EFFECT OF FORTIFIED BISCUIT WITH SOYBEAN FLOUR AS HYPOGLYCEMIC AGENT IN DIABETIC RATS

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ABSTRACT

The present investigation was conducted to investigate the effect of unfortified biscuits, fortified biscuits with soybean flour and school children biscuits on some biological and biochemical parameters such as food intake, percent weight gain, serum glucose, liver functions, kidney functions and iron status in normal and diabetic rats. Biscuits were fortified with 10, 15 and 20% soybean flour. Biscuits samples were evaluated to choose the best samples for the biological part. Sixty albino rats were divided into two main groups (30 rats/each) .The first main group was normal while the second main group was hyperglycemic. Both groups were divided into six subgroups fed on basal diet containing 2.5 or 5% protein from unfortified biscuits, school children biscuits and fortified biscuits with 10% soybean flour. After 5 weeks rats were sacrificed and blood samples were collected to obtain the serum. Results indicated that feeding diabetic groups on basal diet containing soybean flour led to an improvement in serum glucose, liver functions and urea nitrogen. While normal groups fed on basal diet containing 5% protein from school children biscuit led to an improvement in total iron. The healthy groups fed on basal diet containing 5% protein from fortified biscuit with 10% soybean flour led to an improvement in total iron binding capacity. It is recommended to use soybean flour in diabetic foods and diets. Planning and implementing nutrition education programs for diabetic patients in order to explain the important role of soybean in controlling diabetes. Both normal and diabetic children can nutritionally benefit from fortified school biscuits with soybean flour because it is suitable for them and improves their nutritional status.

Key words: Diabetic rats- soybean- glucose- liver functions- kidney functions- total iron and total iron binding capacity.

INTRODUCTION

Diabetes mellitus is a syndrome of metabolic dysregulation, characterized by chronic hyperglycemia, involving carbohydrate, fat and protein metabolism, and resulting from destruction of pancreatic B-cells and/or deficient action of insulin on target tissues. Diabetic subjects show greater liability to long-term complications, such as retinopathy, nephropathy, cardiovascular complications and neuropathy (WHO, 2005).

Teixeira et al., (2004) and Azadbakht et al.,(2008) indicated that isolated soy protein consumption improves several markers that may be beneficial for type 2 diabetic patients with nephropathy. Lu et al., (2008) stated that ingestion of high-isoflavone soy protein not only lowers glucose levels but also reduces the incidence of cataracts in diabetic rats. The beneficial effects of soy isoflavones are attributed to increased insulin secretion, a better glycemic control, and antioxidant protection. In addition, Villegas et al.,(2008) concluded that consumption of soy bean was inversely associated with the risk of type 2 diabetes mellitus.

McCue et al., (2005) suggested that sprouting and dietary fungal bioprocessing of soybean improve the anti-diabetic potential of soybean extracts, potentially through linked to phenolic profile of the extract, and further suggest that enzyme inhibitory activity may be linked to phenolic antioxidant mobilization during sprouting and/or bioprocessing. The significance of food-grade, plant-based enzyme inhibitors for modulation of carbohydrate breakdown and control of glycemic index of foods in the context of preventing hyperglycemia and diabetes mellitus complications such as hypertension in the long-term is hypothesized..

Stephenson et al., (2005) reported that implementation of a soy based diet appears to reduce the glomerular filtration rate (GFR) and total and LDL cholesterol of young adults with type 1 diabetes and glomerular hyperfiltration, thus affecting positively their clinical profile. In addition, Murray-Kolb et al., (2003) reported that soybeans appear to be a good source of nutritional iron in marginally iron deficient individuals.

So, the study was carried out to investigate the effect of fortified biscuits with soybean flour compared with school children biscuits on some biological and biochemical parameters in normal and hyperglycemic rats.

MATERIALS AND METHODS

Materials

- Low fat soybean flour was obtained from Soy Pilot Plant Unit, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.
- School children biscuits were obtained from (6th of October Company for Food Industries).
- Commercial wheat flour (72% extraction rate), eggs, skimmed milk, salt (sodium chloride), baking powder and corn oil were obtained from the local market.
- Sixty weanling female albino rats were obtained from the animal house, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt .

Methods:

Technological methods:

- **Production of biscuits:** Biscuits were prepared according to the method of Abd El Salam, (2000) with some modifications. Biscuits composed of 100 gm flour, 3 gm dried skimmed milk powder, 24 gm egg, 4.5 gm baking powder, 17 gm oil, 4 gm salt. The required amount of eggs and oil were beaten for one min., and then dried skimmed milk was added and mixed for one min. The flour and baking powder were seared together and added to the above mixture and beaten continuously until the blend got smooth. The dough was cut into circles (1/2 cm thick and 2cm diameter) and transferred to greased plate and baked at 180 °C for 12-15minutes.Biscuits were allowed to cool then evaluated chemically and organoleptically.

- Low fat soy flour was used to replace wheat flour at 10, 15 and 20% levels. The fortified biscuits prepared by the same method.

Sensory evaluation:

Thirty three children from AL Cawmia primary school in Hadaec El Koba, Cairo governorate were selected randomely for sensory evaluation of biscuits. Their age ranged between 7 to 12 years. Each child was asked to evaluate biscuits samples fortified with soy bean flour (10, 15, and 20% levels) according to the color; odor, taste, texture, and general acceptability by putting a mark beside the statement that best reflect his/her opinion in the evaluation sheets. The statements used were" like it very much, like it sometimes, don't like or hate it, do not like it sometimes, do not like it very much according to Penfield and Campbell, (1990), and results were analyzed statistically in order to know the best percentage for fortification.

