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STUDIES ON USING OF SOME EGYPTIAN HERBS AS ANTIOXIDANTS IN PRODUCTION OF BISCUIT

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ABSTRACT

Day by day increase the importance of using the natural food additives. Hence in this study, the phenolic extracted from basil leaves, spearmint leaves and fennel seeds, as natural antioxidants, were added at concentrations 200, 400 and 600 ppm from fat weight using in biscuit production. Biscuit was stored at room temperature for 8 months. Induction period was measured by Rancimat apparatus. Total phenolic and phenolic acids were determined. Peroxide value, acid value, thiobarbituric acid and refractive index were measured during storage. Antimicrobial activity of phenolic extraction were examined after 8 months. Sensory evaluation was measured directly after baking. The results showed that total phenolic content in basil leaves, spearmint leaves and fennel seeds were 3.97, 1.91 and 1.53 mg/g as caffeic acid equivalents respectively. Five phenolic acids were found in both basil leaves and spearmint leaves, while four phenolic acids were found in fennel seeds as determined by using HPLC. Rancimat results showed that induction period for phenolic herbs could be ranked as follow: basil leaves > spearmint leaves > fennel seeds. The results elucidated that the best concentration from natural additives as antioxidants activity were 400 and 600 ppm compared with BHT. The results revealed that with increasing the concentration of natural antioxidants increased, the more peroxide value, acid value, thiobarbituric acid and refractive index were decreased. Total bacterial count and (yeast & mold) count were decreased with increasing the concentration phenolic additives. Sensory evaluation indicated that addition of different antioxidants showed no significant differences between control and biscuit samples.

INTRODUCTION

Lipid oxidation causes a decrease in nutritional value of lipids, in their safety and appearance. Nowadays, various synthetic and natural antioxidants are used in prevention or retardation of lipid oxidation. Recently, some negative side effects of the commonly used synthetic antioxidants have been established that has shifted of consumer interest to the natural products as a less harmful alternative to the synthetic ones. So identifying antioxidants that stop or reduce the generation of free radical chain reactions is very important. Synthetic antionxidants,i.e. butylated hydroxy anisol (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinone (TBHQ) and propyl gallate (PG) are used in food industries at low concentration. It was reported that synthetic antioxidants possess carcinogenic effect by stimulation of DNA synthesis and induction of enzymes; producing neoplasms of the non-glandular sequamous cell protein of the stomach and hepatocellular tumors. Thus, the importance of natural antioxidants has been greatly increased, as safer and less adverse reaction **(Williams *et al* 1990).**

Antioxidants can protect a fat or oil from oxidation but cannot rejuvenate an already oxidized fat or oil. Addition of antioxidants can effectively inhibit oxidation and extend the shelf-life of food products. Proper selection of antioxidant(s) and through dispersion into the fat or oil portion of the product can ensure adequate protection against oxidation **(Byrd, 2001)**

Antioxidants are major ingredients which play an important role in manufacturing, packaging and

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storage of lipid containing foods. Synthetic antioxidants are usually used in food industry to reduce deterioration and rancidity of oils and fats. There has been growing concern regarding the possible activity of such synthetic antioxidants to cause liver damage. Therefore development of safer, inexpensive natural antioxidants is essentially **(Yen and Due 1993).**

Phenolic compounds are commonly found in both edible and non-edible plants and they have been reported to have multiple biological effects including antioxidant activity. Crude extracts of vegetables, herbs, fruits, cereals and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antixodant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufactures, and consumers as the trend of the future is moving toward functional food with specific health effects **(Kahkonen *et al* 1999).**

Antioxidant activities of methanol extracts of 180 oriental herbs were studied by determining the peroxide value of linoleic acid during storage at 50ºC. Among to these herbs, basil leaves, spearmint leaves and fennel seeds showed strong antioxidant activities on the oxidation of linoleic acid **(Kim *et al* 1994).**

The purpose of this study was to determine total phenolic and phenolic acids compounds extracted from selected herbs, antioxidant activity, fat stability, antimicrotial effect for phenolic and sensory evaluation of biscuit samples.

MATERIALS AND METHODS

Materials

Basil leaves *(Ocimum basilicum L.)* and spearmint leaves *(Mentha spicata L.)* were obtained from the Experimental Station of Medicinal Plant, Faculty of Pharmacy, Cairo University, Giza, Egypt, while fennel seeds *(Foeniculum vulgare Miller)* was obtained from Medicinal Plant and Agricultural Seeds Harraz Company, Cairo, Egypt.

