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PREPARATION, CHARACTERIZATIONS AND HEALTH EFFECTS OF FUNCTIONAL BISCUITS CONTAINING ISOFLAVONES

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ABSTRACT: *Isoflavones are powerful compounds known as phytoestrogens found in soybean products and sold in a supplement form. The preliminary studies suggest that the regular consumption of soy foods or supplements may protect against cancers, therefore the purpose of this study was to use isoflavones for evaluating the health benefits of it to produce enriched cereal products. Biscuits at different replacement levels 0.50, 1.25 and 2.00 g for each 1000 g wheat flour were prepared and then studied the outcome biscuits chemically, physically, organolyptically and biologically. The results showed that adding isoflavones led to improving the storage stability of biscuits, since it reduced the rate of peroxide value formation during storage for 90 days on room temperature. Also found that adding isoflavones resulted in improving the total cholesterol, reducing the triglyceride, and low density lipoprotein (LDL) cholesterol and increasing the high density lipoprotein (HDL) cholesterol in rat serum feeding on this biscuits for 30 days. On the other hand the sensorial properties, or the chemical composition and the rheological properties did not affected by this adding.*

Key words: *Isoflavones; Cholesterol; Low and High Density Lipoprotein Cholesterol and Triglcerides.*

INTRODUCTION

Isoflavones or phytoestrogens are one of the major phytochemicals they are naturally present in soybean. Daidzin, glycitin, genistin and their respective glucosides are the main isoflavones in unprocessed soy flour. They are responsible of the health functions of the isoflavones particularly in preventing development of heart disease and cancers (Xu *et al.,* 2002). According to Matssura *et al.,* (1999) isoflavone compounds have antihemolytic, antioxidative, antifungal, estrogenic and antitumorial effects. Set Chell *et al.,* (1997) mentioned that about 100 milligrams of Isoflavones are necessary to achieve most of these health benefits. Also Anderson *et al.,* (1995) showed that Isoflavones reduce the risk of cardiovascular diseases by

reducing the level of total cholesterol, low-density lipoprotein (LDL) cholesterol and increasing high density lipoprotein (HDL) cholesterol (Carroll and Kurowska, 1995). Results of Yokota *et al.,* (1996) indicated that isoflavones prevent the progress of atherosclerosis in rabbits by limiting the oxidative modification of LDL cholesterol.

Cassidy *et al.,* (2000) found that isoflavones-rich diets as soybean have a potential role in controlling the hormone-dependent diseases such as cancer and heart disease osteoporosis and menopausal symptoms (Vyn *et al.,* 2002). Demonty *et al.,* (2002) found that isoflavones lowered plasma triglycerides, total and low-density lipoprotein cholesterol concentrations. The same observations stated by Horigome and Cho (1992), and Carroll and Kurowska (1995) in laboratory animals. Laurin *et al.,* (1991) attributed these effects to the ability of isoflavones in the reduction of intestinal absorption of cholesterol and bile acids (Beynen, 1990).

The main aim of this study was to utilize of the above mentioned health effects of isoflavones in preparing functional biscuits. The influence of isoflavones addition on thee rheological characteristics of biscuit dough, chemical and sensorial properties and oxidative stability of biscuits were also evaluated. Also the health effects of feeding of laboratory animals on this prepared product were determined.

MATERIALS AND METHODS

Materials:-

Wheat flour:- (72% extraction) from the south Cairo Mills company, Cairo, Egypt.

Soy isoflavone, product 152-400 Lot # 0006/91 from Arche Daniels Midland company 4666E. Faries were purchased. Milk, corn oil, sugar, fresh eggs, vanilia and baking powder were obtained from the local market, Egypt and twenty four male albino rats 5 weeks old age having 50-60 g. weight were purchased from faculty of veterinary, Cairo, Egypt

Methods:-

1-Technological methods:- The following formula was used to prepare the functional biscuits., 1000 g wheat flour, 300 g sugar, 150 g corn oil, 5 g skim milk powder, 10 g baking powder, 10 g vanilia, 240 g fresh egg, 50 ml water and 0.0, 0.5 g, 1.25 and 2 g isoflavones. Isoflavone blends were prepared, formed and baking at 210°C for 8-10 min. according to the standard procedure for semi hard sweet biscuit (Peti-pier type) according to the formula of Bisco Misr Company, Cairo, Egypt.

2-Rheological Properties: Fairnograph and Extinsograph were carried out according to the methods described in the A.A.C.C (1990).

3-Chemical analysis:

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

B- Evaluation of antioxidant activity of isoflavones: The peroxide value in the extracted crude fat was determined as described in the A.O.C.S (1989).