Chemical analysis:

Moisture content, crude protein, fat, ash, and minerals(Fe, Mg, Na, Mn, Zn and K) were determined in the raw material and all samples of biscuits on dry basis according to the method described in A.O.A.C., (1990).Fiber was determined according to the method described by Pearson, (1970).Total carbohydrate content was determined by differences.

Experimental animal design:

Sixty weanling female albino rats (110±5gm) were housed in well aerated cages under hygienic conditions and fed on basal diet for one week for adaptation. After this period, rats were divided into two main groups (30 rats each):

The first main group was normal (-) and divided into 6 subgroups fed on:

- Basal diet containing 2.5% protein from unfortified biscuits
- Basal diet containing 5% protein from unfortified biscuits
- Basal diet containing 2.5% protein from fortified biscuits with 20% soybean flour.
- Basal diet containing 5% protein from fortified biscuits with 20% soybean flour.
- Basal diet containing 2.5% protein from school children biscuits.

- Basal diet containing 5% protein from school children biscuits.

The second main group was hyperglycemic (+):

Rats were injected with 150 mg/kg body weight of recrystallized alloxan to induce hyperglycemia. Rats were kept fasted for 12 hours but allowed free access to water before the injection of alloxan. After alloxan injection, water contains glucose was introduced to rats for drinking to prevent hypoglycemia according to Buko et al, (1996). All diabetic rats received basal diet prepared according to Reeves et al.,(1993) for 48 hours after injection They were divided into six subgroups fed on the same previous diet scheme.

At the end of experimental period (5 weeks), the rats were fasted overnight then sacrificed. Blood samples were collected from the orbital plexus by means of fine capillary glass tubes. Samples were centrifuged for 10 minutes at 3000 rpm to separate serum from the blood cells. Serum was carefully aspirated into dry clean Wassermann tubes by using a Pasteur pipette and kept frozen till analysis at -20˚C.

Percent Body weight gain (% BWG) and food efficiency ratio (FER) were calculated according to the following formulas:

% BWG = [(Final weight – Initial weight) / Initial weight] × 100.

FER =Gain weight (gm) / Food consumed (gm).

Serum analysis:

- Blood glucose levels were assessed weekly throughout the study. Enzymatic determination of glucose was carried out colorimetrically according to the method of Trinder, (1969).
- Serum urea nitrogen, uric acid and creatinine were determined colorimetrically according to the method of Tabacco et al., (1979), Fossatti and Prencipe, (1980) and Bartels and Bohmer, (1971) respectively.
- Liver function enzymes were determined using the method of Wilkinson (1976) for aspartate amine transferase (AST) and the method of Bergmeyer and Horder (1986) for alanine amine transferase (ALT). Serum protein was determined colorimetetrically according to the method described by Dawson et al., (1986).

- Serum total iron and total iron binding capacity were determined calorimetrically according to the method of Burtis and Edward, (1994).

Statistical analysis:

The statistical analysis was performed according to the methods described by Snedecor and Cochran, (1967) using the computer SPSS software package, version 11, Chicago, USA. Results were expressed as mean ± standard deviation (SD). Significant differences between groups were tested with one-way analysis of variance (ANOVA) followed by the least significant differences test (LSD) as a post hoc test. Two-tailed student t test was used to examine the differences between blood glucose level at the beginning and at the end of the study among various groups.

RESULTS AND DISCUSSION

Chemical composition of wheat flour and soybean flour were presented in table (1). Soybean flour contained 53.43% protein, 6.28% fat, 3.98% fiber, 6.15% ash and 30.16% carbohydrate. Wheat flour composed of 11.19%, 1.40%, 0.69% 0.64 and 86.08%, protein, fat, fiber, ash and carbohydrate, respectively. Soybean flour had the highest percent of protein, fat and ash. While, wheat flour had lowest percent of all determined parameters except for carbohydrate compared with soybean flour.

The obtained results were in agreement with the finding reported by Abd EL-Hady et al., (1997) for soybean flour and with Abo-Zeid (1998) for wheat flour.

Fortification of biscuits either with soybean flour at levels of 10, 15 and 20% caused general increment in protein, fat, fiber, ash and carbohydrate. While, it caused a reduction in carbohydrates content when compared with control biscuits (100% wheat flour).

These results are in agreement with those reported by Dhingra and Jood (2001), who found a significant increase in defatted soybean flours supplemented breads at the 10% level. In addition, Hammad, (2000) found that the addition of defatted soybean flour at levels of 5, 10, 15 and 20% resulted in a remarked increase in crude protein and ash contents of biscuits. Unfortunately, school children biscuits have the lowest value of protein and the highest value in carbohydrate. It contained 10.6±0.36, 13.28±0.16, 0.40±0.03, 0.97±0.15 and 74.75% protein, fat, fiber, ash and carbohydrate, respectively.

Table (1): Chemical composition (g/100g) of wheat flour, soybean flour, unfortified biscuit, fortified biscuit with soybean flour and school children biscuit.

