Egypt. J. Agric. Res., 78 (4), 2000.

EFFECT OF THE ADDITION OF PROPOLIS EXTRACT AS NATURAL ANTIOXIDANT ON THE KEEPING QUALITY OF BISCUIT DURING STORAGE

ABDEL-SALAM, SAMIHA, M.M.

Food Techn. Res. Inst., ARC.

(Manuscript received March, 1999)

Abstract

This investigation was carried out to study the effect of adding propolis extrat, as antioxidant, to the biscuit formula as improving agent to enhance the shelf life. Propolis extract contained 64.1%. total flavonoids. Addition of propolis extract to butter oil resulted in good stability upon storage. The stability was in parallel with propolis concentration which showed 15.5 months at 0.02% to 38 months at 0.1%, addition levels. Meanwhile it was 8 months in respect to control. Peroxide value of treated, untreated butter oil, and that extracted from baked biscuits during 8 months of storage was determined and showed the same trend as that of Rancimat. Organoleptic properties of the baked biscuit showed an improvement due to the addition of propolis extract.

INTRODUCTION

Biscuits are snack food used by children and students because of their high acceptability and contains required nutrients for human nutrition. Biscuits stored at room temperature which ranged from 20 to 40 ^{o}C according to the season for about 2 months, were subjected to rancidity problems which include the production of an off-flavor and other unfavorable components.

Nowadays, there is a tendency for the consumer to reject foods containing synthetic antioxidants, i.e., BHA or BHT, because of their possible toxicity (Prochaika *et al.*, 1986 and Takahashi, 1985) This leads to the interest of preparing antioxidants from natural sources.

In this respect, tocopherol, L-ascorbic acid, purified extract of some herbs aqueous extract of black tea leaves, B-carotene and essential oils were used as natural antioxidants by several investigators.

Recently, flavonoids are taking place as natural antioxidants. In this connection, Vekiari *et al.* (1993) found that the ethyl ether extract from oregano (*Origanum vulagare* L.) leaves was the most effective in stabilizing lard against oxidation. The main antioxidant factors isolated from the ethyl extract consisted of flavonoids. Chromatographic and spectrophotometric analysis demonstrated the presence of the flovone

apigenin, the flovonone, eriodictyol and the dihydroflavonols, dihydrokaempferal and dihydroquercetin. Propolis which consists primarily of plant exudates gathered by bees and mixed with bee wax which they secrete togther with small amounts of sugar for sealing and draught exclusion for beehives was analyzed by several investigators. For example, Johnson *et al.* (1994) found that kaemferal, galangin, 3.3 dimethoxy quercetin, 3-methoxy-kaempferol, quercetin, chrysin, caffeic acid and ferulic acid in the methanolic extract of propolis. Serra-Bonvehi and Ventura-Coll (1994) reported that propolis contained mainly acacetin, isorhamnetin, apigenin and pinocembrin as flavonoids in propolis. The effect of propolis as antioxidant was studied by Dessouki *et al.* (1980) who mentioned that frozen meat in propolis solution showed more pronounced antioxidative effect than using propolis by glazing. Rapta *et al.* (1995) investigated the redox properties of 7 flavonoids and 2 cafferic acid esters, all isolated from propolis, by cyclic voltametry in acetonitirile. Oxidation potentials of the flavonoids ranged from 1.2 to 2.0 and 1.29-2.3 v for the two extracts. Values were influenced mainly by the presence or absence of a double bond in 2.3-position and substituent R in the 3-position. The compounds with lower oxidation potential had the maximum lipid oxidant activity. Some of the radicals obtained on oxidation of the compounds were identified, and anion radicals were found on reduction of chrysin and galangin. Volpert and Elstmer (1996) studied the effect of different ethanolic and aqueous extracts of propolis on leukocytes and some of their most important enzymes, namely myeloperoxidation, NADPH oxidase and lipoxygenase. They found that highly concentration of propolis extracts inhibited these enzymatic activities, but the water soluble derivatives showed a stimulatory effect on the activity of available human myeloperoxidase. Leukocytic myeloperoxidase and NADPH oxidase activites were clearly inhibited by propolis extracts, probably indirectly due to their excellent radical scavening properties.

