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BIOCHEMICAL AND TECHNOLOGICAL EVALUATION OF BISCUITS SUPPLEMENTED WITH NUTRITIONAL PLANT SOURCES RICH IN ANTIOXIDANTS

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ABSTRACT

This study is a trial to produce healthy biscuits supplemented with nutritional sources belonging to several plant families, rich in antioxidants. The investigated sources used for supplementation were thyme (herbs), parsley (vegetables), and kidney bean (legumes) to obtain healthy biscuits. The Statistical analysis of the organoleptic evaluation showed that biscuits supplemented with 3% thyme, 5% parsley and 15% kidney bean of wheat flour (72% extraction) had slight significant differences compared with the control biscuits. Therefore they were chosen to be evaluated chemically and biologically. The results of chemical evaluation showed that biscuits supplemented with 15% kidney bean had the highest value of protein content (6.88%) and crude fiber content (0.77%), while had the lowest value of carbohydrates content (6.88%) , biscuits supplemented with 5% parsley had the highest value of ash content (2.58%), while all samples of supplemented biscuits had nearly the same contents of fat which ranging from 19.55 to19.59% compared with the control biscuits recording 5.59% of protein, 19. 45% fat, 0.40% crude fiber, 2.51% ash and 72.05% carbohydrates. Data also showed that all samples of supplemented biscuits had higher antioxidant activity, compared with control biscuits. The highest antioxidant activity was found in the biscuits supplemented with 3% thyme of wheat flour. It is well known that high antioxidant activity extends the shelf life of produced biscuits. The results of biological evaluation showed that all subgroups of healthy rats fed on supplemented biscuits had higher values of food efficiency and HDL cholesterol, and lower values of glucose, total triglycerides, total lipids, total cholesterol, LDL cholesterol, risk ratio and LDL/HDL ratio compared with subgroup of healthy rats fed on control biscuits (Control C). All subgroups of diabetic rats fed on supplemented biscuits diets had the lower values of glucose, total triglycerides, total lipids, total cholesterol, LDL cholesterol, risk ratio and LDL/HDL ratio compared with those of diabetic rats fed on basal diet (Control B), and diabetic rats fed on control biscuits diet (Control D). The highest significant decreases of these parameters were found in rats fed on biscuits supplemented with thyme. On the other hand, HDL cholesterol value increased in all subgroups of diabetic rats fed on supplemented biscuits diets, and the highest value was found in rats fed on biscuits supplemented with thyme compared with controls B and D. So it could be recommended to incorporate the investigated nutritional sources in bakery products to produce healthy products having good biological effects especially diabetic and hypercholesterolemia patients.

Keywords: Thyme, kidney bean, Parsley, Healthy biscuits, Sensory evaluation, Antioxidant activity and Biological evaluation.

INTRODUCTION

Antioxidants are the compounds that when added to food products, especially to lipids and lipid-containing foods, can increase their shelf life by retarding the process of peroxidation, which is one of the major reasons for deterioration of food products during processing and storage (Valenzuela *et al*., 2004). Great interest has recently been focused on the addition of natural antioxidants to food and biological systems, due to their well known abilities to scavenge free radicals. The generation of free radicals plays an important role in the progression of a great number of pathological disturbances, such as atherosclerosis (Steinberg, 1992), brain disfunction (Gordon, 1996) and cancer (Ames, 1983). Phytochemical compounds such as flavonoids, isoflovonoids, saponins, tannins, minerals, vitamins, tocopherols, some enzymes, sterols, volatile oil and polyunsaturated fatty acids can be considered as natural antioxidants. These phytochemicals (natural antioxidants) can have completely an overlapping mechanisms of action including modulation of detoxification enzyme, stimulation of immune system, modulation of cholesterol synthesis and hormone metabolism, reduction of blood pressure, prevent the onset of chronic diseases, antioxidant control to cystic fibrosis, antihyperilipidemic antihyperglycemic , antibacterial and antiviral effects (Sandak and El-Hadidy, 2004).

Vegetables, fruits, cereals, legumes and herbs are the main sources of natural antioxidants. The herbs of thyme *(Thymus vulgaris, L.)* were known to have high antioxidant capacities, and some methylated flavones were isolated from the thyme herbs as antioxidants. In the essential oil of thyme, thymol and carvacrol were recognized as major components that showed high antioxidant and antimicrobial activity (Damien Dorman *et al*., 2000 and Zheng and Wang, 2001). Parsley *(Petroselinum crispum)* has many health benefits. It is a good source for natural antioxidants, vitamins A, C and K, some volatile oils which used as tumour inhibition agent. It has also been shown to activate the enzyme glutathione-s-transeferase (Justesen *et al.*, 1998; Hirano *et al.*, 2001; Sasaki *et al.*, 2003 and El-Bastawesy *et al*., 2008). Kidney bean *(Phaseolus vulgaris)* contains considerable amounts of phenolic compounds, those posses varying degrees of antioxidative activity (Srisuma *et al*., 1989 and Drumm *et al.*, 1990). It's rich in saponins. Saponins and foods containing saponins have been shown to lower plasma cholesterol concentrations in a number of animal species (Sidhu and Oakenfull, 1986). It's also rich in iron, manganese, phosphrous, copper, magnesium, proteins, vitamin E, and folate (B9). Furthermore it's high in soluble dietary fibres (gums, beta glucans, and pectin) (Hemphill and Jackson, 1982 and Abdel- Gaber *et al.,* 2006).