- Wheat flour (72% extraction) was obtained from Egyptain Milling Company, EL-Malik Fisal St., Giza.
- Shortening (Hydrogenated vegetable oil) was obtained from Safola Egypt Company 10th Ramadan City, Cairo (lable indicated that no antioxidants were added).
- Butylated hydroxy toluene (BHT) as a synthetic antioxidant (Purity 99.9%), standard phenolic acids. Folin–Ciocalteau reagent and caffeic acid standard were obtained from Sigma Chemical CO**.** (St. Louis, MO, USA).

Methods

Extraction of total phenolic

Each sample (20g) fine powder was extracted overnight with 200mL methanol at room temperature. The extracts was filtered through Whatman No.1 filter paper and the residue was re-extracted under the same condition. Both extracts were combined, filtered then evaporated in a rotary evaporator (Rotavapor R-124-BUCHI-Switzerland) at 40ºC to dryness and kept at -4ºC until analysis **(Duch and Yen 1997*).***

Determination of total phenolic content

The methanolic extracts (0.1 mL) from each sample was diluted with glass-distilled water (15mL) in a volumetric flask. Folin-Ciocalteau reagent (5mL) was added. After 3 min., sodium carbonate solution (10mL, 10% W/V) was added and finally quantified to 100mL glass-distilled water and then the mixture was allowed to stand for 30min. The absorbance was measured at 760 nm using spectrophotometer Shimadzu UN-1201. The concentration of total phenolic was expressed as mg caffeic acid/g equivalents by caffeic acid standard calibration curve **(Duch and Yen 1995).**

Identification of phenolic compounds

Identification of individual phenolic compounds of the plant samples was performed on a Hewlett-Packard HPLC (Model 1100) using a hypersil C18 reversed-phase column (250X4.6 mm) with 5µm particle size. Injection by means of a Rheodyne injection valve (Model 7125) with 50µl fixed loop was used. A continous flow rate of 1 ml/min was used with two mobile phase (A) 0.5% acetic acid in distilled water (pH 2.65) and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with (A) buffer and ending with (B) buffer over 35min, using an UV detector set at wavelength 254nm. Comparing their relative retention times with those of the standards mixture chromatogram

identified phenolic compounds of each sample. The concentration of an individual compound was calculated on the basis of peak area measurements. All chemicals and solvents used were HPLC spectral grade **(Zhu *et al* 2004).**

Antioxidant activity

The induction period (IP) was measured with the Rancimat apparatus 679 (Metrohm Ltd. CH-9100, Herisau, Switzerland) at 100 ºC and air flow rate of 20 L/h, whereas (IP) is the time period (hour) from the start until the inflection point in the curve of the increasing conductivity according to the method of **Schwarz *et al* (1996).**

Biscuit preparation

Biscuit formula was consisted of (100g) wheat flour, (33g) shortening (BHT was added at concentration of (200 ppm), phenolic of basil leaves, spearmint leaves and fennel seeds were added at concentration of (200, 400 and 600 ppm) respectively from weight of shortening used), (36g) sugar, (3g) baking powered, (0.25g) vanillia and (18g) water. Shortening, sugar and vanillia were mixed in a dough mixer using the flat beater for 1 minute, then scraped down and continued to mix for 3 minutes at high speed. Wheat flour and baking powder were added to the mixture and mixed at low speed then it was sheeted to 3 mm. thickness. Circle pieces cut of dough were formed by using of templates with an outer diameter of 60 mm. The biscuit were baked at 180 ºC for 12 minutes **(Wade, 1988).** Biscuit samples were packed in bags made from low density polyethylene (Density 0.915 g/cm^3) and stored for 8 months at room temperature.

Extraction of fat

One hundred gram of crushed biscuit was placed in 0 500ml. Closed stopper flask then, 300ml of n-hexane was added, the flask was shaked for 30 min using horizontal shaker and left for 24 hr at room temperature. The homogenated mixture was filtered by suction and the residue was re-extracted as mentioned above. The combined filtrates were evaporated under reduced pressure **AOAC (2000)**.