Components / samples	protein	fat	fiber	Ash	Total Carbohydrate
Wheat flour	11.19±0.14 [A]	1.40±0.04 [A]	0.69±0.07 [A]	0.64±0.07 [A]	86.08 [A]
Soybean flour	53.43±0.86 [C]	6.43±0.21 [C]	3.98±0.07 [B]	6.15±0.06 [B]	30.16 [B]
Unfortified biscuits	13.80±0.29 [b]	12.80±1.77 [a]	0.52±0.06 [a]	1.90±0.06 [b]	70.98 [c]
Fortified biscuits with soybean flour					
10%	16.3±0.01 [c]	14.98±0.07 [c]	1.12±0.04 [b]	2.45±0.04 [cd]	65.15 [b]
15%	18.3±0.03 [d]	15.68±0.03 [d]	1.31±0.03 [bc]	2.66±0.04 [c]	62.05 [a]
20%	19.5±1.38 [d]	16.55±0.18 [f]	1.54±0.34 [c]	2.89±0.032 [d]	59.52 [a]
School children biscuits	10.6±0.36 [a]	13.28±0.16 [b]	0.40±0.03 [a]	0.97±0.15 [d]	74.75 [d]

Results are expressed as mean±SD

Values sharing the same superscript capital letters in column indicate non significant differences between wheat flour and soy bean flour.

Values sharing the same superscript small letters in column indicate non significant differences between various kinds of biscuits

Table (2) showed minerals content of wheat flour, soybean flour fortified biscuits with soybean and school children biscuits. Minerals content of wheat flour were 126.5, 3.6, 0.6, 1.4, 0.9, 29.8 and 123.7 mg/100g for Mg, Na, Zn, Mn, Fe, Ca and K respectively. While, soybean flour contained 130.01, 49.37, 0.40, 5.93, 23.97, 187.11 and 1641 mg/100g for Mg, Na, Zn, Mn, Fe, Ca and K respectively.

In this concept Abo-Zeid, (1998) studied the minerals content (mg/100g) of wheat flour 72% and reported the following values 26.7 for Ca, 122.3 for K, 83.1 for P, 3.4 for Na, 127.8 for Mg, 1.5 for Mn and 0.3 for Zn. In addition, Hussein and El-Akel, (1993) mentioned that mineral content of defatted soybean flour were Fe 25.97, Zn 0.38, Ca 188.32, K 1646.53, Na 50.42, Mg 127.97 and Mn 5.04 (mg/100g).

Faheid and Hegazi, (1991) reported that minerals content was higher in legume flours than in wheat flour, hence, cookies supplemented with legumes flour were favorable than unsupplemented ones probably due to the presence of higher amounts of important minerals they contained.

From the same table it could be noticed that the mineral content of unfortified biscuits and school children biscuits were (32.38,89.15,4.37,0.92,1.21,31.49 and 77.26 mg/100g and

(31.23,70.26,3.82,0.47,4.01,25.06 and 69.12 mg/100g) for Mg, Na, Zn, Mn, Fe, Ca and K, respectively. When soybean flour were used in biscuits fortification at levels of 10, 15 and 20%, gradual increase in most minerals, compared to unfortified or school children biscuits was observed.

Table (2): Minerals content (mg/100 g) of wheat flour, unfortified biscuit, fortified biscuit with soybean flour and school children biscuit.

Components / samples	Mg	Na	Zn	Mn	Fe	Ca	K
Wheat flour	126.5± 130^{A}.	3.6±0.15^{A}	0.60±0.06^{A}	1.40±0.14^{A}	0.90±0.03^{A}	29.8±0.08^{A}	123.70±1.66^{A}
Soybean flour	130.01±0.32^{A}	49.37±0.39^{B}	0.40±0.06^{A}	5.93±0.09^{C}	23.97±0.22^{B}	187.11±0.12^{B}	1641.0±0.11^{B}
Unfortified Biscuits	32.38±0.74^{b}	89.15±1.02^{b}	4.37±0.94^{ab}	0.92±0.07^{b}	1.21±0.10^{a}	31.49±0.60^{b}	77.26± 1.44^{a}
Fortified biscuits with soybean flour							
10%	37.12±0.11^{c}	109.12±0.82^{c}	4.80±0.06^{bc}	2.34±0.04^{c}	2.42±0.03^{b}	71.54±0.17^{c}	519.74±28.12^{c}
15%	50.81±0.07^{d}	121.30±0.14^{d}	4.99±0.10^{c}	2.97±0.04^{d}	2.86±0.07^{c}	82.45±0.05^{d}	604.04±3.82^{b}
20%	62.12±0.14^{e}	130.11±2.61^{e}	5.10±0.15^{c}	3.82±0.06^{e}	3.35±0.03^{d}	100.10±1.59^{e}	787.48±61.17^{d}
School children biscuits	31.23±0.04^{a}	70.26±0.77^{a}	3.82 ±0.03^{a}	0.47±0.02^{a}	4.01±0.04^{e}	25.06±0.06^{a}	69.12±0.09^{a}

Results are expressed as mean±SD

Values sharing the same superscript capital letters in column indicate non significant differences between wheat flour and soybean flour.

Values sharing the same superscript small letters in column indicate non significant differences between various kinds of biscuit.

These results in agreement with El-Shatanovi, (1983) who found that supplementation with 10% soy flour increased minerals content of biscuits, which had an effect on increasing the nutritive value of the product. In addition, Hammad, (2000) revealed that the addition of 5 and 10% defatted soybean flour increased the minerals content such as iron, manganese, zinc, copper, sodium, calcium, potassium and phosphorus.

Table (3) showed the sensory evaluation of fortified biscuits with soybean flour. The control sample of biscuits recorded the following scores: 4.64±0.81, 4.40±0.65, 4.40±0.65, 4.60±0.91 and 8.13±1.54 in color, odor, taste, texture and general acceptability respectively. Results indicated that non significant changes were observed between all characteristics of the control sample and the biscuits fortified with 10% soybean flour. On the other hand, fortified biscuitss with 15 and 20% soybean flour resulted in significant decrease in texture and general acceptability scores as compared to control (non fortified biscuits). Replacement of wheat flour with 20% soybean flour recorded lower scores of all characteristics than that of other samples, except in color

Table (3): Sensory evaluation of fortified biscuits with soybean flour.