Biscuits are a common popular bakery product, for a wide range of consumers as it is consumed in breakfast or tea time. This study was a trial to produce biscuits using different nutritional plant sources accordingly rich in their content of antioxidants, which have chemopreventive effects to avoid the hazard effect of consumption of biscuits. The produced biscuits were organolyptically, chemically and biologically evaluated.

MATERIALS AND METHODS

Materials:-

Wheat flour (soft 72% extraction) was obtained from South Cairo Mills Company, Giza, Egypt.

Thyme *(Petroselinum crispum)* was obtained from Medicine Plants and Agricultural Seeds Haraz Company, Cairo, Egypt.

Parsley *(Petroselinum crispum)* was obtained form Horticultural Research institute, Agricultural Research Center, Giza, Egypt.

Kidney bean *(Phaseolus vulgaris*) was obtained from Legumes Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

All other materials used in dough preparation were obtained from the local market in Dokki, Giza governorate, Egypt.

Experimental animals:

Rats which were used in this assay were obtained according to scientific restriction and housed in the animal house of the Research Institute of Ophthalmology, Giza, Egypt.

Methods:

Preparation of raw material:-

The leaves of parsley was air dried at 40°C for 3hr according to Mudahar *et al.,* (1989), and the dehydrated product was milled to a fine powder, to pass a 100µm sieve , Packaged into polyethylene bags and stored in deep freezer until using.

Thyme and Kidney bean were milled to a fine powder, to pass a100 µm sieve, Packaged into polyethylene bags and stored in deep freezer until using.

Preparation of soft biscuit:-

Biscuits were prepared by using soft wheat flour 72% extraction as a control biscuits , and to prepare the supplemented biscuits wheat flour was replaced with whole meal of thyme at 2, 3, 4% levels; dried parsley leaves at 2.5, 5 and 7.5% levels and whole meal of kidney bean at 12, 15 and 18 % levels.

Biscuits were made according to the Wade (1988) with some modification; the formula is shown in Table (1).

Table (1): The formula of soft biscuits.

Ingredients	**Amounts (gm)**
Soft wheat flour or its blends	100
shortening	33
Sugars	36
Baking powder	3
vanilla	0.25
*Water (ml.)	as required

***water is added in ml. as required**

For making biscuits, the following procedure was followed: - shortening, sugars, vanilla and water 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. Dry ingredients (soft wheat flour or its blends and baking powder were added to the mixture gradually and mixed at low speed for 3 minutes, and the resulted dough was let to rest for 5 min., then sheeted to 3 mm. thickness. Circle pieces cut of dough were formed using the templates with an outer diameter of 50 mm .The biscuits were baked at 170°C for 12 minutes. After baking, biscuit was allowed to cool at room temperature for 1hr before organoleptic evaluation. The produced biscuits were weighed and chemically and biologically evaluated.

Sensory evaluation of biscuits:

Produced biscuits were evaluated for their sensory characteristics after baking by ten panelists from the staff of bread and pastry, research Dep., Food Tech. Res. Institute, Giza. The scoring scheme was established as mentioned by Amer, (2000) as follows; color of crust (20), taste (20), odor (20), crunchiness (20), appearance (20), and the total scores were 100 degrees. Samples of biscuits having the best acceptance of sensory properties were chosen for chemical and biological evaluation.

Chemical analysis:

Moisture, protein, ash, crude fiber contents and ether extract were determined according to the methods described in the A.O.A.C. (2000). Carbohydrates were calculated by difference.

Antioxidant activity measurement:-

The radical scavenging activity of samples was measured using 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging assay as described by Miliauskas *et al.*, (2004).

And also antioxidant activities of the samples were examined by a β-carotene/linoleic acid system as reported by Matthaus, (2002).

Biological investigation:

Biological examination was carried out using samples of biscuits containing 3% whole meal of thyme, 5% dried leaves of parsley and 15% whole meal of kidney bean as well as control biscuits.

Test diets:

The components of formed basal diet were corn starch (70%), casein (10%), corn oil (10%), cellulose (5%), salt mixture (4%), and vitamin mixture (1%) according to the A.O.A.C. (1990). Each experimental diet were formed of 70 % of each sample of investigated biscuits, then the levels of protein, fat, fibre and carbohydrates were completed up to the same ratios as present in the basal diet, by casein , corn oil, Cellulose and, corn starch (was added to complete up to 100 %), respectively.

