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QUALITY ATTRIBUTES OF BEEFBURGER AS AFFECTED BY USING PROPOLIS AND FROZEN STORAGE

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Abstract

Recently, natural substances play an important role in consumer choice especially food products. Natural honey bees products were used in a focussed form on the clinical practices. Lately, the anti-oxidant and anti-microbial properties have drawn more attention. Therefore, in this investigation, beefburger was prepared with different levels of propolis ethanol extract (PEE) as follow: (A) 0.1 %, (B) 0.2 % and (C) 0.3.%, in addition to (control) burger without adding PEE to find-out the effect of using propolis on quality attributes of beefburger.

Chemical composition, mineral content, physico-chemical properties, microbiological properties were determined. Also, ranking method was used to find-out the best product and to test the significance among products immediatelly after processing. Physico-chemical and microbial properties during frozen storage (-18°C) for three months were also investiagated.

Generally, results show that using EPE as a natural substance at level of 0.3% may be a good treatment to improve the quality attributes and accordingly the shelf-life of beefburger.

INTRODUCTION

Propolis is the natural honeybee product collected by the honeybee workers from varios plant sources especially buds and bark. Over all the world, there is a tendency for using the natural substances instead of the synthetic or chemical substances. From the previous studies, propolis was one of the natural substances which was used in focussed form on the clinical practices (Hunang *et al.*, 1999). Propolis contains amounts of vitamins and minerals such as B_1, B_2, B_6, C, A, iron, calcium, aluminium, vandium, silecon, magnesium, manganese and copper in addition to a great number of micro elements (Popravko, 1978). Also, Maciejewicz (1987) reported that propolis contains flavonoids, organic acids, sugars, amino acids, alcohols and hydrocarbons.

With respect to the anti-microbial properties of propolis, Rojas and Alvarez (1996) reported that the propolis showed a strong inhibitory activity in vitro to bacterial species of the Enterobacteriaceae family, Mycobacterium, Micrococcus, Staphylo-

coccus, Bacillus, Pseudomonas and streptomyces. Propolis extracts had a bactericidal effect against gram positive and some gram negative bacteria, fungi and yeast, and it was effective against all pathogens (Hegazi *et al.* 2000).

More-over, the propolis extracts showed better antioxidant behaviour than alfa-tocopherol in polyunsturated lipidic systems (Cengarle *et al.*, 1998). Propolis extracts also retarded the development of rancidity and increase in thiobarbituric acid values and this was due to the anti-oxidant properties of propolis (Hemeida and Abd-Alfattah, 1993).

On the other hand, Han and Park (1996) indicated that the propolis ethanol extract (PEE) or propolis water extract (PWE) were effective in preserving of meat products than potassium sorbate which is currently used in meat products, they suggested that PEE or PWE may become a substitute for chemical preservatives used in meat products.

Therefore, the objective of this work was to study the effect of using propolis on quality attributes of beefgurger. The effect of frozen storag (-18°C) for 3 months on quality attributes of beefburger treated with propolis was also investigated.

MATERIALS AND METHODS

Crude propolis (Honeybees glue) was obtained from the Honeybees Res. Dept., Plant Protection Res. Inst., Agric. Res. Center, Giza, Egypt. Propolis ethanol extract (PEE) was prepared as follow: Propolis was firstly purified from impurities such as wood, straw fragments, insects and wax remains. Then each 15 grams of crude propolis was dissolved in 150 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 filterred by using filter paper Watman No. 4 . The obtained solution of propolis ethanol extract (PEE) was evaporated on water bath (40°C) under vacuum (175 mbar) to obtain a thick mass and then cooling to give a gummy matter of propolis. Fresh beef was obtained from the local market in Giza. Beefburger was prepared by the common method according to the following formulation: 62% beef, 7% onion, 7% egg, 12% extruded Soy, 10% iced water, 1.5% salt and 0.5% spices mixture. Addition levels of PEE added in beefgurger were 0.1% (A), 0.2% (B), 0.3% (C) and 0.0% (Control). The beefburger either control or treatments were evaluated for chemical composition, mineral content, physico-chemical properties, and microbial and sensory properties after processing immediately. Also, physico-chemical, and microbiological attributes were

evaluated during frozen storage at -18°C fror three months.

