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CHEMICAL,SENSORY AND BIOLOGICAL EVALUATION OF BREAD PREPARED USING SOME HERBS AS SOURCE OF BIOACTIVE COMPOUNDS

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ABSTRACT

This study indicate the importace of some herbs as source of bioactive compounds such as thyme , sumac and carob on the chemical, sensory and biological evaluation of bread . Where, bread was prepared by adding thyme, sumac and carob at 1, 2.5% and 5% levels to wheat flour (72% extraction). Chemical composition, phenolic compounds, antioxidant activity (DPPH) and sensory evaluation of prepared bread were determined. Biological evaluation also conducted on hypercholesterolemic rats fed on diets containing thyme, sumac and carob bread for 45 days.The results showed that bread containing these herbs reduced serum total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), very low density lipoproteins (vLDL), while high density lipoproteins (HDL) increased and showed a significant decrease in serum ALT and AST enzymes . So it can be suggested that thyme, sumac and carob can be used in bread preparation at level 2.5% for lipid profil lowering and liver functions improvement .

Keywords: Bread, antioxidants, thyme, carob, sumac, and lipid profile.

INTRODUCTION

Changes in eating habits arising from the development of society in recent decades have led people to search for affordable and healthier foods with satisfactory taste and pleasant appearance. Thus, the food industry continually seeks to adapt and develop new formulations designed to increase shelf life and improve quality and food safety (Darwish *et al*., 2012). Life style of people all over the world have changed in the last century due to income rise, increased leisure time and reduced physical activity. The new life style has considerable impact on health (Venugopal, 2009). Today foods are not intended to only satisfy hunger and provide necessary nutrients for humans but, also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers. In this regard, basic foods play an outstanding role (Hassan, *et al*., 2012).

The bakery industry is growing very fast and the products are increasingly becoming popular among all sections of people. Among ready-to-eat snacks, bread possesses several attractive features including wider consumption base. Bakery products are widely consumed and are becoming a major component of the international food market (Kotsianis *et al*., 2002). Bread is the main staple food meeting nutritional needs of humans in the world. Bread supplies a significant portion of the nutrients required for growth and maintenance of health. It is also one of the sources of proteins, vitamins, minerals, fiber and complex carbohydrates (Azizi and Rao, 2005). An antioxidant effect is observed by scavenging free radicals that are involved in slowing or inhibiting the oxidative chain reaction. The free radical scavenging

activity of phenolic compounds is generally attributed to their ability to donate a hydrogen atom to reduce ROS radicals (Halliwell *et al*., 1995).

Thyme (*Thymus vulgaris L*.) is an ancient herb widely used in folk medicine by the Greeks, the Egyptians and the Romans for the treatment of a variety of diseases. (Rustaiyan *et al*.,2000 ; Aydin et al, 2005). Thyme has been commonly used in foods mainly for the flavour, aroma and preservation (Croteau , 2000).

Sumac (*Rhus coriaria*) is famously used in the Mediterranean region and Middle East as a spic, sauce and drink (Kossah *et al*., 2009). Sumac contains antioxidants, flavonoids and hydrolysable tannins which have anticancer, anti-tumor and hypoglycemic properties (Candan and Sokmen, 2004). The glycoprotein extracted from the fruit of sumac also reduces the total cholesterol, triglycerides, LDL-C and increases the antioxidant capacity (Seeram *et al.,* 2001).

Carob (*Ceratonia siliqua*) is rich in insoluble dietary fiber and polyphenols. In humans, consumption of carob fiber was shown to have a high antioxidant capacity (Kumazawa *et al*., 2002) and to lower serum cholesterol and serum triglycerides (Zunft *et al*., 2003). Furthermore, other studies showed that polyphenols may decrease fat oxidation and energy expenditure in humans (Dulloo *et* al., 1999).

Hyperlipidemia, comprehensive hypercholesterolemia and hypertriglyceridemia, is a main danger factor for the expansion of cardiovascular diseases. The search for new drugs able to decrease and/ or to regularize serum cholesterol and triacylglycerol levels has obtained importance over the years, resulting in varied reports on significant activities of normal agents. (Makni *et al*., 2008).

Thus, the objective of this study was carried out to evaluate the chemically, sensory and biological properties of the bread prepared with addition of thyme, sumac and carob at different levels as source of bioactive compounds.

MATERIALS AND METHODS

Materials:

Wheat flour (72% extraction), thyme (*Thymus vulgaris*) ,carob bean (*Ceratonis siliqua*) and sumac (*Rhus coriaria*L) yeast (*Saccharomyces cerevisiae*) Turkish-made type saf-instant.,salt and sugar were obtained from a local market, Mansoura City, Egypt.

All chemicals and reagents used in this study, were purchased from the local Alamana Company, Cairo City, Egypt.

Male albino rats (Sprague Dawley strain) weighing (140g- 170g) were obtained from the Medical Experimental Research Center, Medicine Faculty , Mansoura University, Egypt.