Animals:

The experimental animals used in this study were 60 male albino rats *(Westerin strain)*, each was eight weeks old and 100 gm weight. The animals were housed individually in well aerated cages, under hygienic conditions and fed on basal diets for two weeks, as an adaptation period. The animals were divided into two main groups, each group included 30 rats. The first group was kept as healthy rats and divided into five subgroups (6 rats each). Rats of the first subgroup continued to be fed on the same basal diet (Control A), and the other subgroups were fed on experimental diets during the whole experimental (4 weeks), one of them was fed on the control biscuits (Control C). Rats of the second group (30 rats) were fasted over night and injected with alloxan solution into the leg muscle (150 mg /100 Kg body weight) to induce hyperglycemia and hypercholesterolemia according to Arbeeny and Bergquist (1991) and Buko *et al*., (1996), then whole groups were fed on basal diet for 48 hr. where hyperglycemia and hypercholesterolemia were developed. After 48 hr. of injection, they were randomly divided into five subgroups 6 rats each. One of them was left as diabetic control; it fed the basal diet (Control B), and the other subgroups were fed on experimental diets during the whole experiment (4 weeks) one of them was fed on the control biscuit (Control D). During the experiment, Food intake and animal weight were recorded twice weekly. At the end of the experimental period, total food intake and gain in body weight were calculated. Food efficiency ratio was determined according to the following equation (Squibb, 1959):-

Food efficiency ratio = Daily body weight / Daily food intake

Biological assay:

Serum total cholesterol, high density lipoprotein (HDL-cholesterol), total lipid, triglycerides (TG) and glucose were determined using colorimetric methods described by Allain *et al.,* (1974); Lopez-Virella *et al.,* (l977); Zollner and Kirsch (1962); Fossati and Preciple, (1982) and Trinder, (1969), respectively.

Low density lipoprotein (LDL-cholesterol) in serum was calculated according to the following equation (Friedewald *et al.*, 1972):-

LDL =Total cholesterol – HDL – TG / 5

Risk ratio was calculated according to the equation of Lopez-Virella *et al.,* (l977):-

Risk ratio = Total cholesterol / HDL

Statistical analysis:

The obtained data from Sensory and biological evaluations were statistically analyzed by the least significant differences value (L.S.D) at 0.05 levels probability procedure to Snedocr and Cochran, (1967).

RESULTS AND DISCUSSION

Cross chemical compositions of the raw materials:

The chemical compositions of materials under investigation are presented in Table (2). It could be noticed that wheat flour (72%) contained the highest value in digestible carbohydrates (88.44%), whereas it was the lowest in values of fat, crude fiber and ash (0.69, 0.70 and 0.49 %, respectively). Whole meal of thyme had the highest values of both of fat (7.13%) and crude fiber (18.48%). Whole meal of Kidney bean and dried parsley leaves contained the highest values of protein content (24.71 and 22.37%, respectively). These results are confirmed by those obtained by Hemphill and Jackson, (1982), Farvili *et al*., (1997); Ensminger *et al*., (1995) and Hamza *et al*., (2001)

Antioxidant activity of the raw materials:

Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu *et al*., 1998). Oxidative damages caused by free radicals to living cells mediate the pathogenesis of many chronic diseases, such as atherosclerosis, Parkinson’s disease, Alzheimer’s disease, stroke, arthritis, chronic inflammatory diseases, cancers and other degenerative diseases (Halliwell and Grootveld, 1987).

Table (2): Cross chemical composition of wheat flour (72% extraction), Thyme, Parsley and kidney bean (% on dry basis).

Components (%)	Wheat flour (72%)	Whole meal of Thyme	Dried parsley leaves	Whole meal of kidney bean
Moisture	13.1	7.21	8.15	10.10
Protein	9.68	9.04	22.37	24.71
Fat	0.69	7.13	4.13	1.80
Crude fiber	0.70	18.48	10.23	5.03
Ash	0.49	11.62	12.49	3.35
*Digestible carbohydrates (DC)	88.44	53.73	50.78	65.11

***Calculated by difference.**
DC=100-%(Protein +Fat +Crude fiber +Ash)

The antioxidant activity of wheat flour 72% extraction, Whole meal of thyme, dried parsley leaves of and Whole meal of Kidney bean were evaluated using two complementary test systems, namely, DPPH free radical scavenging and β- carotene/linoleic acid assays compared with the synthetic antioxidant Tertiary butyl hydroquinone (TBHQ), as control. From the results presented in Table (3), it could be noticed that the order of activity was TBHQ > thyme > Kidney bean> parsley> wheat flour 72% extraction. Thyme was characterized by the highest antioxidant activity compared to the other investigated raw materials. These results are confirmed by those of Damien Dorman *et al*., (2000); Zheng and Wang (2001) and Sasaki *et al.*, (2003).