Fat stability

Peroxide value, acid value, thiobarbituric acid value (absorbance at 535nm) and refractive index (using abbe refractometer with constant temperature at 25 ºC) were analysed at zero, 2, 4, 6 and 8 months according to the methods out lined in **AOAC (2000).**

Microbiological examination

At the end of storage period (8months) total bacterial count was examinated in biscuit samples according to method of **APHA, (1971),** while total (yeast and mold) count were examinated according to the method of **Ranganna, (1979).**

Sensory evaluation

Color, flavor, appearance, taste and texture of biscuit samples were sensory evaluated according to the method of **Smith, (1972).**

Statistical analysis

The data obtained were analyzed using the Statistical Analysis System **SAS, (1996).**

RESULTS AND DISCUSSION

Total phenolic content

Concerning total phenolic content in selected herbs, the data in **Table (1)** showed that total phenolic content in basil leaves, spearmint leaves and fennel seeds were 3.97, 1.91 and 1.53 mg/g as caffeic acid equivalents respectively (on dry weight basis). On the other side **Wei and Wang, (2001)** mentioned that total phenolic content in basil leaves, spearmint leaves and fennel seeds were 2.23, 0.94 and 0.68 mg/g as gallic acid equivalents respectively (on fresh weight basis).

Table 1. Total phenolic content in selected herbs (on dry weight basis)

Samples	Total phenolic*
Basil leaves	3.97
Spearmint leaves	1.91
Fennel seeds	1.53

* Data expressed as mg of caffeic acid per gram..

Phenolic acids compounds

Table (2) and Figures (1), (2) & **(3)** illustrated the high performance liquid chromatography (HPLC) profiles of phenolic acids present in the phenolic extracted from herbs. The results appeared that gallic acid, vanillic acid and chlorogenic acid were the major phenolic acids present

Table 2. Phenolic acids compounds (mg/g) on dry weight basis

Phenolic acid	Peak No.	Basil leaves	Spearmint leaves	Fennel seeds
Gallic acid	1	0.752	0.224	0.100
Vanillic acid	2	0.580	0.706	0.531
Chlorogenic acid	3	0.159	0.118	0.620
Syringic acid	4	0.111	0.405	ND*
Ferulik acid	5	0.152	0.115	0.186

* Not detected

in basil leaves, spearmint leaves and fennel seeds respectively. On the contrary, syringic acid was not detected under the conditions of the experiment in the phenolic extract from fennel seeds.