Samples / Characteristics	Control	Soybean flour		
		10%	15%	20%
Color	4.64±0.81 [a]	4.40±1.00 [a]	4.52±0.71 [a]	4.24±0.93 [a]
Odor	4.40±0.65 [b]	4.52±0.77 [b]	4.64±0.49 [b]	3.92±1.15 [a]
Taste	4.40±0.65 [b]	4.48±0.92 [b]	4.16±0.62 [b]	3.00±1.23 [a]
Texture	4.60±0.91 [c]	4.64±0.70 [c]	4.00±0.76 [b]	2.96±1.15 [a]
General acceptability	8.13±1.54 [c]	7.88±1.64 [c]	6.68±1.79 [b]	4.52±2.40 [a]

Results are expressed as mean±SD
Values sharing the same superscript letters in the same raw are not statistically significant.

The results of feed intake (FI), body weight gain% (BWG %) and feed efficiency ratio (FER) are summarized in table (4). Feed intake values of all experimental groups ranged from 11.75 ± 1.88 gm/day for each rat in the healthy group fed on basal diet containing 2.5% protein from fortified biscuits with 10% soybean flour to 13.87± 3.25 gm/day for each rat in the healthy group fed on basal diet containing 2.5% protein from unfortified biscuits.

No significant differences were observed in feed intake levels among all normal groups fed on basal diets containing unfortified biscuits, school children biscuits and fortified biscuits with soybean flour. Data showed that, BWG% of diabetic groups fed on basal diet containing 2.5 or 5% protein from unfortified biscuits was significantly decreased compared to healthy groups fed on the same diets. These results are in the same line with those obtained by Louz, (1997) who found a significant decrease in daily gain in body weight in case of alloxan-induced diabetic rats fed on basal diet.

Furthermore, BWG% of all healthy groups of rats fed on basal diet containing 2.5 or 5% protein from unfortified biscuits, fortified biscuits with 10% soybean flour and school children biscuits did not significantly differ. Regarding diabetic groups which fed on basal diet containing 2.5 or 5% protein from unfortified biscuits, fortified biscuits with 10% soybean flour and school children biscuits their FER were significantly lower than other healthy rat groups fed on the same diet. Bell and Hye, (1992) found that, there is a decrease in body weight, food intake, food efficiency ratio and digestive efficiency in the injected rats with alloxan compared to non diabetic rats.

Table (4): Effect of unfortified biscuits, fortified biscuits with soybean flour (SBF) and school children biscuits (SCB) on some biological parameters of normal and diabetic rats.

Groups \ Parameters	Feed intake (gm/day)	BWG (%)	FER
2.5% protein from unfortified biscuit (-)	13.87±3.25^{a}	32.87±13.18^{b}	3.61±0.98^{bc}
2.5% protein from unfortified biscuit (+)	12.16±2.24^{b}	2.17±1.64^{a}	0.27±0.18^{a}
5% protein from unfortified biscuits (-)	12.82±1.30^{a}	27.69±7.46^{b}	3.40±0.60^{b}
5% protein from unfortified biscuits (+)	12.04±1.19^{b}	1.35±0.64^{a}	0.18±0.13^{a}
2.5% protein from fortified biscuits with 10% SBF (-)	11.75±1.88^{a}	41.28±11.92^{b}	5.68±2.70^{d}
2.5% protein from fortified biscuits with 10% SBF (+)	12.13±2.45^{b}	1.48±1.02^{a}	0.16±0.08^{a}
5% protein from fortified biscuits with 10% SBF (-)	12.76±2.14^{a}	37.99±21.55^{b}	4.45±1.59^{c}
5% protein from fortified biscuits with 10% SBF (+)	13.08±4.10^{b}	1.45±1.45^{a}	0.11±0.13^{a}
2.5% protein from SCB (-)	12.84±3.10^{a}	32.63±3.04^{b}	4.02±0.51^{bc}
2.5% protein from SCB (+)	12.92±1.90^{b}	2.48±1.45^{a}	0.30±0.31^{a}
5% protein from SCB (-)	13.56±2.60^{a}	30.11±7.46^{b}	3.43±0.71^{b}
5% protein from SCB (+)	13.58±2.10^{b}	1.24±2.46^{a}	0.15±0.21^{a}

Results are expressed as mean±SD BWG: Body Weight Gain FER: Feed Efficiency ratio
Values sharing the same superscript letters in the same column are not statistically significant

Results showed that FER between all healthy groups of rats fed on basal diet containing 2.5 or 5% protein from unfortified biscuits and school children biscuits were not significantly differed. While rats fed on 2.5% protein from fortified biscuits with 10% soybean flour showed significant higher FER than other groups. The best results in FER between all healthy groups were observed in the group fed on basal diet containing 2.5% protein from fortified biscuits with 10% soybean flour.

All normal groups fed on basal diets containing unfortified biscuits, school children biscuits and fortified biscuits with soybean flour showed non-significant differences in serum glucose levels (table 5). This result in same line with Louz, (1997) who found that the healthy group of rats fed on different levels of soybean hull did not show any valuable changes in serum glucose compared with control negative.

Table (5): Effect of unfortified biscuits, fortified biscuits with soybean flour (SBF) and school children biscuits (SCB) on serum glucose levels of normal and diabetic rats.