Table (3): Antioxidant activity of wheat flour (72% extraction), Thyme, Parsley and kidney bean.

Sample	DPPH scavenging (%)	B-carotene (%)
Wheat flour (72% extraction)	48.4	67
Thyme	69.1	74.91
Parsley	51.2	69.57
Kidney bean.	64.9	71.91
***TBHQ**	94.82	90.18

***TBHQ = Tertiary butyl hydroquinone**

Sensory characteristics of biscuits:

From the results presented in Table (4), it could be noticed that sensory characteristics were decreased with increasing the percentage of supplements for thyme and parsley, while in the case of kidney bean the color and appearance were improved with increasing the percentage of supplements, and other sensory characteristics were decreased with increasing the percentage of supplements. Also, it could be noticed that biscuits produced from wheat flour (72% extraction) was supplemented with thyme until level 3 %, parsley until level 5% and kidney bean until level 15% still have very good overall acceptability, so these levels of supplementation were selected with their control to continue the investigation, in order to study their physical properties, chemical composition, antioxidation activity and biological effects on both of healthy, and diabetic rats.

Table (4): Sensory evaluation of biscuits supplemented with the investigated levels of each thyme, Parsley and Kidney bean compared with the control.

Type	%	Color (20)	Taste (20)	Odor (20)	Appearance (20)	Texture (20)	Total Score (100)	Acceptance
Control	0	19.5[a]	20.0[a]	20.0[a]	19.5[a]	20.0[a]	99.5[a]	V
Thyme	2	18.8[b]	19.3[ab]	19.0[ab]	18.5[ab]	19.3[ab]	94.9[ab]	V
	3	18.4[bc]	18.7[b]	18.6[b]	17.9[b]	18.5[bc]	91.4[bc]	V
	4	18.0[c]	17.6[c]	17.4[c]	16.5[c]	17.5[c]	86.5[c]	G
L.S.D		**0.6123**	**1.0231**	**1.0310**	**1.1034**	**1.1220**	**6.6123**	
Parsley	2.5	18.5[b]	19.0[ab]	18.6[b]	18.2[b]	19.0[b]	93.3[ab]	V
	5	17.8[b]	18.6[ab]	18.3[b]	17.5[b]	18.8[b]	91.0[b]	V
	7.5	16.0[c]	17.1[c]	17.0[c]	16.0[c]	18.5[b]	86.10[bc]	G
L.S.D		**0.7697**	**1.1408**	**0.9376**	**1.0893**	**0.9708**	**6.9811**	
Kidney bean	12	19.7[a]	19.5[a]	19.0[a]	19.5[a]	19.0[a]	96.7[ab]	V
	15	19.9[a]	18.5[b]	17.0[b]	19.8[a]	17.5[a]	92.7[bc]	V
	18	20.0[a]	16.5[c]	16.0[bc]	20.0[a]	16.5[bc]	89.0[c]	G
L.S.D		**0.5229**	**0.8829**	**1.2105**	**0.5819**	**1.5110**	**5.5420**	

Each value with the same column is followed by the same letters is not significantly different at level of 0.05.

90-100 Very Good (V). 80-89 Good (G). 70-79 Satisfactory (S). Less Than 70 Questionable (Q).

Physical properties of supplemented biscuits:

From the results presented in Table (5), it could be noticed that all samples of supplemented biscuits products increased in total moisture content 2.82% for biscuits supplemented with 3% thyme of wheat flour, 3.22% for biscuits supplemented with 5% parsley of wheat flour and 2.39% biscuits supplemented with 15% kidney bean of wheat flour compared with the control biscuits (2.20%). It was also observed that expansion factor (D/T) increased for biscuit supplemented with parsley (6.46), while decreased for both of biscuits supplemented with thyme (5.70) and biscuits supplemented with kidney bean (5.71) compared with 6.42 for the control biscuits.

Table (5): Physical properties of biscuits supplemented with 3% whole meal of thyme, 5%dried parsley leaves, and 15% kidney bean of wheat flour compared with the control.

Physical properties	Control biscuits	Biscuits supplemented with		
		3% whole meal of thyme of wheat flour	5% dried parsley leaves of wheat flour	15% kidney bean of wheat flour
Total moisture content (%)	2.20	2.82	3.22	2.39
Diameter (D) (mm)	4.3	4.5	4.2	4.4
Thickness (T) (mm)	0.67	0.79	0.65	0.77
Expansion factor (D/T)	6.42	5.70	6.46	5.71
Volume (V)(Cm^3)	11.0	11.66	10	12.66
Weight (w)(gm)	7.26	7.37	6.86	6.70
V/W ratio (Cm^3/gm)	1.52	1.58	1.46	1.88

This recommends these additions for biscuits producing for technological concepts during processing and packaging. The results also show that the volume/weight ratio increases for both of biscuits supplemented with thyme and supplemented with kidney bean (1.58 and 1.89, respectively) compared with the control biscuits (1.52). While the volume/weight ratio decreased in biscuits supplemented with parsley.