Moisture, fat, protein and ash were determined by the methods described in AOAC (1990). Carbohydrate was expressed as a precentage by the difference. Minerals content were determined according to the method of Anon (1982) using Atomic Absorption Spectrophotometer, Perkin E., Model 2380. Total volatile nitrogen (T.V.N.) was estimated by the method of Winton and Winton (1958). Thiobarbituric acid value (T.B.A.) as an indicator for lipid oxidation was assessed as described by Pearson (1970). The pH Value was measured by a pH-meter according to the method of Krilova and Liskovskaia (1961). Water holding capacity (W.H.C.) and plasticity were measured according to the filter press method of Soloviev (1966). Cooking loss was calculated as a percentage of weight change from the raw to cooked state (in little of oil at 180°C for 3 min. of the side). Freeze thaw stability of beefburger batters was determined according to the method described by Trius *et al.* (1994) as follows: two polyethylen bags per treatment were filled with 100 grams of beefburger batter and frozen at -20°C for 30 days, then samples were thawed at 2°C for 24 hr., thawing loss was calculated by draining released fluids, reweighing samples, and expressing as a percentage of initial weight. With respect to the microbiological properties, total aerobic plate count (T.A.P.C.) was performed according to APHA (1971). Milk-nutrient agar medium was used to grow and count the aerobic proteolytic bacteria according to Nester (1978) while lipolytic bacteria were grown on the nutrient emulsified oil agar (Difco, 1977). Also, Salmonella was detected and counted by using S.S. agar as a selective medium (Difco, 1977). Sensory evaluation of products was carried out using a 20 member panel and ranking method. The ranking method was used to find-out the best product among all the products (which had the lowest sum of ranks), moreover, according to Basker (1988), the critical values of differences among the sum of ranks were used for testing the significant difference between the products, where, the significane is attained when the differences between the rank sum of products are greater than or equal to the critical differences.

RESULTS AND DISCUSSIONS

1. Chemical composition of beefburger as affected by using propolis:

Data presented in Table (1) show the gross chemical composition of beefburger processed with different levels of propolis ethanol extract (PEE). From the results, it could be noticed that beefburger treated with PEE had slight increase in moisture and

ash and slight decrease in protein, fat and carbohydrate when compared to beefburger without adding PEE (control). Also, the same trend was observed with increasing the level of addition PEE in treated beefburger. However, the differences of gross chemical composition between all the products either control or treatments were slight differences and could be neglected.

2. Mineral content:

Mineral content of beefburger as affected by using propolis (PEE) is given in Table (2). From the results, it could be observed that PEE added to beefburger resulted increment of the mineral content of treated beefburger. On whole, mineral content of beefburger manufactured with propolis ethanol extract (PEE) was increased by increasing the level of addition PEE, therefore, product C (beefburger prepared with PEE at 0.3% level) had higher mineral content than products B (0.2% level) and A (0.1% level). On the other hand, adding PEE resulted in a remarkable increase of Ca and Fe contents in treated samples compared to the control. These results were confirmed by the finding of Popravko (1978) and Hegazi *et al.* (2000) who reported that propolis contains a great number of micro elements and amounts of minerals such as calcium, iron, magnesium, manganese, copper, aluminum and others.

3. Physico- chemical properties of beefburger as influenced by using propolis :

Data presented in Table (3) show the values of total volatile nitrogen (T.V.N.), thiobarbituric acid (T.B.A.), pH, water holding capacity (W.H.C.), plasticity, thawing loss % and cooking loss % as indicators of physico-chemical properties of beefburger as affected by using propolis (immediately after processing). From the results, it could be observed that control sample had slightly higher values of T.V.N. and T.B.A. than those treated with PEE and this means that using proplis as PEE in beefburger was effective against microbial activity and lipid oxidation respectively. The reverse was recorded of pH, so the control beefburger had slightly lower value than that of those treated with PEE, this may be due to the chemical composition of propolis whereas by increasing the addition level, the pH value was slightly increased. On the other hand, W.H.C., plasticity, thawing loss % and cooking loss % of beefburger treated with propolis (treatments) were improved than beefburger without adding propolis (control). Also, by increasing the addition level of propolis, T.V.N., T.B.A. thawing loss % and cooking loss % were decreased while pH value was increased in addition to the improvement in the W.H.C. and plasticity. These results were confirmed by the finding of Cengarle *et al.* (1998) and

Table 1. Proximate chemical composition of beef burger as affected by using propolis.