Methods :

Bread preparation:

Bread formulas were processed according to standard No.677.Iraqi year 1983. Formulas of wheat flour with thyme , sumac and carob (Table 1)

were prepared by adding, thyme , sumac and carob, respectively. 1 , 2.5 and 5%.

The ingredients such as wheat flour,yeast , salt and sugar shown in Table (1) were mixed together manually to obtain uniform dough and dough was left to ferment for 30 mintes at room temperature (25 ± 2 ℃), divided dough was allowed to relax for 10 mintes before divicly into pieces.The fermented pieces were shaped to the final form and fermented for additional 30 mintes(25±2 c°) and baked in an oven at 230 ℃ for 2-5 mintes,then left to cool at room temperature and kept in polyethylene bags at room temperature until used.

Table (1): Formula of bread prepared using different herbs

Ingredients%	control	Replacement level		
		1%	2.5%	5%
Wheat Flour	97.5	96.5	95	92.5
Yeast	1	1	1	1
Sugar	0.5	0.5	0.5	0.5
Salt	1	1	1	1
Herbs*	-	1	2.5	5

***Thyme or Carob or Sumac**

Sensory evaluation of bread:-

Bread samples were evaluated organoleptically, by well panelists from Food Industries Dept., Fac of Agriculture Mansoura University according to the method described by Jaber(1981) for face color(20) , back color (10) , homogeneity of color (10) , rubber and portability chewing (10) , odor (20) , taste (20) , regular shape (5) , the fraction of edges (5) .

Analytical Methods:

Gross chemical composition:-

Moisture, protein, fat, crude fibers and ash contents of thyme, carob, sumac and bread were determined according to AOAC (2007). While carbohydrates percentage was calculated by difference : carbohydrates = 100 – [% protein + % fat + % ash + % crude fiber] Determination and Identification of phenolic compounds :-

Phenolic compounds fractionated and identified, by HPLC according to the method of Goupy *et al*., (1999) as follow: 5 g of sample were mixed with methanol and centrifuged at 10000 rpm for 10 min and the supermant was filtered through a 0.2 pm Millipore membrane filter, then 1-3 ml was collected in avail for injection in to HPLC (Agilent 1200 series) auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series 1100). The column temperature was maintained at 35°c. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of l ml/min. Phenolic acid standard from Sigma Co were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used for calculation of phenolic compounds concentration by the data analysis of HEWLLET packared software .

Free radical scavenging activity (DPPH):-

Free radical scavenging activities of the extracts were measured by the DPPH method proposed by Brand-Williams *et al.* (1995). Each extract was dissolved and diluted in 99% methanol. One milliliter of extract was added to 2 ml of DPPH (5.9 mg in 100 ml methanol) solution. The mixture was incubated at room temperature for 30 min. and
then the absorbance was measured at 517 nm. The DPPH radical scavenging activity was calculated according to the following equation :
DPPH radical scavenging activity (%) = [(A0-A1)/A0] x 100 where Ao was the absorbance of the control, and A1 was the absorbance of the tested sample .

Biological assay:-

Animals and experimental design :

Fifty four adult albino rats (140 - 170 g) were kept under normal healthy conditions, all animals were housed in bottomed cages, fresh and clean drinking water was supplied through specific nipple. Rats were kept at a constant environmental and nutritional conditions throughout the period of the experiment (Temp 24 ± 2C°) and 12 hr light- dark cycle). Rats were fed on basal diet for acclimatization, for 10 days. The composition of basal diet (gm/ 100 gm) as described by AOAC(1990) was given in Table (2) ,salt and vitamin mixtures were described by Abo-El Naga(2002).

Table (2) Composition of basal diet and hypercholesterolemic diets (g/100g)

Ingredients	Basal diet %	Hypercholesterolemic%
Corn starch	66.5	49
Casein	11.2	11.2
Corn oil	13.3	9.3
Salt mix	4	4
Vitamins mix	1	1
Cellulose	4	4
Cholesterol	-	1
Choline chloride	-	0.25
Bile salt	-	0.25
Beef tallow	-	20
Total	100	100

*** The composition of basal diet (gm/100gm) according to.A.O.A.C.,(1990).**

*** The composition of different experimental hypercholesterolemic diets according to (Osman, 2001).**

After adaptation period, rats were fed for eight weeks according to the following schems :

G1 - =Rats fed on basal diet(negative control).
G2+ =Rats fed on hypercholesterolemic diet (positive control group).
G3 = Rats fed on (Bread 100%wheat flour).
G4 = Rats fed on (bread 1% thyme).
G5 = Rats fed on (bread 2.5% thyme).
G6 = Rats fed on (bread 1%carob).
G7 = Rats fed on (bread 2.5% carob).

G8 = Rats fed on (bread 1% sumac).
G9 = Rats fed on (bread 2.5% sumac).

After hypercholesterolemic period (14 days) and at the end of experiment (64 days) , rats were fasted overnight and anesthetized using diethyl ether and blood samples were collected from the vein plexus eye by capillary tube into a clean dry centrifuge tubes. The serum was separated by centrifuge at 4000 rpm for 10 minutes and kept at- 18℃ until analysis.