Cross chemical composition of biscuits:

Data in Table (6) show that biscuits supplemented with 15% kidney bean of wheat flour had the highest values of both protein and crude fiber 6.88 and 0.77%, respectively while it had the lowest value of digestible carbohydrate content (6.88%). Biscuits supplemented with 5% parsley of wheat flour had the highest value of ash content (2.85%).

All samples of supplemented biscuits were nearly the same in contents of fat which ranged from 19.55 to19.59% compared with those of control biscuits which recorded 5.59, 19.45, 0.40, 2.51, 72.05 % for protein, fat, crude fiber, ash, digestible carbohydrates, respectively. These results would be due to the highest nutritional values of used raw materials.

Antioxidant activity of supplemented biscuits:

The antioxidant activity of supplemented biscuits products compared with the control biscuits and the synthetic antioxidant TBHQ are presented in Table (7). It could be noticed that all types of supplemented biscuits had the higher antioxidant activities compared with control biscuit and the highest antioxidant activity was found in the biscuits supplemented with 3% thyme of wheat flour.

Table (6): Cross chemical composition of biscuits supplemented with 3% whole meal of thyme, 5%dried parsley leaves, and 15% kidney bean of wheat flour compared with the control.

Component (%)	Control biscuits	Biscuits supplemented with		
		3% whole meal of thyme of wheat flour	5% dried leaves of parsley of wheat flour	15% kidney bean of wheat flour
Protein	5.59	5.57	5.95	6.88
Fat	19.45	19.59	19.55	19.56
Crude fiber	0.40	0.71	o.68	0.77
Ash	2.51	2.70	2.85	2.76
*Digestible carbohydrates (DC)	72.05	71.43	70.97	70.03

***Calculated by difference. DC=100-%(Protein +Fat +Crude fiber +Ash)**

Table (7): Antioxidant activity of biscuits supplemented with 3% whole meal of thyme, 5%dried leaves of parsley, and 15% kidney bean of wheat flour compared with the control biscuits.

Antioxidant activity	*TBHQ	Control biscuits	Biscuits supplemented with		
			3% whole meal of thyme of wheat flour	5% dried leaves of parsley of wheat flour	15% kidney bean of wheat flour
DPPH scavenging (%)	94.82	45.73	60.17	51.17	54.40
B-carotene (%)	90.18	60.76	65.28	61.35	63.49

***TBHQ = Tertiary butyl hydroquinone**

Biological evaluation of the experimental diets of biscuits:

The experimental diets of biscuits containing 3% whole meal of thyme or 5% parsley or 15% kidney bean of wheat flour were biologically evaluated using two groups of rats the first was healthy (normal) rats, and other group was diabetic rats.

1- Effect of experimental diets on healthy rats:

Gain in body weight, food intake and food efficiency for healthy rats.

From data presented in Table (8), it could be noticed that all subgroups of healthy rats fed on supplemented biscuits diets had the higher values of gain in body weight and food efficiency compared with rats fed on control biscuits diet (Control C), but all these subgroups of rats showed lower values of the same parameters comparing with those fed on basal diet (Control A), that had the highest gain in body weight.

Table (8): Gain in body weight, Food intake (gm) and Food efficiency for healthy rats fed on diets of biscuits supplemented with each of thyme, parsley, and kidney bean compared with the controls A and C.

Group of rats fed on		Initial body weight	Final body weight	Gain in body weight (gm)	Daily gain in body weight	Food intake (gm)	Daily food intake	Food efficiency
Basal diet (Control A)		*115^{a} ±11.15	197.7^{b} ±27.31	82.70^{a} ±17.13	2.76^{a} ±0.57	283.8^{a} ±35.09	9.46^{a} ±1.17	0.2918^{a} ±0.0393
Control biscuit (Control C)		117^{a} ±8.04	151.2^{a} ±23.94	34.20^{c} ±16.25	1.14^{c} ±0.54	272.4^{a} ±34.96	9.08^{a} ±1.17	o.1256^{c} ±0.0513
Biscuits supplemented with	3% of thyme of wheat flour	114^{a} ±10.93	170.57^{ab} ±25.59	56.57^{b} ±15.32	1.88^{b} ±0.52	282.9^{a} ±21.84	9.43^{a} ±0.73	0.1994^{b} ±0.0515
	5% of parsley of wheat flour	114^{a} ±9.75	160.48^{a} ±28.29	46.48^{bc} ±18.85	1.55^{bc} ±0.63	285.3^{a} ±51.12	9.51^{a} ±1.71	0.1630^{bc} ±0.0561
	15% of kidney bean of wheat flour	118^{a} ±6.73	174.95^{ab} ±25.59	56.95^{b} ±18.96	1.9^{b} ±0.63	275.2^{a} ±42.64	9.17^{a} ±1.43	0.2072^{b} ±0.0929
LSD		**11.27**	**31.15**	**12.65**	**0.6900**	**41.42**	**1.5257**	**0.0726**

*** Mean ± standard deviation of six rats in each group.**
Significant at 0.05 Probability level (means with the same letters are not significantly different).