Samples*	Moisture %	Protein %	Fat %	Ash %	Carbohydrate %
Control	63.30	23.20	5.40	3.10	5.00
A	63.47	23.10	5.40	3.12	4.91
B	63.54	22.91	5.38	3.25	4.92
C	63.69	22.71	5.34	3.28	4.88

* = Propolis was used as propolis ethanol extract (PEE).

Control = Beef burger without adding propolis

A = Beef burger prepared with propolis at level of 0.1 %

B = Beef burger prepared with propolis at level of 0.2 %

C = Beef burger prepared with propolis at level of 0.3 %

Table 2. Mineral content of beef burger as affected by using propolis (mg/100gm).

Samples*	Ca	P	Fe	Z	Mg	K	Na
Control	61.00	89.10	3.47	2.12	17.00	76.00	602.00
A	63.13	89.31	3.99	2.30	17.51	76.18	602.15
B	65.28	90.10	4.85	2.82	17.93	76.41	602.28
C	70.61	90.81	6.10	2.96	18.40	76.59	602.39

* For explanation, see table (1).

Hegazi *et al.* (2000) who reported that propolis extracts had antimicrobial and antioxidant properties.

4. Microbial properties of beefburger as affected by using propolis:

Data presented in Table (4) show the microbial properties of beefburger as affected by using propolies. The results indicate that beefburger treated with PEE recorded lower total counts for total aerobic bacteria, proteolytic bacteria, lipolytic bacteria, and psychrophilic bacteria compared to the control sample. Product C which was formulated with PEE at level of 0.3% recorded the lowest counts of tested bacteria compared to the other added two PEE levels or control. It might be due to the antimicrobial effect of propolis which had bactericidal effect against gram positive and some gram negative bacteria, fungi, yeast and it was effective against all pathogens (Rojas and Alvarez, 1996 and Hegazi *et al.*, 2000).

5. Sensory evaluation :

Data presented in Table (5) show the results of ranking method and critical differences to find-out the best prodcut and testing the significance among all the products of beefburger as affected by using propolis (immediately after processing) . The lowest sum of ranks means the best product, accordingly the best product was C i.e. beefburger treated with PEE 0.3 % (rank sum = 48) followed by B and A (rank sum for both = 49) then control (rank sum = 54) which were treated with 0.1%, 0.2% and 0.3%, respectively. On the other hand, according to the critical differnces and the differences between products (in rank sum), signifcant differences were not recorded between all the products either at significance level of 0.05 or 0.01 whereas the products differs signifcantly when the rank sum differnces are greater than or equal to the critical difference.

6. Effect of using propolis on phsico-chemical properties of beefburger during frozen storage :

Physico-chemical properties of beefburger as infuenced by using PEE and frozen at -18°C for three months are given in Table (6). From these results, it could be observed that by increasing the period of storage till the end of storage, the values of T.V. N., T.B.A. and pH were gradually increased, but the increment ratio was lower in the beefburger treated with PEE than the control, as by the end of storage period of 3 months, the increment ratios of T.V.N., T.B.A. and pH value of control were 167, 59, 12% compared to 48, 57 and 10 % of treatment A ; 76, 54 and 9 % of treatment B

Table 3. Physico-chemical properties** of beef burger as affected by using propolis.

Samples*	T.V.N	T.B.A	pH	W.H.C	Plasticity (cm/03mg)	Thawing loss %	Cooking loss%
Control	8.8	0.54	5.28	1.6	1.2	1.7	9.5
A	8.8	0.42	5.29	1.2	1.5	1.2	7.6
B	8.5	0.35	5.31	1.2	2.0	1.0	7.4
C	7.6	0.22	5.35	0.1	2.2	0.3	5.3

* For explanation, see table (1).

** - T.V.N. = Total volatile Nitrogen (mg/100 gm sample).

T.B.A = Thiobarbituric Acid (mg malanaldehyd/kg sample).

W.H.C = Water Holding Capacity (cm/0.3 gm sample).

Table 4. Microbial ** properties of beef burger as affected by using propolis (cfu/gm).