The present work was carried out to study the effect of ethanolic extract of propolis as antioxidant on the shelf of biscuits.

MATERIALS AND METHODS

Wheat flour (72% extraction rate) was obtained from Egyptian Milling Company, El-Malik Fisal St., Giza. Pure butter oil (Haloub) a product of European Economic Committee (E.C.C.) and skimmed milk powder spray dried, admixture grade, low heat, USA origin were purchased from the supper market. Butylated hydroxy toluene (BHT) as synthetic antioxidant (99.9 purity) was obtained from the Naarden International Company. Propolis was obtained from Giza and Fayoum and mixed in one homogenous

mass.

Chemical analysis of propolis

Propoils analyzed for total lipids, total sugars waxes according to the methods outlined in A.O.A.C. (1990) while total flovonoids were determined according to the method described by Snell and Snell (1954).

Preparation of ethanolic extract of propolis

The biologically active substance was extracted from the propolis with ethyl alcohol according to the method described by Ohta and Yagishita (1970) as modified by Meresta and Meresta (1982). Propolis (100 g) was covered with n-hexane overnight, then filtered. The residue was treated with hot ethanol (70%, v/v) at 70 ^{o}C for 10 hr. The alcoholic extract was fitered and evaporated under vacuum. Absolute ethanol was used to obtain ethanolic of propolis. The solvent was removed by lyophillization.

Test of flavonoids

Flavonoids were detected using the method of Well *et al* (1954) as follows: concentrated HCl (2ml) was added drop wise to the alcohlic extract (3 ml) of propolis containing a fragments of magnesium ribbon. The produced pink color indicated the presence of flavonoids.

Determination of total flavonoids

Total flavonids were determined according to the method described by Snell and Snell (1954) as follows: Five ml of the ethanolic extract, 3 ml of aluminium chloride hexhydrate (2.4% w/v) (AICl 3.6 H2O) solution were added followed by the addition of potassium acetate (5 ml, 9.8, w/v). After 5 min, the resulting yellow color was measured at 420 nm using spectronoic 20. A blank determination was carried out. Total flavonoids were calculated as quercetin.

Determination of peroxide value of butter oil and those extracted from biscuit during storage

Peroxide value of treated or untreated butter oil with BHT or ethanolic extract of propolis and those extracted from biscuit during storage were determined according to the method oulined in A.O.C.S. (1993).

Determination of oxidative rancidity of butter oil Rancimat

The Rancimat method was developed by Hadorn and Zurcher (1974). Rencimat 679 which can accommodate 6 test samples, was used in the present study. A known weight (5 g) of sample (treated or untreated butter oil with propolis extract) was placed in the reaction vessel, then placed into the heating block for 10 min to preheat the sample. After that, air is supplied by the built-in pump at flow rate of 20 L/hr, the temperature was adjusted at 100^{o}C and absorption vessels were connected after being filled with 60 ml distilled water for each. Measuring electrodes were immersed into water insuring that each one is in its correct opposition and recording the conductivity curves starts, two tangents for both sides of the induction curve were drawn, the intercept of which meet the time axis perpendicularly at the point known as induction time, which is defined as the time elapsed from the beginning up till the oil starts rancidity at the conditions of the determination.

Preparation of biscuit

Before biscuit dough preparation, lyophilized propolis extract was mixed with the butter oil (Haloub) at 0.02, 0.04, 0.06, 0.08 and 0.1% levels as natural antioxidant, while BHT was mixed at 0.02% as synthetic antioxidant. The treated and untreated oil were added to the biscuit formulas 50 g of each.

The biscuit formula consists of :

1 Wheat flour (180 g)

2. Sucrose (60 g)

3. Skimmed milk (7.5 g)

4. Baking powder (8.0 g)

5. Sodium metabisulfite (0.2 g)

6. Sodium bicrabonate (1.25 g)

7. Water (65 ml)

8. Butter oil (50 g)

The ingredients were mixed well for 20 min., shaped, cut, and baked at 240^{o}C for 10 min. After baking biscuits were cooled for 15 min. before packing in cellophane

and aluminum foil and stored for 8 months.