Groups / parameters	Initial level (mg/dl)	Final level (mg/dl)
2.5% protein from unfortified biscuits(-)	88.88±3.57 [a]	90.57±2.74 [a]
2.5% protein from unfortified biscuits(+)	230.42±39.11 [b]	250.64±2.94 [b]
5% protein from unfortified biscuits (-)	90.68±3.36 [a]	91.53±2.66 [a]
5% protein from unfortified biscuits (+)	222.21±21.85 [b]	246.41±5.37 [b]
2.5% protein from fortified biscuits with 10% SBF (-)	88.89±4.51 [a]	90.08±2.46 [a]
2.5% protein from fortified biscuits with 10% SBF (+)	229.12±42.13 [b]	134.93±6.25 [d]
5% protein from fortified biscuits with 10% SBF (-)	89.35±2.49 [a]	92.44±5.75 [a]
5% protein from fortified biscuits with 10% SBF (+)	232.89±.29.02 [b]	118.74±27.66 [c]
2.5% protein from SCB (-)	89.94±1.57 [a]	91.78±4.15 [a]
2.5% protein from SCB (+)	221.12±13.25 [b]	231.53±16.20 [b]
5% protein from SCB (-)	88.29±4.96 [a]	92.95±1.66 [a]
5% protein from SCB (+)	230.02±16.01 [b]	230.37±22.08 [b]

Results are expressed as mean±SD
Values sharing the same superscript letters in the same column are not statistically significant

The injection of some groups of rats (positive control) with alloxan caused; as expected; significant increase in serum glucose levels when compared with negative control groups. No significant differences were observed between positive groups concerning serum glucose levels at the initial level. The same trend was noticed also between negative control groups.

Final serum glucose levels of diabetic rats fed on basal diet containing 2.5 or 5% protein from fortified biscuits with 10% soybean flour (134.93 ± 6.25 and 118.74 ± 27.66 mg/dl) respectively, were significantly lower than their initial levels (229.12 ± 42.13 and 232.89 ± 29.12 mg/dl) respectively.

Nutritional intervention studies performed in humans suggest that the ingestion of soy protein associated with isoflavones and flaxseed rich in legnans improves glucose control and insulin resistance (Bhathena and Valesquez, 2002; Rochofort and panozzo, 2007).

No significant differences were found between the initial and final serum glucose levels of normal or diabetic rats fed on basal diet containing 2.5 or 5% protein from school children biscuits. Moreover, final serum glucose levels of the later groups were significantly higher than that of other diabetic groups fed on basal diet containing 2.5 or 5% protein from 10% soybean flour.

Results in table (6) demonstrated the effects of unfortified biscuits, school children biscuits and fortified biscuits with soybean flour on aspartate amino transferase (AST), alanine amino transferase (ALT) and total protein level of healthy and diabetic rats. There were non-significant differences in serum AST between all healthy groups fed on basal diets containing unfortified biscuits, school children biscuits and fortified biscuits with 10% soybean flour. Similar results were observed in ALT and total protein levels.

The obtained results are in agreement with those reported by Louz, (1997) who found that no significant differences were observed in GOT and GPT between rats fed on balady bread and the other groups fed on bread containing 5 and 10% soybean hull.

It is worth to be mentioned that, the control positive groups which were fed on basal diet containing 2.5% and 5% protein from unfortified biscuits, revealed a significant increase in the levels of AST and ALT enzymes compared with those of the negative control groups, fed on the same diets. Diabetic rats fed on basal diets containing 2.5% as well as 5% protein from school children biscuits showed significant increase in the level of AST and ALT enzymes as compared with normal groups fed on the same content of diets. In this respect, Anthony et al., (2004) found that markers of liver injury, including AST and ALT were significantly associated with risk of incidence of type2 diabetes.

Feeding diabetic rats on basal diet containing fortified biscuits with 10% soybean flour provided the diets with 2.5% protein led to significant increase in AST and ALT enzymes compared to healthy group fed on the same diet. On the other hand, diabetic groups fed on basal diets containing fortified biscuits with 10% soybean flour which provided the diet with 5% protein showed non-significant changes in AST and ALT enzymes as compared to normal groups fed on the same diets.

Table (6): Effect of unfortified biscuits, fortified biscuits with soybean flour and school children biscuits on liver functions of normal and diabetic rats.

Groups \ Parameters	AST (U/L)	ALT (U/L)	Total protein (Mg/dl)
2.5% protein from unfortified biscuits (-).	30.55±5.96 ^{a}	19.74±2.95 ^{a}	6.01±0.1 ^{a}
2.5% protein from unfortified biscuits (+).	47.1±10.73 ^{b}	36.01±0.08 ^{c}	5.22±0.08 ^{b}
5% protein from unfortified biscuits (-).	29.37±7.97 ^{a}	23.44±2.26 ^{ab}	6.17±0.73 ^{a}
5% protein from unfortified biscuits (+).	45.04±3.84 ^{b}	37.27±2.56 ^{c}	5.23±0.10 ^{b}
5% protein from fortified biscuits with 20% CP (+).	27.35±4.19 ^{a}	26.31±2.17 ^{b}	6.00±0.02 ^{a}
2.5% protein from fortified biscuits with 10% SBF(-).	29.87±3.64 ^{a}	22.47±1.23 ^{ab}	6.06±0.21 ^{a}
2.5% protein from fortified biscuits with 10% SBF (+).	43.61±8.81 ^{b}	34.59±3.77 ^{c}	5.23±0.05 ^{b}
5% protein from fortified biscuits with 10% SBF (-).	30.68±3.44 ^{a}	23.61±2.26 ^{bd}	5.70±0.27 ^{ab}
5% protein from fortified biscuits with 10% SBF (+).	34.72±3.82 ^{a}	26.73±4.75 ^{b}	5.16±0.10 ^{b}
2.5% protein from SCB(-).	27.01±5.24 ^{a}	21.27±2.11 ^{ad}	5.92±0.06 ^{a}
2.5% protein from SCB (+).	47.19±7.37 ^{b}	35.37±1.32 ^{c}	5.20±0.01 ^{b}
5% protein from SCB (-).	29.38±3.54 ^{a}	21.25±3.21 ^{ad}	5.89±0.04 ^{a}
5% protein from SCB (+).	43.58±4.88 ^{b}	35.81±0.49 ^{c}	5.30±0.02 ^{b}

Results are expressed as mean±SD
AST:aspartate amino transferase. ALT: alanine amino transferase
Values sharing the same superscript letters in the same column are not statistically significant.