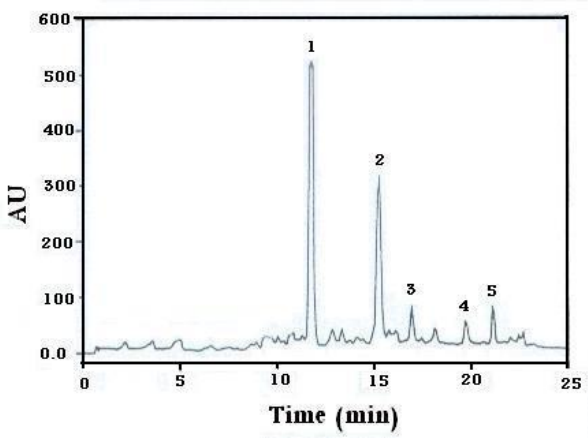

Fig.1. HPLC profile of basil phenolic compounds

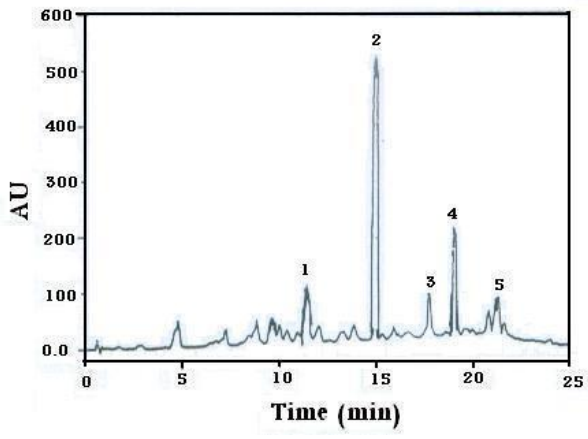

Fig. 2. HPLC profile of spearmint phenolic compounds

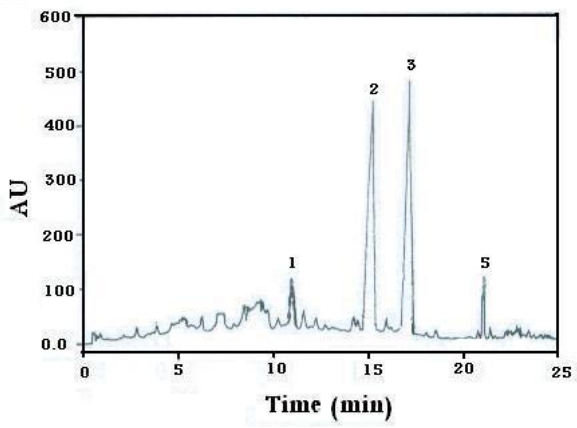

Fig. 3. HPLC profile of fennel phenolic compounds

Antioxidant activity

With respect to antioxidant activity, the data in **Table (3)** showed that addition of phenolic extracted from herbs led to longer induction peried than control (untreatment). From the same table it is obvious that induction period for phenolic herbs could be ranked as follow: basil leaves > spearmint leaves > fennel seeds. It is evident from the same table that the best concentration from natural additives as antioxidants activity were 400 and 600 ppm compared with BHT. These results are in full agreement with those obtained by **Velioglu *et al* (1998).** They investigated correlation coefficient between total phenolic content and antioxidant activity in 28 plant and proved that, there was a positive and a highly significant (P<0.001) relation between total phenolic content and antioxidant activity. As well **Wei and Wang (2001*)*** found a positive linear correlation between the phenolic content and antioxidant capacity of the herbs. They also found that antioxidant capacity

Table 3. Effect of addition different antioxidants on induction period

Treatments	Concentration (ppm)	Induction period (hr)	Protection factor*	Antioxidant activity (%)**
Control	-	6.21	1	-
BHT	200	8.30	1.36	33.65
Basil phenolic	200	7.67	1.23	23.51
	400	9.15	1.47	47.34
	600	12.30	1.98	98.06
Spearmint phenolic	200	7.53	1.21	21.25
	400	8.72	1.40	40.41
	600	11.17	1.79	79.87
Fennel phenolic	200	7.41	1.19	19.32
	400	8.60	1.38	38.48
	600	9.86	1.58	60.38

* Protection factor = induction period of fat with additive / induction period of control.
** Antioxidant activity (%) = induction period of sample – induction period of control / induction period of control x100

expressed as oxygen radical absorbance capacity (ORAC) were 14.27, 8.10 and 5.88 μ mol of TE/g of fresh weight basis in basil, spearmint and fennel respectively whereas TE (trolox equivalent) is (6-Hydroxy-2,3,7,8-tetramethylchroman – 2 – carboxylic acid).

Fat stability

The results in Tables (4), (5), (6) and **(7)** showed the changes in peroxide value (PV), acid value (AV) thiobarbituric acid value (TBA), and refractive index (RI). The results revealed that with increasing the concentration of phenolic extraction, the values of PV, AV, TBA and RI were decreased during biscuit storage. From the same tables it could be observed that, phenolic basil leaves had the highest retard fat oxidation in biscuit followed by phenolic spearmint leaves then fennel seeds. It could be also seen that biscuit control sample gave the higher values of PV, AV, TBA, and RI than biscuit with different additives as antioxidant. With reference to peroxide value **Allen and Hamilton (1983)** reported that the peroxide value is a good index for the quality of fat. A refined fats should have peroxide value of less than 1 milliequivalent / kg fats that have been stored for some period of time after refining may be found to have peroxide value of up to 10 milliequivalent / kg. In relation to TBA **Ke *et al* (1984)** reported that TBA less than 0.576 mg kg^{-1} sample are considered not rancid , whereas values of 0.65-1.44 mg kg^{-1} sample are regarded as rancid but still acceptable , and values grater than 1.5 mg kg^{-1} sample are said to be rancid and unacceptable. These results in **Table (4), (5), (6)** and **(7)** can be explained by some theories as follow: The first step in lipid oxidation is the abstraction of hydrogen atom from a fatty acid and oxygen involvement gives a peroxy radical. Generally, the antioxidants suppress the hydrogen atom abstraction from the fatty acid which leads to the decrease of hydroperoxide formation. It is well known that the phenolic compounds act as hydrogen donors to that reaction mixture and therefore, the formation of hydroperoxides is decreased. One would except that the increase in the number of phenolic OH groups leads to an increase the antioxidant phenomenon. In general, the phenolic OH has to be in the free form and if these groups are attached to other groups it would prevent their antioxidant power due to the lack of hydrogen atom donates to a fatty acid radical, **(Asakura *et al* 1989).** The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides **(Osawa, 1994).** Phenolics are able to act as antioxidants in different ways. Hydroxyl phenols are good metal ion chelators. The implication of this is that metal-catalyzed non-enzymaztic free radical generation

Table 4. Changes in peroxide value (milliequivalent peroxides/kg fat) during storage period.*