Biochemical analysis of serum:

Serum total cholesterol was determined according to the method described by Allain, *et al.*,(1974). Triglycerides were determined according to the method described by Fossati and Principe (1982). Serum HDL-cholesterol was determined by the method of Lopez-Virell *et al*., (1977). Serum Low Density Lipoprotein (LDL-cholesterol) was determined by the method of Wieland and Seidel (1982). Serum alanine aminotransferase (A1T) and aspartate aminotransferase (AST) activites were measured according to the method described by Reitman and Frankel (1957).

Statistical analysis:

Data were ststistically analyzed according to the technique of analysis variance (ANOVA), the least significant difference (L.S.D) and Duncan's methods was used to compare the deference between the means of treatment values to the methods described by Gomez and Gomez (1984). All statistical analyses were performed using analysis of variance technique by means of Co STATE Computer Software.

RESULTS AND DISCUSSION

Gross chemical composition of thyme,sumac and carob powders :

Gross chemical compositions of thyme, sumac and carob powders were determined and the results are presented in Table (3).

Table (3): Chemical composition of thyme, sumac and carob powders (on dry weight)

Samples	Moisture	Protein%	Fat%	Fiber%	Ash%	Total carbohydrate%
Thyme	8.66 ^{c}	9.7 ^{a}	12.48^{a}	30.33 ^{b}	7.84 ^{a}	39.65
Sumac	9.57 ^{b}	3.42^{c}	4.07 ^{b}	42.76 ^{a}	3.79 ^{b}	45.96
Carob	11.23 ^{a}	4.91 ^{b}	1.73 ^{c}	9.17 ^{c}	3.44 ^{c}	80.75
LSD	0.2657	0.2457	0.2218	1.1987	1.4984	--

It can be noticed that moisture contents were 11.23, 9.57 and 8.66 % for carob, sumac and thyme powder, respectively. Thyme powder had high contents of protein (9.7%) and fat (12.48%) compared with carob and sumac powders. Also, the results show that the herbs contain considerable amounts of fibers , which sumac had the highest content (42.76%) followed by thyme (30.33%). The results also show that the total carbohydrates content was higher in carob powder (80.75 %), compared with those of sumac (45.96 %)

and thyme (39.65 %).These results are in accordance with those of Ayman, (2000); Kossah *et al.*, (2009); Kamal *et al*., (2013).

Phenolic compounds and antioxidant activity of thyme, sumac and carob powders:-

Phenolic compounds are known as antioxidants which have long been recognized to have protective function against oxidative damage in diet they may provide health benefits associaticl with reduced rsik of chronic disease (Karppinen *et al.,* 2003).

Phenolic compounds were determined and identified in studied herbs and the results are presented in Table (4). From these results, it is evident that these herbs have considerable amounts of phenolic compounds (261.3-1277.1 mg/100g). The highest content was observed in sumac powder (1277.13mg/100g), followed by carob (407.83mg/100g). The lowest amount (261.34mg/100g) was detected in thyme powder.

Table (4): Phenolic compounds and antioxidant activity of thyme, carob and sumac powder (mg/100g)

Phenolic compound	Samples			
	Thyme	Carob	Sumac	LSD
syringic	9.37 b	195.93 a	2.62 c	2.32
gallic	0.32 c	4.94 b	15.67 a	2.32
pyrogallol	25.87 c	122.02 b	338.90 a	2.32
4-aminobenzoic	0.16 b	1.57 b	8.50 a	2.32
protocatechuic	4.46 b	1.31 c	50.01 a	2.32
catechein	4.81 b	4.38 b	19.89 a	2.32
chlorogenic	1.76 b	2.34 b	13.00 a	2.32
catechol	14.65 c	35.29 b	95.23 a	1.93
epicateechein	11.46 c	--	231.53 a	2.32
caffeine	5.19 a	3.22 ab	2.56 b	2.32
p.oh.benzoic	6.38 c	10.45 b	62.21 a	2.32
caffeic	2.58 b	4.45 b	22.46 a	2.32
vanillin	5.70 b	2.33 c	15.79 a	2.32
p.coumaric	18.79 b	2.57 c	217.83 a	2.32
ferulic	16.20 b	2.17 c	39.11 a	1.93
Iso-ferulic	25.11 a	0.21 c	21.01 b	1.93
ellagic	6.26 b	1.78 c	81.30 a	1.93
a-coumaric	17.42 a	0.12 b	1.75 b	1.93
benzoic	22.21 a	4.40 b	21.36 a	2.32
salycilic	56.47 a	7.85 b	8.49 b	2.32
coumarin	3.68 a	0.27 b	5.27 a	1.93
3,4,5.methoxy cinnamic	2.38 a	0.19 b	2.64 a	1.93
cinnamic	0.13 a	0.05 b	0.03 c	0.02
Total	261.34 c	407.83 b	1277.13 a	2.32
Antioxidant activity% (as DPPH)	85.66	78.13	94.46	-

Pyrogallol, Iso-frulic acid and catechol were the major free phenolic compounds existed in thyme powder(25.87, 25.11and 14.65mg/100g) respectively.Syringic(195.93mg/100g), pyrogallol(122.02mg/100g) and Catecol (35.29 mg/100g) were the predominant phenolic compounds in Carob powder. Whereas, many phenolic compounds were detected in high amounts in sumac powder such as pyrogallol (338.9mg/100g), p-coumaric(217.83mg/100g),catechol(95.23mg/100g),ellagic (81.3mg /100g) and protocatechuic (50.01 mg/100g).These data are in agreement with those reported by Yizhong *et al*.,(2004) ; Hussein *et al*., (2014) .