Xu et al.,(2001) suggested that soy flour has the function of decreasing blood sugar, blood lipid and improving their metabolism, and of protecting liver and kidney of diabetes mellitus mice. These results were confirmed withthose of Louz, (1997) who found that the addition of 5 and 10% soybean hull to balady bread decreased significantly the GOT and GPT levels in hyperglycemic rats.

Treating diabetic groups with basal diet containing 5% protein from fortified biscuits with 10% soybean flour led to significant decrease in AST and ALT enzymes, as compared to diabetic groups fed on basal diet containing unfortified biscuits and school children biscuits which provided the diets with the same amount of protein.

Concerning total protein, results revealed that, all normal groups fed on basal diets containing unfortified biscuits, school children biscuits and fortified biscuits with soybean flour showed non-significant differences in total protein. Diabetic rats fed on basal diets containing unfortified biscuits provided the diets with 2.5 or 5% protein have a significant decrease in the mean values of total protein when compared with those of the control negative groups fed on the same content of diets. The obtained results are in the same line with those obtained by Allam, (2001) observed that total protein was reduced from 5.87 mg/dL in the normal control to 5.33mg/dL (90.80% relative to normal control) for the hyperglycemic rats.

Furthermore, significant decrease in the mean value of total protein in diabetic groups fed on basal diets containing school children biscuits which provided the diet with 2.5 or 5% protein, compared with normal groups fed on the same diets.

Results in table (7) revealed that, all normal groups fed on basal diets containing unfortified biscuits, school children biscuits and fortified biscuits with soybean flour showed non-significant differences in uric acid, createnin and urea nitrogen. Positive control groups fed on basal diets containing 2.5% or 5% protein from unfortified biscuits, showed significant increase in the mean value of uric acid, createnin and urea nitrogen when compared with those of the negative control groups fed on the same diets.

There were a significant increase in the mean value of uric acid, createnin and urea nitrogen in diabetic groups fed on basal diets containing 2.5% or 5% protein from school children biscuits, compared with normal groups fed on the same diets.

Rockel, (2001) reported that inadequate adjustment of blood sugar and blood pressure levels in diabetic patients tended to cause abnormal protein metabolism, an impairment of kidney functions and eventually diabetic glomeruloscloerosis. This is caused by hyperfusion and hyperfiltration of glomruli which could consequently lead to microalbuminuria and acute renal failure.

Feeding diabetic groups on basal diet containing 2.5 or 5% protein from fortified biscuits with 10% soybean flour resulted in significant increase in uric acid and createnin values in comparison with healthy groups fed on the same diet. Diabetic rats fed diet containing 2.5 or 5% protein from fortified biscuits with 10% soybean flour showed significant decrease in createnine levels compared to the diabetic groups fed on basal diet containing the same amount of protein from unfortified biscuit and school children biscuit.

Table (7): Effect of unfortified biscuits, fortified biscuits with soybean flour (SBF) and school children biscuits (SCB) on kidney functions of normal and diabetic rats.

Groups \ Parameters	Uric acid (mg/dl)	Createnin (mg/dl)	Urea nitrogen (mg/dl)
2.5% protein from unfortified biscuits (-).	1.68±0.10 [a]	0.51±0.06 [a]	56.70±1.73 [ab]
2.5% protein from unfortified biscuits (+).	2.46±0.23 [b]	1.64±0.05 [c]	67.54±2.28 [c]
5% protein from unfortified biscuits (-).	1.61±0.02 [a]	0.54±0.03 [a]	57.56±1.06 [ab]
5% protein from unfortified biscuits (+).	2.42±0.11 [b]	1.65±0.04 [c]	65.61±4.04 [c]
2.5% protein from fortified biscuits with 10% SBF (-).	1.48±0.04 [a]	0.58±0.03 [a]	55.10±6.56 [a]
2.5% protein from fortified biscuits with 10% SBF (+).	2.47±0.36 [b]	0.69±0.02 [b]	66.47±1.54 [c]
5% protein from fortified biscuits with 10% SBF (-).	1.49±0.15 [a]	0.55±0.05 [a]	58.92±1.51 [ab]
5% protein from fortified biscuits with 10% SBF (+).	2.40±0.31 [b]	0.64±0.05 [b]	62.56±3.25 [bc]
2.5% protein from SCB (-).	1.63±0.03 [a]	0.53±0.02 [a]	58.39±2.34 [ab]
2.5% protein from SCB (+).	2.52±0.26 [b]	1.68±0.04 [c]	68.84±3.40 [c]
5% protein from SCB (-).	1.63±0.05 [a]	0.53±0.02 [a]	58.68±1.46 [ab]
5% protein from SCB (+).	2.57±0.24 [b]	1.71±0.02 [c]	66.47±3.13 [c]

Results are expressed as mean±SD
Values sharing the same superscript letters in the same column are not statistically significant

These results are in good agreement with Kontessis et al., (1995) who found that soy protein consumption reduced urinary protein excretion in type 1 diabetic patients with diabetic nephropathy. In addtion, Azadbakht et al., (2003) found that, soy protein inclusion in the diet fed diabetic patients with nephropathy can modify the risk factors of heart disease and improve kidney function in these patients.