Treatments	Concentration (ppm)	Storage period (month)				
		Zero	2	4	6	8
Control	-	0.81	1.23	4.90	10.62	15.45
BHT	200	0.81	1.17	3.87	6.79	11.23
	200	0.81	1.18	4.00	7.12	11.36
Basil phenolic	400	0.81	1.12	3.51	6.40	8.71
	600	0.81	1.10	2.40	3.77	5.37
	200	0.81	1.19	4.12	7.93	11.85
Spearmint phenolic	400	0.81	1.15	3.62	6.46	10.88
	600	0.81	1.11	3.13	5.10	7.58
	200	0.81	1.21	4.33	8.00	11.96
Fennel phenolic	400	0.81	1.16	3.80	6.63	10.93
	600	0.81	1.14	3.35	5.86	8.00

* Each value is mean of duplicate determination.

Table 5. Changes in acid value (mg KOH/g fat) during storage period*

Treatments	Concentration (ppm)	Storage period (month)				
		Zero	2	4	6	8
Control	-	0.59	1.42	1.50	1.91	2.37
BHT	200	0.59	1.38	1.44	1.83	2.20
	200	0.59	1.40	1.45	1.84	2.23
Basil phenolic	400	0.59	1.30	1.40	1.79	2.13
	600	0.59	1.28	1.39	1.75	2.11
	200	0.59	1.38	1.47	1.87	2.25
Spearmint phenolic	400	0.59	1.36	1.42	1.80	2.15
	600	0.59	1.33	1.40	1.77	2.13
	200	0.95	1.39	1.47	1.89	2.26
Fennel phenolic	400	0.95	1.37	1.43	1.82	2.19
	600	0.95	1.33	1.42	1.78	2.18

* Each value is mean of duplicate determination

Table 6. Changes in thiobarbituric acid value (mg malonaldehyde/ kg sample) during storage period*

Treatments	Concentration (ppm)	Storage period (month)				
		Zero	2	4	6	8
Control	-	0.07	0.39	0.76	1.97	2.15
BHT	200	0.07	0.22	0.68	1.00	1.46
Basil phenolic	200	0.07	0.24	0.71	1.12	1.50
	400	0.07	0.18	0.65	0.89	1.15
	600	0.07	0.15	0.63	0.80	0.92
Spearmint phenolic	200	0.07	0.26	0.73	1.13	1.52
	400	0.07	0.19	0.66	0.93	1.23
	600	0.07	0.17	0.64	0.87	1.03
Fennel phenolic	200	0.07	0.26	0.75	1.15	1.53
	400	0.07	0.20	0.67	0.98	1.38
	600	0.07	0.18	0.65	0.95	1.25

* Each value is mean of duplicate determination.

Table 7. Changes in refractive index during storage period*

Treatments	Concentration (ppm)	Storage period (month)				
		Zero	2	4	6	8
Control	-	1.4711	1.4717	1.4724	1.4733	1.4749
BHT	200	1.4711	1.4714	1.4719	1.4727	1.4740
Basil phenolic	200	1.4711	1.4715	1.4720	1.4729	1.4742
	400	1.4711	1.4712	1.4716	1.4723	1.4734
	600	1.4711	1.4710	1.4715	1.4720	1.4730
Spearmint phenolic	200	1.4711	1.4716	1.4721	1.4730	1.4745
	400	1.4711	1.4713	1.4718	1.4725	1.4737
	600	1.4711	1.4712	1.4717	1.4721	1.4733
Fennel phenolic	200	1.4711	1.4716	1.4723	1.4730	1.4747
	400	1.4711	1.4714	1.4718	1.4726	1.4739
	600	1.4711	1.4713	1.4718	1.4724	1.4737

* Each value is mean of duplicate determination

Table 8. Effect of antioxidants on total bacterial count and (yeast & mold) count after storage period (8 months)

Treatments	Concentration (ppm)	Bacteria (1000cell/gm)	Yeast & mold (10cell/gm)
Control	-	7	2.5
BHT	200	4.5	1.5
Basil phenolic	200	4.0	1
	400	3.5	0.5
	600	2.5	0.5
Spearmint phenolic	200	5.0	1.5
	400	4.5	1
	600	3	0.5
Fennel phenolic	200	5.5	1.5
	400	5.5	1
	600	4.5	1

Table 9. Effect of antioxidants on the sensory evaluation of biscuit after directly baking*