Sensory evaluation

The organoleptic properties of the baked biscuits were measured by ten personal trained judges. The judges were asked to give a score from zero to ten for taste, color, odor, texture and shape as reported by Amerine *et al.* (1965).

Statistical analysis

All sensory parameters were statistically analyzed according to the method described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Chemical composition of propolis

Results in table 1 show the chemical composition of raw propolis. The data revealed that it contains high amount of flovonoids (64.1%), waxes (27%), total sugar (2%), total lipids (1.5%) and other substances (5.3%). The obtained data are in agreement with the findings of Johnson *et al.* (1994) who mentioned that the methanol extractable resin in propolis collected from Ohio and Georgia ranged from 24 to 79 % by weight.

Table 1: Chemical analysis of propolis.

Component	Percentage (%)
Total flavonoids	64.1
Waxes	27.0
Total sugars	2.0
Total lipids	1.5
Other substances*	5.3

* By differences

Effect of propolis extract on the peroxide value of butter oil and that extracted from biscuit during storage

Results in table 2 show that the peroxide value of butter oil and those extracted from biscuit during storage period of 8 months. The data reveal that slight gradual in-

Table 2. Effect of antioxidants on the peroxide value of butter oil and that extracted from biscuit during storage periods.

Storage period (month)		Control	B.H.T at 0.02%	Concentrations of propolis extract %				
				0.02	0.04	0.06	0.08	0.10
Zero time	B.O*	1.62	1.62	1.62	1.72	1.62	1.62	1.62
	B**	1.72	1.72	1.72	1.62	1.72	1.72	1.72
1	B.O*	2.41	1.81	1.65	1.80	1.62	1.72	1.62
	B**	2.50	1.83	1.80	1.63	1.72	1.62	1.72
2	B.O*	3.56	1.96	1.70	1.83	1.63	1.72	1.62
	B**	3.17	2.00	1.83	1.65	1.72	1.62	1.72
3	B.O*	4.11	2.22	1.88	1.96	1.66	1.83	1.62
	B**	5.80	2.43	1.96	1.68	1.83	1.62	1.72
4	B.O*	4.83	2.37	2.40	2.11	1.68	1.94	1.62
	B**	6.70	2.58	2.11	1.72	1.94	1.64	1.72
5	B.O*	7.15	4.71	2.91	2.37	1.70	2.15	1.68
	B**	9.12	5.11	2.37	1.77	2.15	1.64	1.77
6	B.O*	10.10	5.96	4.10	4.44	1.73	2.30	1.72
	B**	12.70	6.85	4.44	1.91	2.30	1.68	1.88
7	B.O*	12.66	7.14	5.00	5.96	2.74	2.37	1.78
	B**	14.50	10.60	5.96	2.11	2.37	1.88	1.94
8	B.O*	14.40	9.40	6.60	6.81	2.78	1.96	1.91
	B**	17.32	14.22	6.81	3.55	2.50	2.27	2.00

* B.O = butter oil
** B= soils extracted from biscuit

crease in peroxide value till 5 months then increased rapidy to reach its maximum value being 15.4 and 17.32 meq/kg for the butter and the extracted one from biscuit, respectively after 8 months of storge. Meanwhile addition of BHT (0.02% w/v) delayed the increase in the peroxide value of the butter oil untill 7 months and then markedly increased after 8 months of storage of biscuit. Propolis extract, showed good stability of either butter oil or that extracted from biscuit during storage periods. It seems that the lower peroxide value was in parallel with the increase in propolis extract. According to the Egyptian standard which limited the peroxide value of edible oil at 10 meq/k, it could be concluded that propolis extract at 0.02% could be recommended to keep biscuit containing butter oil more than 8 months. In this respect, Vekiari *et al.* (1993) found that the ethyl ether extract from Oregano leaves which contains flavoinds was the most effective for the stability of lard against oxidation. Also, Khalil *et al.* (1997) reported that the broad bean hull extracts can be used as natural antioxidant to improve the oxidative stability of soybean oil as a result of phenolic and flavonoid compounds.