Samples*	T.A.P.C.	P.B.	L.B.	P_S.B.	Salmonella
Control	4.2×10^5	7.1×10^4	3.9×10^4	9.8×10^3	- -
A	3.8×10^5	6.3×10^4	3.5×10^4	9.3×10^3	- -
B	3.2×10^5	5.9×10^4	3.0×10^4	9.0×10^3	- -
C	1.8×10^5	4.3×10^4	2.2×10^4	8.1×10^3	- -

* For explanation, see table (1).

** - T.A.P.C. = Total Aerobic plate Count.

P.B. = Proteolytic Bacteria.

L.B = Lipolytic Bacteria.

Ps .B. = Psychrophilic Bacteria.

cfu = Colonies forming units.

Table 5. Results** of ranking method and critical differences used for sensory evaluation of beefburger as affected by using propolis (after processing immediately).

Samples*	Control	A	B	C
Rank sum	54	49	49	48
Difference Vs.				
Control	- -	5	5	6
A	- -	- -	- -	1
B	- -	- -	- -	1

Significance level	P = 0.05	P = 0.01
Critical difference	21.00	25.40
Preferred products		
C	a	a
B	a	a
A	a	a
Control	a	a

* For explanation, see table (1).

** - The lowest sum of ranks means the best product.
- The products differs significantly (different letters) when the rank sum differences are greater than or equal to the critical difference .

Table 6. Physico-chemical** properties of beefburger as affected by using propolis and storage at -18°C for 3 month.

Samples*	Storage period (in month)	T.V.N	T.B.A.	PH	W.H.C.	Plasticity (cm^2/0.3mg)	Thawing loss %	Cooking loss%
Control	0	8.8	0.54	5.28	1.6	1.2	1.7	9.5
	1	12.6	0.60	5.47	1.8	1.6	1.9	9.9
	2	17.0	0.75	5.71	2.5	2.0	2.5	10.6
	3	23.5	0.86	5.90	3.7	2.8	3.0	11.5
A	0	8.8	0.42	5.29	1.2	1.5	1.2	7.6
	1	10.9	0.47	5.45	1.4	1.6	1.3	8.1
	2	12.8	0.55	5.65	1.7	1.9	1.7	8.5
	3	17.5	0.66	5.83	1.9	2.7	2.2	9.3
B	0	8.5	0.35	5.31	1.2	2.0	1.0	7.4
	1	9.3	0.36	5.46	1.4	2.2	1.2	7.4
	2	11.0	0.43	5.61	1.8	2.6	1.5	7.9
	3	15.0	0.54	5.80	2.2	2.9	2.0	8.6
C	0	7.6	0.22	5.35	0.1	2.2	0.3	5.3
	1	7.8	0.25	5.50	0.3	2.3	0.4	5.8
	2	8.6	0.28	5.60	0.6	2.4	0.6	6.2
	3	12.2	0.33	5.71	1.1	2.6	0.8	6.5

* and ** = For explanation, see tables (1) and (3)

and 61, 50 and 7 % of treatment C respectively. This means that the increment ratios of T.V.N, T.B.A. and pH during storage were decreased by increasing the addition level of PEE in treated beefburger, accordingly keeping quality and extending the shelf life might be occurred for the products treated with propolis. On the other hand, with respect to W.H.C., plasticity and thawing and cooking loss %, the same previous trend was recorded whereas the PEE reduced the thawing loss % and cooking loss % of treatments which recrded 0.3 – 1.2 % and 5.3 – 7.6 % when compared to control which had 1.7 and 9.5 %, respectively at zero time, also PEE improved the W.H.C. and plasticity of treatments. During storage for three months, W.H.C., plasticity, thawing loss % and cooking loss % of treatments were better than that of control. These results are confirmed by the finding of Han and Park (1996) and Cengarle *et al.* (1998).

7. Effect of using propolis on microbial properties of beefburger during frozen storage :

Data presented in table (7) show the total counts of total aerobic, proteolytic, lipolytic, psychrophilic bacteria and salmonella of beefburger as affected by using propolis ethano1 extract. These data indicated that beefburger treated with PEE had lower counts of tested microorganisms than control sample at zero time of storage, as well as, the total counts of tested bacteria were decreased by increasing the addition level of PEE in treated beefburger. On the other hand, during storage period at -18°C for three months, the same previous trend was recorded for control and beefburger treated with PEE. Also, from the results, it was evident that using PEE in processing of beefburger was effective against the tested bacteria. Salmonella was not detected either for control or beefburger treated with PEE . These results were confirmed by the finding of many researchers such as Rojasand and Alvarez (1996) and Hegzi *et al.* (2000) who reported that the propolis extracts had a bactericdal effect against gram positive, some gram negative, fungi and yeast in addition to its effective against all pathogens.