Treatments	Conc. (ppm)	Color (20)	Flavor (20)	Appearance (20)	Taste (20)	Texture (20)
Control	-	17.95±0.63a	18.91±0.42a	18.45±0.16a	19.00±0.79a	18.13±0.81a
BHT	200	18.24±0.41a	19.13±0.23a	18.51±0.28a	18.97±0.10a	18.26±0.73a
Basil phenolic	200	18.27±0.58a	19.10±0.31a	18.38±0.13a	19.10±0.21a	18.30±0.85a
	400	18.25±0.60a	19.18±0.18a	18.40±0.20a	19.12±0.28a	18.23±0.66a
	600	18.29±0.76a	19.20±0.73a	18.37±0.27a	19.16±0.35a	18.27±0.70a
Spearmint phenolic	200	18.24±0.30a	19.13±0.68a	18.40±0.54a	19.20±0.19a	18.25±0.62a
	400	18.26±0.47a	19.14±0.92a	18.29±0.33a	19.24±0.30a	18.29±0.90a
	600	18.30±0.95a	19.16±0.28a	18.27±0.30a	19.25±0.96a	18.24±0.77a
Fennel phenolic	200	18.28±0.34a	19.17±0.62a	18.35±0.37a	19.15±0.39a	18.21±0.49a
	400	18.25±0.53a	19.22±0.36a	18.33±0.41a	19.18±0.51a	18.16±0.51a
	600	18.20±0.57a	19.26±0.87a	18.36±0.28a	19.21±0.13a	18.19±0.80a

* values followed by the same letters within the same column were not significantly different (P >0.05).
values are mean ± standard error

is thus suppressed in the presence of suitable phenolics. Also, phenolic structures often have the potential to interact strongly with proteins, mediated both by their hydrophobic benzenoid rings and the hydrogen-bonding potential of the phenolic hydroxyl groups. As mentioned, this gives phenolics the potential to act as enzyme inhibitors. Sometimes, as in the case of various tannins and other polyhydroxy-substitutes phenolics, this interaction can be largely non-specific and there is a relatively general inhibitory effect. In other cases, discrete interaction occurs between individual phenolics and the active site of enzymes, and a more specific type of inhibition results. This ability of certain phenolics to modify selected enzyme activities is presumed to have a role in the physiological action of these phenolics, though the picture is yet incomplete, since the multiple interrelated action of phenolics, some structure-specific, some non-specific, make it very difficult to obtain a detailed understanding of their mode of action **(Aruoma *et al* 1996).** Phenolic may also inhibit oxidation by chelating divalent metal ions and thus reducing the formation of free radicals induced by fenton reactions **(Robards *et al* 1999).**

Antimicrobial activity

With regard to total bacterial count and (yeast & mold) count (cell/gm) in biscuit samples, the results in **Table (8)** revealed that the total bacterial count and (yeast & mold) count were decreased at the end of storage period (8 months) with increasing the concentration phenolic. These decrease could be explained by **Branen *et al* (1980).** They mentioned that Phenolic antioxidants have antimicrobial activity against bacteria, molds, viruses and protozoa. Moreover, they revealed that the mechanism of microorganisms decreasing might be related to the destruction or inactivation of essential enzyme and /or genetic material. Also inhibition of DNA, RNA and protein synthesis. They also added that the monoglycerides depressed the NADH oxidase system by acting on the o_2 side of flavine of NADH dehydrogenase. Also, **Deans and Ritchie, (1987)** reported that many naturally spices have been shown to possess antimicrobial functions and could serve as a source of antimcrbial agents food pathogens. And so, **Kubo *et al* (1993)** referred that phenolic compound and their subclasses, such as flavonoids, tannins, saponins and essential oil have antimicrobial function.

Sensory evaluation

Regarding sensory evaluation of biscuit samples after directly baking the data in **Table (9)** declared that addition of different antioxidants showed no significant of differences between control and biscuit samples. These results were confirmed by **Mohamed and Awatif, (1998)**. They mentioned that addition of antioxidants has become popular as a method of increasing the shelf-life of food products and improve the loss of sensory and nutritional quality. Furthermore, **Lee and Shibamoto, (2002)** indicated that herbs and spices possessing high antioxidant activity, such as basil, would not only be very useful to maintain food freshness, flavor, taste and color, but also to alleviate diseases by preventing oxidative deterioration. Moreover, **Pszola, (2001)** noted that the shelf-life of food is becoming more and more important due to the increasing amount of manufactured foods needed for feeding the growing world population and to the demographic shift from rural to areas.

From the above-mentioned results, it could be concluded that the phenolic compounds extracted from herbs, can be used as a natural antioxidants, in order to produce a good quality of food and to increase the shelf-life of food products.

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