The antioxidant activities of thyme,carob and sumac are tabulated in Table (4). The data in Table (4) indicate that sumac powder has the highest level of antioxidant activity (94.48%), while the thyme powder recorded the antioxidant activity (85.66%) followed by carob powder that recorded (78.13%).This high antioxidant activity in sumac is due to high content of phenolic compounds present in sumac.

These data are in agreement with those reported by Lee *et al.,* (2003) ; Bashasha *et al*., (2014).

These results are confirmed with those of Hajaji *et.al*., (2010),who found that carob contain high amounts of polyphenols with strong anti-radical, antioxidant capacity and reducing properties,which might constitute an important source of natural antioxidants.Darwish *et al*., (2012),reported also that thyme contain high amounts of phenols with strong antioxidant capacity.

Chemical composition of bread samples:-

Data in Table (5) show that the moisture content of bread was significantly affected by addition of herbs the highset content of moisture (9.8%) was deteced in sumac bread (1%). Fat of different bread treatment were nearly,the highest fat content was observed in carob bread (2.5%). Results of protein content (Table 5) indicate that bread made from wheat flour only had the highest content (14.65%) followed by 2.5% sumac bread (14.42%).The ash content of bread was differed significantly according the addative. The highest ash content was observed in thyme bread (2.5%) (Hamza *et al*., 2001; Hussein *et al*., 2014).

Table (5) Chemical composition of bread samples (% dry weight).

Constituents Sample	Moisture	Fat	Protein	Fiber	Ash	Total carbohy
A 100%wheat flour	7.87^{d}	4.59^{e}	14.65a	1.03^{f}	0.69^{bc}	79.05a
A1 100% wheat flour +1% thyme	7.57^{e}	5.37^{bcd}	14.22bc	1.81^{a}	0.80^{bc}	77.80a
A2 100% wheat flour +2.5% thyme	7.86^{d}	5.52^{ab}	14.15dc	1.77^{b}	1.04^{a}	78.02a
B1 100% wheat flour +1% carob	8.24^{c}	5.28^{cd}	13.89d	1.53^{e}	0.64^{c}	78.66a
B2 100% wheat flour +2.5% carob	8.12^{c}	5.65^{a}	13.50e	1.53^{e}	0.86^{ab}	78.46a
C1 100% wheet flour +1% sumac	9.8^{a}	5.19^{d}	14.41abc	1.67^{d}	0.64^{c}	78.09a
C2 100% wheat flour +2.5% sumac	9.42^{b}	5.44^{abc}	14.42ab	1.71^{c}	0.74^{bc}	77.70a
LSD	0.19	0.22	0.26	0.035	0.21	1.80

Sensory evaluation of bread samples :-

Sensory evaluation of bread samples was illustrated in Table (6) .The results show that control bread sample recorded the highest score (88.5)compared with other bread samples .

Bread samples containing thyme had the highest over all acceptability (86.4 and 82.8%) at a level 1 and 2.5%, respectively. followed by carob bread (76.6 and 71.7 %) at a levels 1 and 2.5% and sumac bread recorded 71.5 and 69.00% at a level 1 and 2.5%, while at a level (5%) had the lowest score (49.1%). From data in Table (6),it could be observed that increasing the level of added herbs reduced overall acceptability of bread. Bread of thyme the highest score for face color (17.6).This may be attributed to the higher content of volatile aromatic or essential compounds and oils in thyme.

On the other hand addition of sumac at 5% reduced all sensory attributes relative to other herbs. This reduction may be due to the dark red color of sumac but still acceptable at other levels . Thyme bread (5%) has been excluded because of the lack of acceptablility. Generally, it could be observed that the addition of selected plants to bread did not affect greatly on sensory properties (Khorshid *et al*., 2011 ; Basuny *et al.*2012). Jinshui *et al.,* (2002) found through sensory evaluation that consumer panellists judged these fibre-enriched breads as acceptable. Therefore, the use of these herbs, especially carob, allows an increase of the daily intake of herbs without promoting negative effects on the rheological properties of doughs or quality and overall acceptability of the resulting breads.

6-

Phenolic compounds of bread samples:

Phenolic compounds account for a major partion of the antioxidant capacity in many plants (Duthie and Crozier, 2000). The strong correlations were observed between antioxidant activities and phenolic content indicate phenolic compounds were a major contributor to the antioxidant activity in samples. Phenolic compounds are know as antioxidants which have long been recognized to have protective function against oxidative damage in diet they may provide health benefits associaticl with reduced rsik of chronic disease(karppinen et al., 2003).