From table (8), it could be noticed that, the control negative groups that fed on basal diets containing 2.5 or 5% protein from unfortified biscuits, revealed a significant increase in the mean values of total iron compared with those of the positive control groups fed on the same diets.

Table (8): Effect of unfortified biscuits, fortified biscuits with soybean flour (SBF) and school children biscuits (SCB) on total iron (TI) and total iron binding capacity (TIBC) levels of normal and diabetic rats.

Groups \ Parameters	TI (µg/dl)	TIBC (µg/dl)
2.5% protein from unfortified biscuits(-).	114.60 ± 6.693 [c]	375.3 ± 8.715 [f]
2.5% protein from unfortified biscuits (+).	96.00 ± 3.807 [b]	308.2 ± 5.805 [b]
5% protein from unfortified biscuits (-).	111.60 ± 4.774 [c]	345.6 ± 3.493 [d]
5% protein from unfortified biscuits (+).	81.420 ± 2.175 [a]	295.4 ± 5.549 [a]
2.5% protein from fortified biscuits with 10% SBF (-).	129.80 ± 3.962 [d]	369.4 ± 5.594 [f]
2.5% protein from fortified biscuits with 10% SBF (+).	109.00 ±1.414 [c]	324.8 ± 3.271 [c]
5% protein from fortified biscuits with 10% SBF (-).	159.00 ± 5.656 [f]	413.4 ± 10.38 [h]
5% protein from fortified biscuits with 10% SBF (+).	128.40 ± 1.673 [d]	332.3 ± 6.519 [c]
2.5% protein from SCB(-).	160.60 ± 6.426 [f]	403.8 ± 11.05 [g]
2.5% protein from SCB (+).	148.20 ± 2.774 [e]	386.4 ± 7.232 [e]
5% protein from SCB (-).	171.00 ± 6.855 [f]	419.2 ± 4.549 [h]
5% protein from SCB (+).	146.40 ± 5.177 [e]	396.0 ± 8.944 [g]

Results are expressed as mean±SD
TI: Total iron TIBC: Total Iron Binding Capacity
Values sharing the same superscript letters in the same column are not statistically significant

Significant increase in the mean value of total iron in normal groups fed on basal diets containing 2.5 or 5% protein from school children biscuits was observed when compared with diabetic groups fed on the same content of diet. Moreover, there is a significant increase in the value of total iron in normal groups fed on basal diet containing 2.5% or 5% protein from fortified biscuits with 10% soybean flour when compared with diabetic groups fed on the same content of diet

There were non significant differences between healthy groups fed on basal diet containing 2.5 or 5% protein from unfortified biscuits and between diabetic groups fed on basal diet containing 2.5% protein from fortified biscuits with 10% soybean flour. Feeding healthy groups on basal diet containing 2.5 or 5% protein from fortified biscuits with 10% soybean flour showed non significant changes in total iron, compared to diabetic group fed on basal diet containing 2.5% from school children biscuits. On the other hand, feeding healthy rats on basal diet containing 2.5% protein from fortified biscuits with 10% soybean flour showed non significant differences, compared to diabetic groups fed 5% protein from fortified biscuits with 10% soybean flour. The best result of total iron was observed in healthy group fed on 5% protein from school children biscuits.

Results of total iron binding capacity (TIBC) indicated that feeding normal groups on basal diet containing 2.5 or 5% protein from unfortified biscuits, revealed a significant increase in the mean values of total iron when compared with those of the control positive groups fed on the same diets. There was significant increase in the mean value of total iron binding capacity in healthy groups fed on school children biscuits provided the diet with 2.5 or 5% protein when compared with the diabetic groups fed on the same content of diet

There was significant increase in the mean value of TIBC in normal groups fed on basal diet containing 2.5 or 5% protein from fortified biscuits with 10% soybean flour when compared with diabetic groups fed on the same diets. Non significant differences were observed in the mean value of TIBC between healthy groups fed on basal diet containing 2.5% protein from unfortified biscuits, fortified biscuits with 10% soybean flour and the healthy group fed basal diet containing 5% protein from fortified biscuits with 10% soybean flour.

Anuradha and Sangeetha, (2001), reported that soy malt product (biscuits) significantly improved the iron profile (hemoglobin, serum ferritin and iron, and total iron binding capacity) of experimental group subjects.

From the obtained results, both normal and diabetic children can nutritionally benefit from replacement of some wheat flour used in making school biscuits with soybean flour because it is suitable for them and improves their nutritional status especially iron status. It is recommended to use soy bean flour in diabetic foods and diets through planning and implementing nutrition education programs for diabetic patients in order to explain the important role of it in controlling diabetes. Combination of wheat flour with another protein sources like soybean can offers economical improvement in protein quality of wheat flour biscuits and increase its protein content. Finally, further studies are needed to investigate the effect of soybean flour fortification on other biological and biochemical parameters.

REFERENCES

Abd El Hady, E. A.; Mostafa, G. A. and El-Samahy, S.K. (1997): Production of rice extrudates with high nutritional value. Egypt. J. Appl. Sci., 12 (12): 172-186.

Abd El Salam, S. M., (2000): Effect of the addition of propolis extract as natural antioxidant on the keeping quality of biscuit during storage. Egypt. J. Agric. Res., 78(4): 1659-1671.

