6% or 8% (WEP)still induced significantly higher flavor scores after 8 weeks of storage than other treatments indicating a potent effect of 6% (WEP) on the flavour quality of the prepared bastarami samples. On the other hand, adding the (WEP) during processing of bastarami samples improved significantly ($P < 0.05$) the colour and texture quality over the control ones (Table 3). Besides, adding 6% (WEP) followed by 8% (WEP) caused significantly ($P < 0.05$) higher color and texture quality than other treatments, during storage period. In addition, it could be concluded that, the control samples were not accepted by the panelists after only 4 weeks of refrigerated storage. This indicates that treated bastarami samples with 6% (WEP), was the most acceptable sample during storage, and the (WEP) seemed to cause more improvement in sensory attributes and keeping quality and extended the shelf-life of this product up to 12 weeks of refrigerated storage.

CONCLUSION

As a result of this study, the main chemical constituents present in the Egyptian bee propolis are flavonoids, phenolics and various aromatic compounds, which are believed to contribute significantly to the chemical properties, antioxidant and antimicrobial effects of propolis. Therefore, it could be concluded that, the addition of 6% (WEP), during processing of bastarami product as a natural substances, may be substituted for chemical preservatives used in meat products, and this addition recommended to extend the shelf life of bastarami, and keeping its quality over long-term storage at 6°C.

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التركيب الكيمياوى والمركبات الفعالة فى صمغ نحل العسل(البروبوليس) المصرى واستخدامها كمواد طبيعية فى حفظ البسطرمة

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من الأتجاهات الحديثة فى مجال التصنيع الغذائى هو محاولة استخدام مواد الحفظ الطبيعية فى حفظ الأغذية بدلا من استخدام المواد الكيميائية التى تسبب مشاكل وأضرار صحية للمستهلك . ويعتبر صمغ نحل العسل (البروبوليس) من المواد التى تحتوى على مركبات طبيعية، قد عرف أن لها تأثير حافظ ضد نشاط الأحياء الدقيقة و تأثير مضاد الأكسدة . وهو يعتبر منتج طبيعى يتم جمعه بواسطة شغالات نحل العسل .

وفى هذا البحث تم الحصول على عينات البروبوليس من قسم الحشرات الاقتصادية والمبيدات بكلية الزراعة – جامعة القاهرة . حيث تم دراسة التركيب الكيمياوى والتعرف على كمية ونوعية المركبات الفعالة فيه .

تم تحضير المستخلص المائى لعينة البروبوليس بتركيزات مختلفة ٢، ٤، ٦، ٨%، واستخدمت العضلة الظهرية للحم البقرى الطازج لتصنيع عينات البسطرمة باستخدام هذه التركيزات اثناء التصنيع بالإضافة لعينة الكنترول (بدون إضافات) . وتم تخزين جميع العينات بالتبريد على ٦ ° لمدة ١٢ أسبوع . وقد تم تقدير وتقييم الخواص الكيميائية والحسية والميكروبية للعينات بعد التصنيع مباشرة وعند ٢، ٤، ٨ ، ١٢ أسبوع اثناء التخزين ، وكانت نتائج الدراسة ما يلى : -

بالنسبة لمحتوى كل من الرطوبة ، والرماد ، والشموع ، والراتنجات ، الزيوت الطيارة ، والمركبات الفيولية الكلية لعينة البروبوليس : ٢,٤٦، ٢١,٥٢، ٢,٤٥، ٧١,٦٦، ١,٩١، ٩,١٤% على التوالى .

وبالنسبة للتركيب الكيمياوى للبسطرمة المصنعة، وجد أن نسبة الرماد فى العينات المعاملة أعلى من العينة الضابطة (الكنترول) ، أما باقى المكونات كانت مشابهة فى جميع العينات . وأثناء التخزين بالتبريد وجد أن قيمة كل من المركبات القاعدية النتيروجينية الطيارة (TVBN) ، وحامض الثيوبار بيوتريك (BA) ، والـ P^H بالإضافة للعد الميكروبى لكل من الميكروبات الهوائية والمحبة للبرودة وكذلك عدد الفطريات والخمائر فى العينة الضابطة كانت أعلى معنويا عن مثيلتها فى باقى العينات الأخرى عند أى فترة من فترات التخزين بالتبريد . وكانت العينات المعاملة بإضافة ٦% أو ٨% من المستخلص المائى للبروبوليس (WEP) كانت أفضل العينات معنويا خاصة فى محتواها الميكروبى وخلوها من أى روائح غير مرغوبة وانخفاض قيمة (TBA) بالإضافة إلى تحسن اللون وباقى خواص الجودة الأخرى اثناء فترة التخزين لمدة ١٢ أسبوع، وهذا يؤكد التأثير الفعال لهذه المعاملة (٦% WEP) فى حفظ البسطرمة وزيادة فترة صلاحيتها.

وبذلك يمكن التوصية باستخدام مستخلص البروبوليس المائى فى حفظ منتجات اللحوم (البسطرمة) بالتبريد لفترة طويلة تصل إلى ثلاث شهور .