Serum glucose level and lipids profile of healthy rats.

The increase in HDL-cholesterol and decrease in LDL-cholesterol achieved by the diets may be of particular importance in the light of the evidence that the occurrence of cardiovascular diseases is strongly related to decrease in HDL-cholesterol (Assmann and Schult, 1992) and increase in LDL-cholesterol (Grundy, 1990). Results in Table (9) show that all subgroups of healthy rats fed on supplemented biscuits diets had lower values of glucose, total cholesterol, triglycerides, total lipids, LDL-cholesterol, risk ratio and LDL/HDL ratio compared with control C. The lowest values for these parameters were found in rats fed on biscuit supplemented with thyme. On the other hand HDL-cholesterol increased in all subgroups of rats fed on supplemented biscuits diets, and the highest value of its found in rats fed on biscuit supplemented with thyme compared with control C. Our results are in agreement with those of Ibrahim *et al.*, (2000) who found that the thyme (*Thymus vulgaris, L.*) in its crude herb form is indicated for Low immunity, anemia. It improved growth rate, blood picture, enzymatic activity, metabolic process. It had lower values of blood total lipids, total cholesterol content and liver function enzymes (El-Mallah, 2003 and Tollba, 2003).

Table (9): Serum glucose, total cholesterol, total triglycerides, total lipids, HDL and LDL-cholesterol (mg/100ml.), Risk ratio and LDL/HDL ratio for healthy rats fed on diets of biscuits supplemented with each of thyme, parsley, and kidney bean compared with the controls A and C.

Group of rats fed on		Glucose	Total cholesterol	Total triglycerides	Total lipid	HDL-cholesterol	LDL-cholesterol	Risk ratio	LDL / HDL ratio
Basal diet (Control A)		*102.80[a] ± 17.83	98.58[a] ± 17.10	74.79 [b] ± 12.96	761[a] ± 132.02	47.1[a] ± 8.17	36.52[a] ± 22.49	2.09 [b] ± 0.73	0.78 [b] ± 0.62
Control biscuit (Control C)		114.8[a] ±19.92	124.56[a] ±21.62	142.8[a] ± 24.77	841[a] ±145.90	32.9[b] ± 5.71	63.10[a] ± 22.21	3.79 [a] ± 1.33	1.92[a] ± 1.02
Biscuits supplemented with	3% of thyme of wheat flour	107.6[a] ±18.40	113.81[a] ±19.74	130.20[a] ± 22.59	728[a] ± 126.29	41.88 [a] ± 7.26	45.89[a] ± 22.31	2.72[ab] ± 0.95	1.10[ab] ± 0.74
	5% of parsley of wheat flour	109.8[a] ±19.05	120.43[a] ± 20.89	141.70[a] ± 24.58	794[a] ± 137.74	35.42[ab] ± 6.14	56.67[a] ± 10.24	3.40[ab] ± 0.16	1.60[ab] ± 0.13
	15% of kidney bean of wheat flour	112.9[a] ± 19.39	117.46[a] ± 20.38	139.60[a] ± 24.22	778[a] ± 134.97	39.12[ab] ± 6.79	50.42[a] ± 22.14	3.00[ab] ± 1.05	1.29[ab] ± 0.80
L.S.D.		**85.54**	**32.19**	**35.85**	**218.09**	**11.05**	**32.91**	**1.50**	**1.14**

*** Mean ± standard deviation of six rats in each group.**
Significant at 0.05 Probability level (means with the same letters are not significantly different).

2- Effect of experimental diets on diabetic rats:

Gain in body weight, food intake and food efficiency for diabetic rats.

From the data presented in Table (10), it could be noticed that diabetic rats fed on diet of biscuits supplemented with 15% kidney bean of wheat flour had the highest value for gain in body weight and food efficiency compared with the diabetic rats fed on basal diet (Control B), and diabetic rats fed on the control biscuit diet (Control D). This may be due to the high protein content of kidney bean and its high nutritional value as shown in the chemical evaluation in Table 2. Our results also agree with Hemphill and Jackson (1982), who reported that leguminous plants, especially beans (*Phaseolus vulgaris*) are considered a food with high nutritional value and one of the richest sources of plant protein. Bean protein was found to contain different essential amino acids plus high concentrations of niacin, lysine and folic acid.

Table (10): Gain in body weight, Food intake (gm) and Food efficiency for diabetic rats fed on diets of biscuits supplemented with each of thyme, parsley, and kidney bean compared with the controls B and D.