Effect of propolis extract on the oxidative stability of butter oil by using 679 Rancimat

Results in table 3 show the induction time of butter oil separately treated with propolis extract at 0.02, 0.04, 0.08 and 0.1% w/w compared to the control. The data revealed that the untreated butter oil showed induction time of 8 hr (one hr equal one month), Hill (1994). Meanwhile addition of propolis resulted in 15.3 hr, 24.15, 30 hr, 34 hr and 38 hr which was in line with the concentration.

From the above mentioned data, it could be noticed that the induction time was in accordance with the peroxide value (Table 2). For delaying peroxide and keeping quality of baked goods especially that contains oils, propolis extract as natural and safe antioxidant could be recommended to keep quality of the baked good and also to increase its shelf life.

Effect of propolis extract, as antioxidant, on the organoleptic properties of biscuit during storage

The organoleptic properties of baked biscuits contained different concentrations of ethanolic extract of propolis, i.e, 0.02, 0.04, 0.06, 0.08 and 0.1% (w/w) compared to BHT (0.02% w/w) and untreated ones during storage (8 months) are presented in Table 4. Biscuits taste, odor, color, texture and shape are expressed by numerical values relative to that is considered as the best. The maximum score of these parameters

was 10. From the obtained data, it could be observed that additions of propolis extract resulted in improving the biscuit organoleptic properties during storage and it depend upon its concentration compared to the BHT as synthetic antioxidant or the untreated biscuit. The data revealed also that the addition of propolis extract increases the shelf life of produced biscuits which is relative to its concentration, compared to BHT. The statistical analysis (Table 5) show significant differences in all parameters due to treatments, storage periods and the interaction between treatments and storage propolis. The improvement occured when extracts were used may be due to their flavonoids content which act as natural antioxidant and delay the peroxidation process as found in Rancimat experiment.

Table 3. Effect of propolis addition on the inductione time of butter oil by 679 Rancimat.

Treatment	Induction time (hr)
Control	8
Propolis extract %:	
0.02	15.30
0.04	24.15
0.06	30.00
0.08	34.00
0.10	38.00

Table 4. Effect of propolis extract as natural antioxidant on the organolpetic properties of biscuit during storage periods.