The phenolic compounds were determined and identified in studied bread samples and the results are presented in Table (7).From these results, it is evident that these bread samples have considerable amounts of phenolic compounds (0.467-18.347 mg/100g).The highest content was observed in sample 2.5% sumac bread (18.347 mg/100g) followed 2.5% thyme bread (16.951 mg/100g), then 2.5% carob bread (4.613 mg/100g).

The samples 1% sumac bread, 1% thyme bread and 1% carob bread, content of phenolic compounds was (3.615, 2.094 and 1.454 mg/100g), respectively.The lowest amount (0.467mg/100g) was detected in control bread. Pyrogallol was the major free phenolic compound existed in bread control and at a low rate (0.292mg/100mg). Thyme bread (1%) Pyrogallol (1.398 mg/100g), e-vanillic(0.088 mg/100g), benzoic (0.084 mg/100g), and ferulc (0.076 mg/100g) were the predominant phenolic compounds. Also, phenolic compounds were detected in high amounts in 2.5% thyme bread such as pyrogallol (8.369 mg/100g), protocatechuic (1.654 mg/100g), benzoic (0.833 mg /100g) ,chlorogenic (0.804 mg /100g) and ferulc(0.719 mg/ 100g). Carob bread (1%) the major free phenolic compound were pyrogallol (1.313 mg /100g) and catechein (0.041 mg/100g). While, phenolic compounds were detected in high amounts in 2.5% carob bread such as pyrogallol (2.745 mg/100g), e-vanillic (0.718 mg/100g) and catechein (0.087 mg /100g). Sumac bread 1% were pyrogallol (1.263mg/100g), benzoic (0.758 mg/100g) and e-vanillic (0.241 mg/100g) the major free phenolic compounds. Also, phenolic compounds were detected in high amounts in 2.5% sumac bread such as pyrogallol ,salycilic, chlorogenic e-vanillic,and catechein were (15.162, 0.587, 0.372, 0.336 and 0.299 mg / 100g), respectively. These data are in agreement with those reported by Selimović *et al.,* (2014), who found the reason for the rise or fall of some phenolic compounds from samples of bread was result of manufacturing processes that take place on bread (kneading, fermenting, bread). Some of phenolic compounds are active on the degree of fermentation temperature and others irresistible high temperatures experienced by the bread. Baking temperature influenced significantly more the loss of total phenols in wheat flour.Also, Dietrych-Szostak and Oleszek, (1999) found by using different temperature regimes resulted in drastic reductions of the total phenolic and flavonoid concentration in the wheat flour bread.

Table (7) : Phenolic compounds and antioxidant activity of bread samples (mg/100g).

Phenolic compounds mg/100g	samples							LSD
	controlA	A1	A2	B1	B2	C1	C2	
gallic	0.010 d	0.025 c	0.053 b	0.011 d	0.022 c	0.012 d	0.095 a	0.004
pyrogallol	0.292 g	1.398 d	8.369 b	1.313 e	2.745 c	1.263 f	15.162 a	0.012
4-aminobenzoic	0.002 e	0.008 d	0.021 a	0.001 e	0.010 cd	0.013 c	0.036 a	0.003
protocatechuic	0.008 f	0.027 d	1.654 a	0.005 f	0.056 c	0.020 e	0.135 b	0.004
chlorogenic	0.012 f	0.015 e	0.804 a	0.017 e	0.027 d	0.189 c	0.372 b	0.004
catechol	0.008 f	0.022 d	0.469 a	0.007 f	0.157 c	0.068 d	0.213 b	0.004
catechein	0.010 f	0.047 e	0.384 a	0.041 e	0.087 c	0.089 c	0.299 b	0.004
caffeine	0.015 d	0.003 f	0.294 a	0.001 f	0.054 b	0.010 e	0.021 c	0.003
p.oh.benzoic	0.007 e	0.008 e	0.482 a	0.003 f	0.063 c	0.042 d	0.230 b	0.003
caffeic	0.002 e	0.016 c	0.126 b	0.001 e	0.005 d	0.016 c	0.133 a	0.003
vanillic	0.001 e	0.019 d	0.445 a	0.002 e	0.017 d	0.041 c	0.077 b	0.003
ferulic	0.009 e	0.076 b	0.719 a	0.009 e	0.012 e	0.030 d	0.044 c	0.003
Iso-ferulic	0.004 f	0.010 e	0.080 a	0.003 f	0.032 b	0.026 c	0.013 d	0.003
e-vanillic	0.013 f	0.088 e	0.708 b	0.007 g	0.718 a	0.241 d	0.336 c	0.005
reversetrol	0.023 d	0.028 c	0.201 a	0.001 e	0.022 d	0.020 d	0.039 b	0.004
ellagic	0.004 e	0.051 c	0.343 a	0.003 e	0.059 b	0.055 c	0.045 d	0.003
alpha-coumaric	0.002 e	0.011 d	0.090 a	0.001 e	0.025 c	0.044 b	0.043 b	0.003
benzoic	0.019 f	0.084 e	0.833 a	0.016 f	0.371 c	0.758 b	0.248 d	0.006
salycilic	0.011 f	0.067 d	0.195 c	0.009 f	0.036 e	0.394 b	0.587 a	0.004
3,4,5.methoxy cinnamic	0.004 f	0.062 b	0.158 a	0.001 f	0.011 e	0.053 c	0.035 d	0.003
coumarin	0.003 ef	0.005 e	0.064 c	0.001 f	0.045 d	0.191 a	0.148 b	0.003
p- coumaric	0.004 ef	0.007 e	0.407 a	0.001 f	0.021 d	0.040 b	0.032 c	0.003
cinnamic	0.003 de	0.016 c	0.051 a	0.001 e	0.019 b	0.003 d	0.005 d	0.003
Total	0.467 g	2.094e	16.951 b	1.454 f	4.613 c	3.615 d	18.347 a	0.086
Antioxidant activity%	47.61	51.1	54.96	53.12	53.30	64.80	65.88	-