Group of rats fed on		Initial body weight	Final body weight	Gain in body weight (gm)	Daily gain in body weight	Food intake (gm)	Daily food intake	Food efficiency
Basal diet (Control B)		*115^{a} ±17.5	140.2^{a} ±22.5	25.2^{a} ±5.33	0.84^{a} ±0.18	282.6^{a} ±45.73	9.42^{ab} ±1.53	0.0892^{b} ±0.0066
Control biscuit (Control D)		116^{a} ±14.63	149^{a} ±19.54	33.0^{a} ±7.33	1.1^{a} ±0.25	314.8^{a} ±33.09	10.5^{a} ±1.11	0.1048^{ab} ±0.0195
Biscuits supplemented with	3% of thyme of wheat flour	116^{a} ±15.88	146.78^{a} ±23.81	30.78^{a} ±9.07	1.03^{a} ±0.30	270.5^{a} ±30.01	9.02^{ab} ±0.9938	0.1142^{ab} ±0.0241
	5% of parsley of wheat flour	115^{a} ±11.65	141.72^{a} ±21.27	26.72^{a} ±10.63	0.89^{a} ±0.35	272.4^{a} ±83.62	8.48^{b} ±1.55	0.1050^{ab} ±0.0496
	15% of kidney bean of wheat flour	118^{a} ±13.23	152.8^{a} ±21.42	34.80^{a} ±11.01	1.16^{a} ±0.37	279.0^{a} ±48.2	9.30^{ab} ±1.61	0.1247^{a} ±0.0222
LSD		**17.51**	**25.87**	**10.62**	**0.35**	**61.58**	**1.64**	**0.0335**

*** Mean ± standard deviation of six rats in each group.**
Significant at 0.05 Probability level (means with the same letters are not significantly different).

Serum glucose level and lipids profile of diabetic rats:

Results in Table (11) show that all subgroups of diabetic rats fed on supplemented biscuits diets had the lowest values of glucose, total cholesterol, total triglycerides, total lipids, LDL-cholesterol, risk ratio and LDL/HDL ratio compared with the controls B and D .

Table (11): Serum glucose, total cholesterol, total triglycerides, total lipids, HDL and LDL-cholesterol (mg/100ml), Risk ratio and LDL/HDL ratio for diabetic rats fed on diets of biscuits supplemented with each of thyme, parsley, and kidney bean compared with the controls B and D.

Group of rats fed on		glucose	Total cholesterol	Total triglycerides	Total lipid	HDL-cholesterol	LDL-cholesterol	Risk ratio	LDL / HDL ratio
Basal diet (Control B)		*342.53^{a} ± 59.39	318.46^{a} ± 55.25	217.30^{a} ± 37.70	2352^{a} ± 408.03	22.7 ^{b} ± 3.94	252.30 ^{a} ± 51.51	14.03^{a} ± 4.92	11.11^{a} ± 4.25
Control biscuit (Control D)		294.5^{b} ± 51.09	289.41^{a} ± 50.21	188.1^{ab} ± 34.36	1122^{bc} ± 194.65	25.80^{ab} ± 4.48	225.99^{a} ± 47.70	11.22^{a} ± 3.94	8.76^{a} ± 3.41
Biscuits supplemented	3% of thyme of wheat flour	126.75^{c} ± 21.99	132.18^{b} ± 22.93	146.7^{b} ± 25.45	874^{c} ± 151.62	30.94^{a} ± 5.37	71.9^{c} ± 23.06	4.74^{b} ± 1.50	2.32^{b} ± 1.17
	5% of parsley of wheat flour	137.7^{c} ± 21.81	159.29^{b} ± 27.64	162.1^{b} ± 28.12	1034^{c} ± 179.38	26.55^{ab} ± 4.61	100.32^{c} ± 17.65	6.00^{b} ± 0.29	3.78^{b} ± 0.23
	15% of kidney bean of wheat flour	140^{c} ± 22.17	154.87^{b} ± 26.87	157.4^{b} ± 27.31	950^{c} ± 164.81	28.11^{ab} ± 4.87	95.28^{c} ± 16.80	5.51^{b} ± 0.27	3.39^{b} ± 0.21
L.S.D.		**76.02**	**62.67**	**49.78**	**762.34**	**7.52**	**96.89**	**4.67**	**4.02**

*** Mean ± standard deviation of six rats in each group.**
Significant at 0.05 Probability level (means with the same letters are not significantly different).

The highest rate of decrease for these parameters was found in rats fed on biscuit supplemented with thyme. On the other hand HDL-cholesterol increased in all subgroups of diabetic rats fed on supplemented biscuits diets, and the highest value was found in rats fed on biscuit supplemented with thyme compared with the controls B and D. The hypolipidemic effect of thyme can be explained as a result of direct reduction in the blood glucose concentration. In addition the antioxidant properties may reduce the susceptibility of lipids to oxidation and stabilize the membrane lipids by reducing oxidative stress. The volatile oil of thyme is known to have a hypoglycemic effect (Ibrahim *et al.*, 2000; El-Mallah, 2003 and Tollba, 2003).