4- Biological evaluation of functional biscuits:- The 24 rats were divided into 4 experimental groups of bout equal weight. The rats were housed individually in stainless steel cages at temp. (25 ±1°C) and fed feed consisted of 4.25 % casein, 0.23 corn oil, 1% Vit. mixture, 4% salt mixture, 5% cellulose, 18.85% corn starch and 66.67% biscuits free and containing different levels of isoflavones for 30 days according to AOAC (2000). All rats groups were checked daily, and their body weights recorded weekly.

5- Serum analysis: The rats were weighed and fasted over night before sacrificing. The blood was collected from the neck region in 15 ml. centrifuge tubes, clotted and centrifuged at 10.000 xg for 30 min. at 4 °C. Serum was promptly removed from the separated cells with a Pasteur pipette and analyzed for total Serum Cholesterol, HDL, LDL and Serum triglycerides according to Bestein *et al.*, (1970), Tietz and Dryer (1970), Bucolo and David (1973), Allian, *et al.*, (1974), Varely *et al.*, (1980). Tietz (1991).

6- Sensory evaluation of Biscuits: Ten panelists of the bread and Pastry, Dept. of the Agric. Res. Center, Giza., Egypt, were asked to evaluate the sensorial properties of biscuits using the following scores for each property described by Smith (1972) as follows: appearance (20), color (20), texture (20), flavor (20) and taste (20).

The product had a total scores from 90-100 was described as: very good, 80-89 good, 70-79 satisfactory and less than 70 questionable.

7-Statistical analysis of biological evaluation:

The data was statistically analyzed using analysis of variance and Duncan's Multiple Range Test according to the SAS program (1986).

RESULTS AND DISCUSSION

Rheological properties of biscuit doughs:

Results presented in Table (1) show the effect of supplementation wheat flour with different levels of isoflavone on fairnograph and extinsograph parameters. From the results of farinograph parameters, it could be notice that the water absorption and dough development times of doughs were not affected, while dough stabilities increased and weakening of doughs decreased slightly with increasing the isoflavone levels from 1.25 to 2.00 gm. Also the results of extinsograph showed that the Extensibility (E) of doughs was slightly decreased, while the resistance to extension (R) of dough was marked increased with increasing the isoflavones added levels. This led to increase the proportional ratio of (R/E) of doughs with increasing the levels of isoflavones addition. Energy values of dough were noticeably increased due to isoflavones addition. These results indicated that supplementation of wheat flour with isoflavones strengthened the gluten network.

Sensory properties of biscuits:

The results presented in Table (2) showed that addition of isoflavounes did not affect the color, taste, odor, appearance, texture and total acceptability of biscuits.

Chemical composition:

As shown from Table (3) the proximate chemical composition of prepared biscuits did not change due to its supplementation with the different added levels of isoflavones.

Storage stability of biscuits:

Results in Table (4) showed that the peroxide value of biscuits increases gradually with extending of storage period. The rate of this increase was less in products containing isoflavones than control. This is mainly due to the antioxidant potency of isoflavones.

Biological and physiological effects of functional biscuits:

The results in Table (5) showed that among the prepared functional biscuits, product contained 1.25gm isoflavone/1000 gm flour was similar with the control, free from isoflavones, in feed intake and the increment in weight gain of rats through feeding period days. The other types of functional biscuit products were similar in their determined parameters.

The feeding on biscuits containing isoflavones did not significantly affected the total serum cholesterol (P= 0.05, Table 4). On the other hand triglyceride was significantly dropped due to feed on the biscuit containing isoflavones. This effect increased with increasing the addition level of

isoflavones (P= 0.05, Table 4). Therefore product had 2 gm isoflavones /1000 gm flour had the strong effect among the other samples.

A contrary relationship between HDL and LDL was observed due to feed the rats on biscuits containing isoflavones. Feeding on such products caused a significant (P= 0.05, Table 4) increase in HDL, good cholesterol, and a significant (P= 0.05, Table 4) reduction in LDL. Such changes were more obvious with increasing the addition level of isoflavones from 0.5 to 2.0 g/Kg. biscuit flour.

Table (1) Effect of soy isoflavones addition on rheological properties of biscuit doughs

Samples	Farinograph				Extinsograph			
	W.A	D	S	W	R	E	P.N	Energy
0.0 (control)	52.5	1.0	3.0	130.0	120.0	133.0	0.90	32.0
0.50 g /Kg flour	52.5	1.0	3.0	130.0	170.0	125.0	1.36	38.0
1.25 g/K g flour	52.5	1.0	3.5	125.0	170.0	122.0	1.39	38.0
2.00 g /Kg flour	52.5	1.0	3.5	125.0	180.0	120.0	1.50	38.0

W.A = Water absorption %
D= Development time (min) S= Stability (min)
W= Weakening of dough (BU) R= Resistance to extension (BU)
E= Extensibility (BU) P.N = Proportional number (R/E)