Storage period (month)	Control	B.H.T at 0.02%	Concentrations of propolis extract %				
			0.02	0.04	0.06	0.08	0.10
			Taste				
Zero time	8.75	8.75	8.90	8.92	8.81	8.75	8.72
1	8.60	8.75	8.90	8.90	8.80	8.75	8.70
2	8.10	6.62	8.88	8.90	8.80	8.74	8.70
3	7.20	8.11	8.77	8.90	8.80	8.71	8.69
4	6.82	7.76	8.73	8.87	8.80	8.70	8.66
5	6.46	7.15	8.67	8.68	8.77	8.63	8.65
6	6.10	7.00	8.56	8.81	8.71	8.60	8.55
7	5.42	6.13	8.40	8.80	8.70	8.58	8.53
8	5.13	5.70	8.30	8.75	8.70	5.59	8.50
			Odor				
Zero time	9.00	8.80	9.00	9.40	9.50	9.50	9.25
1	8.50	8.72	9.00	9.36	9.50	9.50	9.20
2	8.50	8.61	9.00	9.14	9.43	9.46	9.20
3	7.30	8.10	8.87	9.00	9.27	9.32	9.10
4	7.10	7.25	5.56	8.91	9.10	9.26	8.96
5	7.00	7.00	8.37	8.87	9.00	8.82	8.87
6	6.53	6.66	8.00	8.75	8.85	8.80	8.85
7	6.10	6.62	7.77	8.64	8.80	8.75	8.71
8	5.80	6.50	7.62	8.60	8.72	8.70	8.58
			Color				
Zero time	8.36	8.40	8.53	8.60	8.21	8.71	8.31
1	8.35	8.33	8.53	8.55	8.20	8.65	8.28
2	8.21	8.22	8.50	8.53	8.19	8.58	8.20
3	8.00	8.17	8.41	8.50	8.16	8.50	8.12
4	7.90	8.12	8.40	8.44	8.12	8.43	8.10
5	7.83	8.12	8.36	8.36	8.00	8.11	7.93
6	7.70	8.00	8.30	8.35	7.96	8.97	7.87
7	7.50	8.00	8.00	8.30	7.91	7.90	7.75
8	7.50	8.00	8.00	8.28	7.87	7.90	7.71
			Texture				
Zero time	7.93	8.00	8.00	8.00	8.10	7.87	7.85
1	7.75	8.00	8.00	8.00	8.10	7.81	7.84
2	7.33	8.00	8.00	8.00	8.10	7.77	7.81
3	7.10	7.87	8.00	7.87	8.00	7.71	7.80
4	7.10	7.81	7.90	7.83	7.92	7.68	7.77
5	7.10	7.62	7.88	7.80	7.88	7.60	7.70
6	7.00	7.51	7.78	7.68	7.75	7.60	7.66
7	7.00	7.48	7.75	7.65	7.69	7.58	7.54
8	6.90	7.45	7.73	7.61	7.65	7.32	7.50
			Shape				
Zero time	8.70	8.72	8.76	8.75	8.75	8.75	8.90
1	8.50	8.72	8.75	8.75	8.73	8.72	8.66
2	8.45	8.70	8.75	8.70	8.72	8.70	8.80
3	8.40	8.65	8.73	8.70	8.70	8.66	8.73
4	8.30	8.58	8.70	8.65	8.61	8.61	8.69
5	8.10	8.41	8.66	8.58	8.57	8.42	8.61
6	8.00	8.36	8.61	8.51	8.55	8.31	8.85
7	8.00	8.30	8.60	8.48	8.51	8.11	8.50
8	7.70	8.30	8.60	8.48	8.00	8.00	8.50
			Average total score				
Zero time	8.55	8.25	8.64	8.73	8.67	8.72	8.61
1	8.34	8.50	8.64	8.71	8.66	8.69	8.57
2	8.12	8.43	8.63	8.65	8.65	8.65	8.54
3	7.60	8.18	8.56	8.69	8.59	8.58	8.49
4	7.44	7.90	8.46	8.54	8.51	8.54	8.44
5	7.30	7.66	8.39	8.49	8.44	8.32	8.35
6	7.07	7.51	8.25	8.42	8.36	8.26	8.30
7	6.80	7.31	8.10	8.37	8.32	8.18	8.20
8	6.61	7.19	8.05	8.34	8.19	8.10	8.16

Table 5. Least significant differences at P>0.05 of organoloeptic properties of biscuit during storage.

Properties	Taste	Odor	Color	Texture	Shape	Average score
Treatments	0.48	1.10	0.23	0.44	0.21	0.13
Storage	1.57	2.05	0.71	0.98	0.92	0.54
Treatment x storage period	0.78	2.41	0.63	0.64	0.93	0.70

REFERENCES

1. Amerine, M.A.; R.M. Paglorn and E.B. Roessler (1965). Principals of sensory evaluation of foods. Academic Press, New York.

2. A.O.A.C. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th Ed., lington, Virginia, USA.

3. A.O.C.S. (1993): Official Methods and Recommended Practices of the American Oil Chemists Society. 4th Ed. Pub. American oil Chemists Society, 1608, Broadmorr Drive, Champaign, Illionis 68126-3489.

4. Dessouki, T.M.; A.A. El-Dashlouty, M.M. El-Ebzary and H.A. Meikal (1980). Propolis and some natural antioxidants for fat of frozen meat. Agric. Res. Rev., 58 (3): 311-321.

5. Hadorn, H. and K. Zurcher (1974). Dtsch Lebensn, Rundsch 70:577. c.f. Determination of oxidative stability of some edible vegetable oil Rancimat. Mohamed A. Madkour (ed.). Egypt. J. Nutr.X (1): 175-184.

6. Hill, S.E. (1994). Comparison : measuring oxidative stability. AOCS, 5 (1): 104-109.

7. Johnson, K.S., F.A. Fischer and D.E. Giannasi (1994). Chemical composition of North American bee propolis and biological activity towards larvae of greater wax noth (Lepidoptera: Pyralidae). J. Chemical Ecology, 20 (7) 1783-1792.