The data in Table (7) indicate that (2.5%) sumac bread sample and (1%) sumac bread sample have the highest level of antioxidant activities (65.88%and 64.8%), while the (2.5%) thyme bread sample and (1%) thyme bread sample recorded the antioxidant activity (54.96% and 51.1%) followed by (2.5%) carob bread sample and (1%) carob bread sample that recorded (53.30 % and 53.12%), respectively.

While the control sample had the lowest percentage in the content of antioxidants active .High antioxidant activity in these samples is due to high content of phenolic compounds present in them (Owen *et al*., 2003; Gabr *et al* 2014).

Biological evaluation

Lipid profile :

The results in Table (8) show the effect of different bread diets on serum lipid profile of normal and hypercholesterlemic rats. From the obtained data, it could be observed that, the initial level of triglycerides in blood serum recorded 110.66 mg/dL blood serum for negative group. While, after feeding of experimental groups on hypercholesterolemic diet for two weeks, the levels of triglycerides in blood serum increased to 163.31 mg/dL. At the end of experimental period, it could be observed that the hypocholesterolemic group and the group which fed on hypercholesterolemic diet of control bread (prepared by using only wheat flour 72%) recorded higher blood triglycerides levels (144.40 and 128.17 mg/dL, respectively) compared with control group fed only on basal diet which recorded 111.00 mg/dL. The feeding of hypercholesterolemic rats on bread sample which prepared by using 1% and 2.5% of thyme, sumac and carob powder significantly reduced blood triglycerides levels. Also it was observed that feeding on deit containing 2.5% sumac bread (G9) resulted in the lowest percentage of triglycerides (116.93mg/dl), followed by 2.5% thyme bread (G5) (117.53 mg/dl), 2.5% carob bread, (G3)(128.17 mg/dl) control bread. The decrease in blood triglycerides levels in these groups may be due to the higher dietary fiber and antioxidants content of bread samples.

Concerning the results of total cholesterol, HDL-cholesterol,LDL-cholesterol and vLDL-cholesterol presented in the same table, it could be observed that, the initial levels of total cholesterol, LDL-cholesterol and vLDL-cholesterol recorded of 121.56, 48.38 and 22.13 mg/dL blood serum respectively for all groups. While, after feeding of experimental groups on hypercholesterolemic diet for two weeks.The levels of LDL-cholesterol and vLDL-cholesterol were increased to record 167.31, 98.07 and 35.66 mg/dL blood serum respectively.This may be due to feeding on hypercholesterolemic diet. These results are in agreement with those of Mott *et al*., (1992),who reported that the high-cholesterol diet increased the cholesterol content of normal adult baboon bile resulting in a 15% higher cholesterol than in baboons fed a very low-cholesterol diet. Morever, Ahmed (2009) found that, rats which fed on high fat and cholesterol diet had a highly significant increase ($p<0.001$) in all lipid parameters namely, total lipids, triglycerides, total cholesterol and LDL cholesterol, moreover HDL cholesterol showed highly significant decrease ($p<0.001$) compared to the normal group. At the end of experimental period, it could be observed that, the hypocholesterolemic group which fed only on hypercholesterolemic diet during all experimental period and the group which fed on hypercholesterolemic diet of control bread (prepared using only wheat flour 72%) still recorded the higher blood total cholesterol, LDL-cholesterol plus vLDL-Cholesterol levels while HDL-cholesterol was decreased compared with control group fed only on basal diet.

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On the contrary, the groups which fed on hypercholesterolemic diet of bread samples prepared by using 1% and 2.5% of thyme, sumac and carob powder significantly reduced blood total cholesterol, LDL-cholesterol plus vLDL-cholesterol levels. It was the lowest decline appeared in the G9 fed on 2.5% sumac bread followed by G5 fed on 2.5% thyme bread then G7 fed on 2.5% carob bread and G6 fed on 1% carob bread, respectively.While HDL-cholesterol was increased compared with hypercholesterolemic group to reach its highest level in G5 fed on 2.5% bread thyme, reaching 51.03mg/dl.The decrease in blood total cholesterol, LDL-cholesterol and vLDL-cholesterol levels in these groups that may be due to the higher dietary fiber and antioxidants content of bread sample compared with control bread sample. For dietary fiber effect.These results are in agreement with those of Ali *et al*. (2012) ;Anwer *et al*. (2012); Madihi *et al*., (2013).