On the whole, "propolis" the natural product of honeybess which has antioxidant and antimicrobial effect could be used as a natural preservative instead of chemical or synthetic preservatives in meat products to improve and keep the quality and extend the shelf life of these products such as beefburger. Finally, beefburger treated with propolis ethanol extract at addition level of 0.3 % was suggested for production on commercial scale.

Table 7. Microbiogical** properties of beefburger as affected by using propolis and storage at – 18°C for 3 month(cfu/gm).

Storage period (in month)	Samples*	T.A.P.C.	P.B.	L.B.	Ps .B.	Salmonella
0	control	4.2×10^5	7.1×10^4	3.9×10^4	9.8×10^3	-
	A	3.8×10^5	6.3×10^4	3.5×10^4	9.3×10^3	-
	B	3.2×10^5	5.9×10^4	3.0×10^4	9.0×10^3	-
	C	1.8×10^5	4.3×10^4	2.2×10^4	8.1×10^3	-
1	control	5×10^5	8.3×10^4	4.6×10^4	1.2×10^4	-
	A	2.7×10^5	5.4×10^4	3.3×10^4	8.9×10^3	-
	B	1.8×10^5	4.3×10^4	1.7×10^4	8.2×10^3	-
	C	8.0×10^4	2.0×10^4	9.1×10^3	6.3×10^3	-
2	control	6.9×10^5	1.0×10^5	7.3×10^4	3.5×10^4	-
	A	4.0×10^5	6.8×10^4	3.7×10^4	1.0×10^3	-
	B	2.8×10^5	5.5×10^4	1.9×10^4	9.0×10^3	-
	C	8.5×10^4	2.8×10^4	9.3×10^3	6.8×10^3	-
3	control	9.8×10^5	4.3×10^5	9.6×10^4	5.3×10^4	-
	A	5.5×10^5	8.5×10^4	4.9×10^4	2.1×10^3	-
	B	4.0×10^5	6.6×10^4	3.0×10^4	9.9×10^3	-
	C	9.3×10^4	3.6×10^4	1.0×10^4	7.5×10^3	-

* and ** = For explanation, see tables (1) and (4)

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تأثير استخدام البروبلس والتخزين بالتجميد على خصائص جودة برجر اللحم

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في السنوات الحديثة تلعب المواد الطبيعية دوراً هاماً في تحديد اختيار المستهلك للمنتجات الغذائية ، وقد استخدمت منتجات نحل العسل بشكل مركز في مجال التجارب الكلينيكية وحديثاً فإن خواصها المضادة للميكروبات والمضادة للأكسدة قد شدت الانتباه بصورة كبيرة . ولذلك ، في هذا البحث تم تصنيع برجر اللحم البقري المعامل بمستويات مختلفة من البروبلس المستخلص بالإيثانول Propolis Ethanol Extract (PEE) وكانت مستويات الاضافة هي ١,٠ ٪ (أ) ، ٢,٠ ٪ (ب) ، ٣,٠ ٪ (ج) بالإضافة إلى تصنيع برجر اللحم البقري بدون اضافة البروبلس (كنترول) وذلك لمعرفة تأثير استخدام البروبلس على خصائص جودة المنتج.

وقد تم تقدير التركيب الكيماوي ، المحتوى المعدني ، خصائص الجودة الطبيعية والكيماوية ، الخصائص الميكروبيولوجية ، كما استخدمت طريقة الـ Ranking لتقييم المعاملات حسياً واختبار المعنوية بينها وذلك بعد التصنيع مباشرة . كما تم تقدير خصائص الجودة الطبيعية والكيماوية والميكروبيولوجية أثناء التخزين بالتجميد (- ١٨°م) لمدة ٣ شهور.

وقد أوضحت النتائج بصورة عامة أنه يمكن إضافة مستخلص البروبلس كمادة طبيعية بنسبة ٣,٠ ٪ للمحافظة على خصائص جودة برجر اللحم وبالتالي إطالة مدة صلاحيته.