Table (2) Effect of isoflavones addition on sensory properties of biscuits

Treatments	Color (20)	Taste (20)	Odor (20)	Appearance (20)	Texture (20)	Total Score (100)
0.0 (control)	18.80	18.50	18.30	18.45	17.65	91.70
0.50g/kg flour	18.90	18.30	18.20	18.30	18.10	91.80
1.25g/kg flour	18.90	18.55	18.30	18.40	17.80	91.95
2.00g/kg flour	18.70	17.80	18.20	18.20	17.30	90.20
L..S..D at 0.05 level	1.2046 N.S	1.5299 N.S	1.6239 N.S	1.6021 N.S	2.0706 N.S	7.1233 N.S

Scores 90 – 100 very good
80 – 90 good
70 – 80 satisfactory
Less than 70 Questionable

Table (3) Effect of isoflavones addition on proximate chemical composition of biscuits

Biscuits	Crude Protein %	Total Carbohydrate %	Fat %	Ash %	Crude fiber %
0.0 (control)	8.94	74.22	14.66	0.86	1.32
0.50 g/Kg flour	8.96	74.31	14.52	0.85	1.36
1.25g/kg flour	8.93	74.12	14.71	0.82	1.42
2.00g/Kg flour	8.93	74.11	14.70	0.81	1.45

Table (4) Some biological and physiological effects of free and isoflavones containing biscuits

Supplementation Level	Feed Intake (gm)	Weight gain (gm)	Cholesterol (mg/100 gm)	Triglyceride (mg/100gm)	HDL[1] (mg/100gm)	LDL[2] (mg/100 gm)
0.0 (control)	387.5	110.0	82.67	87.667	44.517	20.627
	±25.12 2	±34.16 2	± 5.65 a	± 11.35 a	± 6.65 c	± 2.44 a
0.5g/ kg flour	367.50	94.17	79.50	66.00	49.120	17.925
	±30.30 2	± 17.90 2	± 5.74 a	± 10.42 b	±5.94 c	± 2.13 a
1.25g/ kg flour	385.83	124.17	78.00	56.50	55.567	11.143
	±24.12 2	± 21.70 2	± 2.082 a	± 3.73 bc	±2.56 b	± 1.19 b
2g/ kg flour	368.33	105.00	80.17	49.667	62.470	7.697
	± 27.20 2	± 18.84 2	± 2.732 a	± 5.15 c	±2.43 a	± 2.34 c

[1]High density Lipoprotein

[2]Low density Lipoprotein

Table (5) Effect of isoflavones (ISF) addition on storage stability of biscuits

Storage period (days)	0.0 (control)	0.50g ISF/ kg flour	1.25g ISF/ kg flour	2.0g ISF/ kg flour
Zero	2.05	2.05	2.05	2.05
15	2.96	2.32	2.19	2.22
30	3.79	2.88	2.37	2.45
60	7.06	3.95	3.04	3.33
90	5.71	4.45	3.56	3.95

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إعداد وخواص والتأثيرات الصحية للبسكويت الموظف المحتوى على الأيزوفلافونات

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الملخص العربي

تعتبر الأيزوفلافونات مركبات قوية ، وتعرف على أنها فيتوستيروجينات موجودة فى مركبات فول الصويا وتباع في منتجات مدعمة بها.

ولقد اقترحت الدراسات المبدئية أن الاستهلاك المنتظم من أغذية فول الصويا أو مدعماتها يمكن أن يعطى وقاية ضد السرطان ، ولهذا كان الغرض من تلك الدراسة هو استخدام الأيزوفلافون بغرض تقييم الفوائد الصحية لها لأنتاج منتجات حبوب مدعمة. ولقد تم تجهيز البسكويت على مستويات استبدال مختلفة ٠,٥ ، ١,٢,٢٥جرام لكل ١٠٠٠ جرام دقيق قمح ثم دراسة البسكويت الناتج حسيا وطبيعياً وكيميانياً وبيولوجيا. ولقد أظهرت النتائج إن إضافة الأيزوفلافون تؤدى إلى تحسين الثباتية التخزينية للبسكويت، حيث أنها تقلل من معدل تكوين رقم البيروكسيد خلال التخزين لمدة ٩٠ يوم على درجة حرارة الغرفة. وقد وجد أيضا إن أضافه الآيزوفلافون تؤدى إلي تحسين الكوليسترول الكلى وتقليل الجليسريدات الثلاثية والليبوبروتينات القليلة الكثافة LDL للكوليسترول، وزيادة الليبوبروتينات العالية الكثافة HDL للكوليسترول في سيرم الفئران المغذاة على هذا البسكويت لمدة ٣٠ يوم.على الجانب الأخر لم تتأثر الخصائص الحسية أو التركيب الكيماوي والخصائص الريولوجية بهذه الإضافة.