Conclusion and recommendations:

From this study, it could be concluded that using of these raw materials (i.e. kidney bean, parsley and thyme) as natural sources had several desirable biological effects (antioxidant, hypocholesterolic, hypoglycemic, hypolipidaemic) to produce affordable healthy bakery products that would de preferable and accepted.

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التقييم البيوكيميائي والتكنولوجي للبسكويت المدعم بمصادر غذائية نباتية غنية بمضادات الأكسدة

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هذه الدراسة محاولة لإنتاج بسكويت صحي عن طريق الإستفادة من الدور الحيوي الذي تلعبه مضادات الأكسدة الطبيعية في الوقاية من مدى واسع من الأمراض الشائعة مثل إرتفاع الكولسترول والسكر وتصلب الشرايين ومرض القلب هذا علاوة عن دورها المعروف في إطالة مدة الحفظ وخاصة الأغذية المرتفعة في محتواها الدهني لتفادى الخطر الناتج من إستهلاك البسكويت وإكسابه صفه صحية وقائية وإطالة مدة حفظه . وتحقيقا لذلك إختيرت مصادر طبيعية غذائية غنية في محتواها من مضادات الأكسدة من مصادر نباتية مختلفة توابل(زعتر)- بقول(فاصوليا بيضاء) - خضر(بقدونس) , لدمجها ضمن مكونات البسكويت الناعم و بحث إمكانية إكساب المنتج صفه صحية وقائية وإطالة مدة حفظه وذلك بتقييم البسكويت الناتج حسيا وطبيعيا وكيميائيا وبيولوجيا وأظهرت نتيجة الدراسة الأتي:- بإجراء التقيم الحسى للبسكويت الناتج أوضحت نتيجة التحليل الإحصائي أن أنواع البسكويت المصنعة من دقيق (إستخلاص ٧٢ %) و المدعم ب ٣ % زعتر،٥ % بقدونس ، ١٥% فاصوليا بيضاء يظهرعندها أقل فرق معنوى بينها وبين عينة بسكويت المقارنة ولذا تم إختيارها لتقييمها كيميائيا و طبيعيا و حيويا و أوضحت نتائج التقييم الكيميائي أن البسكويت المدعم ب ١٥% فاصوليا بيضاء أعلى فى محتواه من البروتين (٨٨, ٦%) و الالياف الخام (٧٧, ٠%) وأقل في الكربوهيدرات (٠٣, ٧٠%) بينما كان البسكويت المدعم ب ٥% بقدونس أعلى فى الرماد (٨٥, ٢%) و كانت كل عينات البسكويت المدعمة الناتجة متقاربة فى محتواه من الدهن (٥٥, ١٩- ٥٩, ١٩%) و بينما سجل بسكويت المقارنة ٥٩, ٥% بروتين و ٤٥, ١٩% دهن و٤٠, ٠% ألياف خام و٥١, ٢% رماد و٠٥, ٧٢% كربوهيدرات. وكان النشاط المضاد للأكسدة لكل عينات البسكويت المدعمة أعلي من بسكويت المقارنة وكان البسكويت المدعم بالزعتر الأعلي تأثيرا من حيث النشاط المضاد للأكسدة ، ممايعطي بعدا إيجابيا علي إطالة مدة الحفظ لعينات البسكويت الناتجة. وبإجراء التقييم الحيوى على مجموعتين من الفئران إحداهما تركت سليمة والأخرى إصيبت بالسكر فأظهرت كل المجاميع الفرعية للفئران السليمة والمغذاه على البسكويت المدعم تحسن فى كفاءة إستخدام الغذاء وإرتفاع مستوى HDL لسيرم الدم بينما سجلت إنخفاض في الجليسريدات الثلاثية الكلية و اللبيدات الكلية و الكوليستيرول الكلى و LDL وratio Risk و LDL/HDL ratio فى السيرم لدم الفئران السليمة مقارنة بالمجموعة الفرعية للفئران السليمة المغذاه على البسكويت الغير مدعم بينما أظهرت كل المجاميع الفرعية للفئران المصابة بالسكر والمغذاه على البسكويت المدعم إنخفاض فى الجلوكوزوالجليسريدات الثلاثية الكلية و اللبيدات الكلية و الكوليستيرول الكلى و LDLو LDL/HDL ratioوRisk ratio و أرتفاع لقيمة HDL مقارنة بمجموعتى المقارنة الفرعية للفئران المصابة بالسكر و المغذاه على العليقة القياسية أو البسكويت غير المدعم وكان البسكويت المدعم بالزعتر ألأفضل تأثيرا . وتوصى الدراسة بدمج هذه المصادر فى منتجات المخابز لإنتاج مخبوزات صحية عالية القيمة الحيوية و خاصة لمرضى أرتفاع السكرو كوليستيرول الدم.