8. Khalil, M.F., R.N. Sandak and A.Y. Girgic (1997). Effect of broad bean (*Vicia faba* hull extracts as natural antioxidants on stability of soybean oil. Egypt. J. Appl. Sci., 12 (8): 506-524.

9. Meresta, L. and T. Meresta (1982). Use fullness of various solvents for extracting biologically active substances from propolis. Bull. of the Veterinary Ints. In Pulaway, 25 (1-4):12.

10. Ohta, N. and K. Yagishita (1970). Comparative study for different solvents with polyphenolic components. Agric. Biol. Chem. 34:900.

11. Prochaska, H.J., H.S. Bregman, M. J. Delong and P. Talalay (1986). Specificity of induction of cancer protective enzymes by analags of tert-butyl-4-hydroxyanisol (BHA). Biochem. Pharmacol., 34 (21):3909-3914.

12. Rapta, P.; V. Misik; A. Stasko and I. Vrabel (1995). Rodex intermediates of flavonoids and caffic acid esters frm proples in EPR spectroscopy and cyclic valtametry study free-radical. Biology and Medicine, 18 (50):901-908.

13. Serra- Bonvehi, J. and F. Ventura-Coll (1994). Phenolic composition from China and from South America. Zeitschrift Fur Naturorschung "Section C". Bioscience 49 (11):11-12, 712-718.

14. Snell, F.D. and G.T. Snell (1954). Colorimetric methods of analysis. 3rd Ed. D. Van Nostrand and Co. New York.

15. Steel, R.G. and J.H. Torrie. (1980). Principle and Procedure of Statistical and Biometrics Approach. McGrow Hill-Book Company, 2nd Ed.

16. Takahashi, O. (1985). Reduced aggregation capacity of washed platelets and dysfibrinogenamia in rats ginen butylated hydroxotluene. Food and Chemical Toxicol. 23 (10):937-939. c.f. FSTA 18:5T 70 (1986).

17. Vekiari, S.A., V. Oreopolou, C. Tzia and C.D. Thomopoulos (1993). Organo flavonoids as lipid antioxidants. J. Amer. Oil. Chem. Soc., 70 (5): 483-487.

18. Volpert, R. and E.F. Elstner (1996). Interaction of different extracts of propolis with leucocytes and leukocytic enzymes. Arzeimittel Forschung 46 (1):47-51.

19. Wall, M.E., M.M. Kreider, C.F. Kremson, G.R. Eddy. J.J. Willian, D.S. Covell and H.S. Gentry (1954) Survey of plants for steroridal saponins and other constituents. J. Pharm. Soc., 43: 1-5.

تأثير إضافة مستخلص صمغ النحل كمضاد أكسدة طبيعى على صفات البسكويت أثناء التخزين

سميحة محمد محمد عبد السلام

معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية

أجرى هذا البحث بإستخدام مستخلص صمغ النحل فى صناعة البسكويت لتحسين فترة التخزين . ولقد أوضح التحليل الكيميائى لصمغ النحل أنه يحتوى على ٦٤,١٪ فلافونيدات ، كذلك أجرى اختبار الثبات للدهن المستخدام فى صناعة البسكويت بإستخدام جهاز الرانسيمات فى وجود التركيزات المختلفة لمستخلص صمغ النحل ، اوضحت النتائج أن صمغ النحل أدى إلى إطالة فترة ثبات الدهن متمشيا مع التركيز حيث كان أعلاها ٣٨ شهر عند إضافة ٠,١٪ مستخلص صمغ النحل فى حين أن إضافة ٠,٠٢٪ مستخلص صمغ النحل أدى إلى ثبات الدهن لفترة ١٥ شهر ونصف مقارناً بالدهن غير المضاف إليه مضاد إكسدة حيث كانت ٨ أشهر فقط.

كذلك تم تقدير رقم البيروكسيد للدهون المخزنة وكذلك المستخلصة من البسكويت أثناء فترات التخزين المختلفة وكانت متمشية مع نتائج الرانسيمات . وأوضحت الصفات الحسية للبسكويت تحسناً ملموساً نتيجة إضافة التركيزات المختلفة من مستخلص صمغ النحل.