Al Badr (2011), found that the thyme powder showed a significant decrease in final weight and increase in serum ALT and AST enzymes activity and showed a significant decrease in hemoglobin , and significant increase in the values of liver cholesterol compared with control (-ve) group. The all treated rat groups showed a significant increase in serum total bilirubin, A/G ratio and liver MDA, triglyceride & total lipid and a significant decrease in body weight gain,FER, serum globulin and liver GPX compared with control (-ve) group.

Liver functions:

Liver function tests in the present study included the determination of liver alanine amino-transferase enzyme (ALT) and aspartate amino-transferase enzyme (AST). All analysis were performed after feeding of experimental groups on hypercholesterolemic diet for two weeks(zero time), and finally after feeding for 64 days. The results in Table (9) showed the effect of bread diets on serum alanine amino-transferase enzyme (ALT) of normal and hypercholesterlemic rats. From the obtained data, it could be observed that, the initial level of ALT in blood serum recorded 47.50mg/dl blood serum for all groups. While, after feeding on hypercholesterolemic diet for two weeks, the levels of ALT in blood serum increased to record 101.30 mg/dl blood serum in all groups, except basal diet group which was 47.50 mg/dl, blood serum. Concerning the blood ALT levels of all groups at the end of experimental period, it could be observed that the hypercholesterolemic group fed only on hypercholesterolemic diet during all experimental period and the group which fed on hypercholesterolemic diet of control bread (prepared using only wheat flour 72% extraction rate) still recorded the higher blood ALT levels being 71.60 mg/dl and 64.00 mg/dl respectively, compared with control group fed only on basal diet which recorded 48.51 mg/dl. While, hypercholesterolemic rat fed on diet of bread samples prepared by using 1% and 2.5% of thyme, sumac and carob powders significantly reduced blood ALT levels. And the lowest level of ALT was observed in group 9 (57.10mg/dl), followed by the group5 (58.20mg/dl).

Table (9): Liver functions of normal and hypercholesterlemic rats fed on different prepared bread diets.

Group / Treatment	G1(-)	G2(+)	G1(-)	G2(+)	G3	G4	G5	G6	G7	G8	G9
Time	Time0	Time0	End time								
ALT	47.50 gh	101.30a	48.51 d	71.60 b	64.00 c	60.10 de	58.20 ef	62.30 cd	59.60 ef	63.60 c	57.10 f
AST	61.00 h	106.70a	63.60 f	91.00 b	83.20 c	75.10 d	60.40 h	71.30 e	67.90 g	70.30 e	69.40 e

Means followed by different letters in the same column are significantly different by Duncan's multiple test (p<0.05).ALT=alanin amino transferase.AST=aspartate amino transferase.

For aspartate amino-transferase (AST) levels, it could be observed that, the initial AST level in blood serum recorded 61.00 mg/dl blood serum for negative group. While, after feeding of experimental groups on hypercholesterolemic diet for two weeks, the levels of AST in blood serum was increased and recorded 106.70mg/dl blood serum in all groups, except group which fed with basal diet (61.00 mg/dl) .At the end of experimental period it could be observed that the hypocholesterolemic group which fed only on hypercholesterolemic diet during all experimental period and group fed on hypercholesterolemic diet of control bread still recorded the higher blood AST levels being 91.00 mg/dl and 83.20 mg/dl respectively, compared with control group fed only on basal diet which recorded 63.60. While, the groups which fed on hypercholesterolemic diet of bread samples which prepared by using 1% and 2.5% of thyme, sumac and carob powder significantly reduced blood AST levels compared with hypocholesterolemic group. It was the lowest drop level in the group 5 (60.40 mg/dl), followed by group 7 (69.40 ml/dl). These results are in agreement with Al Badr (2011), Ali *et al*., (2012), Anwer *et al*. (2012).

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التقييم الكيميائي والحسي والبيولوجي للخبز المصنع باضافة بعض الاعشاب الغنيه بالمركبات النشطه حيويا

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هذه الدراسه توضح اهمية التقييم الكيمائي والحسي والبيولوجي للخبز المصنع باضافة بعض الاعشاب الغنيه بالمركبات النشطه حيويا مثل الزعتر والسماق والخروب حيث تم تصنيع الخبز باضافة كمية من مسحوق (الزعتر والسماق والخروب) بنسبة ١ ، ٢.٥ ، ٥٪ إلى دقيق القمح (استخلاص٧٢٪). وتم تقدير التركيب الكيميائي والمركبات الفينولية، والنشاط المضادة للأكسدة (DPPH) وكذلك اجراء التقييم الحسي للخبزالمصنع. وأظهر التقييم البيولوجي أن الفئران التي تغذت على وجبات مفرط كوليستيرول الدم تحتوي على علائق بالسماق والزعتروالخروب لمدة ٤٥ يوما , انخفاض بشكل ملحوظ (بمستوى معنويه ٠.٠٥%) كل من الكوليسترول الكلي (TC)، الدهون الثلاثية (TG)، البروتينات الدهنية منخفضة الكثافة (LDL)، البروتينات الدهنية منخفضة الكثافة جدا (VLDL)،في مصل الدم في حين زادت البروتينات الدهنيه عالية الكثافة (HDL). وأظهرت انخفاضا كبيرا في انزيمات الكبد ALT و AST .

توصي الدراسة بامكانية استخدام مسحوق الزعتر والسماق والخروب في إعداد الخبز لخفض مستوى الدهون في الدم ولغرض تحسين وظائف الكبد.

Table (6): Sensory evaluation of bread samples.

Characters / Sample	Face Color (20)	Color bac (10)	Homogeneity of color (10)	Rubber and portability chewing (10)	Odor (20)	Taste (20)	Irregular shape (5)	The proportion of edges (5)	Total (100)
A	17.70 a	9.10 a	8.50 a	8.40 a	18.00 a	17.90 a	4.70 a	4.20 ab	88.50 a
A1	17.70 a	7.80 ab	7.90 ab	8.30 a	17.40 ab	18.10 a	4.50 ab	4.70 a	86.40 ab
A2	17.60 a	7.70 ab	7.30 ab	7.60 ab	17.20 ab	16.90 ab	4.30 ab	4.20 ab	82.80 abc
A3	13.40 bc	6.10 ab	5.40 ab	5.60 ab	13.70 cd	13.10 cd	3.10 ab	1.90 b	62.30 de
B1	16.20 a	6.80 ab	7.40 ab	7.30 ab	15.00 abc	16.20 abc	4.00 ab	3.70 ab	76.60abcd
B2	15.00 a	7.00 ab	7.20 ab	6.90 ab	15.10 abc	13.10 cd	3.90 ab	3.50 ab	71.70 abcd
B3	10.20 bc	4.60 b	4.40 b	4.60 b	12.00 cd	12.00 cd	2.60 b	2.40 ab	52.80 e
C1	15.70 a	6.40 ab	5.80 ab	6.80 ab	14.10 cd	15.00 abc	4.00 ab	3.70 ab	71.50 bcd
C2	14.50 ab	6.60 ab	6.40 ab	6.20 ab	14.50abcd	14.40 abc	3.60 ab	2.80 ab	69.00 cd
C3	9.60 e	4.90 b	4.60 b	4.50 b	10.90 d	9.60 d	2.90 ab	2.10 b	49.1 e

A = Bread 100%wheat flour. A1 = bread 1% thyme. A2 = bread 2.5% thyme. A3=bread 5% thyme . B1 = bread 1%carob. B2 = bread 2.5% carob. B3 = bread 5% carob . C1 = bread 1% sumac. C2 = bread 2.5% sumac. C3= bread 5% sumac

Table(8): Serum lipid profile of rats fed on bread prepared with different herbs.

Group	Time	Triglyceridees (mg/dl)	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-Cholesterol (mg/dl)	Vldl-cholesterol (mg/dl)
G1 negative control	Time0	110.66ef	121.56^{h}	51.05^{a}	48.38^{h}	22.13^{d}
G2 positive control	Time0	163.31a	167.31^{a}	33.58^{g}	98.07^{a}	35.66^{a}
G1 negative control	End time	111.00 ^{ef}	123.58^{h}	48.19^{cd}	53.19^{g}	22.20^{d}
G2 positive control		144.40^{b}	158.07^{b}	35.33^{g}	93.49^{a}	29.88^{b}
G3100%wheat flour		128.17^{c}	153.6^{c}	43.67^{f}	84.30^{b}	25.63^{b}
G4 100% wheat flour +1% thyme		125.53^{c}	145.77^{ed}	47.00^{de}	73.65^{c}	25.12^{c}
G5 100% wheat flour +2.5% thyme		117.53^{de}	141.76^{f}	51.03^{a}	67.22^{f}	23.51^{cd}
G6 100% wheat flour +1% carob		125.53^{c}	146.27^{d}	46.00^{e}	75.15^{d}	25.12^{c}
G7 100% wheat flour +2.5% carob		121.65^{cd}	143.25^{f}	50.03^{ab}	68.89^{d}	24.33^{c}
G8 100% wheet flour +1% sumac		125.03^{c}	143.74^{ef}	47.00^{de}	71.73^{e}	25.01^{c}
G9 100% wheat flour +2.5% sumac		116.93^{de}	139.16^{g}	48.33^{bcd}	67.44^{e}	23.39^{cd}
LSD	6.97		2.05	1.80	1.94	2.72

*** Means followed by different letters in the same column are significantly different by Duncan's multiple test (p<0.05).TG=triglycerides.TC=total cholesterol.HDL=high density lipoprotein.LDL=low density lipoprotein. vLDL=TG/5.**