Abo-Zeid, F. K., (1998): Physico, chemical and biochemical studies on muffins fortification with protein and some minerals. Ph. D. Thesis, Food Sci. and Technol. Dept., Fac. of Agric., Cairo Univ.

Allam, S. O. A. (2001): Biochemical studies on chickpea (cicer arietinum L.) utilized in African food products. Ph. D. Thesis, Natural Rrecorces Dept., Institute of African Research and Studies, Cairo Univ.

Anthony, J. G.; Hanley, K. W.; Andereas, F.; Lynne, E.W.; Ralph, B. D.; Judy, K. B. and Steven, M. H. (2004): Elevations in markers of liver injury and risk of type 2 diabetes. Diabetes, 53: 2623-2632.

Anuradha, V. and Sangeetha, K. (2001): Development and impact of soymalt product in improving the iron status of anaemic adolescent girls (16-18 years). Indian J. Nutr. and Diet., 38(5): 141-145.

A. O. A. C. (1990): Official methods of analysis of the association of official analytical chemists. 12^{th} ed .Washington, D. C

Azadbakht, L.; Shakerhosseini, R.; Atabact, S.; Jamshdian, M.; Mhrabi, Y. and Esmaill-Zadeh, A. (2003): Beneficiary effect of dietary soy protein on lowering plasma levels of lipid and improving kidney function in type II diabetes with nephropathy.Europ. J. Clin. Nutr., 57(10): 1292-1294.

Azadbakht L, Atabak S, and Esmaillzadeh A. (2008): Soy protein intake, cardiorenal indices, and C-reactive protein in type 2 diabetes with nephropathy: a longitudinal randomized clinical trial.Diabetes Care,31(4):648-654.

Bartels, H. and Bohmer, M. (1971): Cretinine standard and measurement of serum creatinine with picric acid.Clin. Chem., Acta, 32: 81-85.

Bell, R. H. and Hye, R. J. (1992): Animal models of diabetes mellitus, physiology and pathlology. J. Surg. Res.; 35: 433-440.

Bergmeyer, H. U. and Horder, M. (1986): A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. J. Clin. Chem., and Clin. Biochem., 24: 481-486.

Bhathena, S. J. and Valesquez, M. T. (2002): Beneficial role of dietary phytoestrogens in obesity and diabetes. Am. J. Clin. Nutr., 76(6): 1191-1201.

Buko, V.; Lukivskaya, O.; Tarasov, Y.; Zavodnik, L.; Borodassky, A.; Goren, S. B.; Janz, B. and Gundermann, K. J. (1996): Hepatic and pancreatic effect of polyenoyl phatidyl cholin in rats with alloxan-induce diabetes. Cell Biochem. Funct.; 14(2): 131-137

Burtis, C. A and Edward A. (1994): Clinical chemistry.2nd Ed., W.B.Saunders company, p2195

Dawson, R. C.; Elliot, W. H. And Jones, K. (1986): Data for biochemical research.3rd ed. Clarendon Press. Oxford

Dhingra, S and Jood, S. (2001): Organoleptic and nutritional evaluation of wheat breads supplemented with soybean and barley flour. Food Chem., 77(4):479-488.

El-Shatanovi, G. A. T. (1983): Studies on soybean products other than oil. M.Sc. Thesis, Food Sci. and Technol. Dept., Fac. of Agric., Ain-Shams Univ., Egypt.

Faheid, M. M. and Hegazi, A. (1991): Effect of adding some legume flours on the nutritive value of cookies. Egypt J. Food Sci., 19 (12): 147-159.

Fossati, and Prencipe, M. (1980): Standard methods of clinical chemistry. Edited seligson, D., Chin: Chem., 28: 227-231.

Hammad, A. A. (2000): Chemical and technological studies on soy bean proteins.Ph. D. Thesis, Food Sci. and Technol. Dept., Fac. of Agric., Moshtohor, Zagazig Univ., Banha Branch.

Hussein, W. A. and El-Akel, A. T. (1993): Production of high protein quality snacks: Chemical composition and sensory evaluation of snacks. Egypt. J. Food Sci., 21: 103-111.

Kontessis, P. A.; Bossinakou, I.; Sarika, L.; Iliopoulou, E.; Papantoniou, A.; Trevisan, R.; Roussi, D.; Stipsanelli, K.; Grigorakis, S.; and Souvatzoglou, A. (1995): Renal, metabolic and hormonal responses to proteins of different origin in normotensive, nonproteinuric type I diabetic patients. Diabetes Care, 18: 1233-1240.

Louz, S. L. (1997): Biochemical study on soybean products.M. Sc. Thesis, Biochem. Dept., Fac. of Agric., Cairo Univ. Egypt.

Lu M., Wang R., Song X., Chibbar R., Wang X., Lingyun WU and Meng Q.H. (2008): Dietary soy isoflavones increase insulin secretion and prevent the development of diabetic cataracts in streptozotocin-induced diabetic rats. Nutrition Research 28: 464–471.

McCue, P.; Kwon, Y. l.; Shetty, K. (2005): Anti-diabetic and anti-hypertensive potential of sprouted and solid-state bioprocessed soybean Asia Pacific J. Clin. Nutr., 14(2): 145-152.

Murray-Kolb, L. E.; Welch, R.; Theil, E. C. and Beard,J. L.(2003): Women with low iron stores absorp iron from soybeans.

Am. J. Clin. Nutr.; 7(1): 180-184.

Pearson, P. (1970): The chemical analysis of food. 6th ed. J. An. Phurbhili, London. J. P 380: 385, 520: 515.

Penfield, M. and Campbell, A. M. (1990): Experimental food science. 3rd Ed. Academic press Inc. London, P.33-34.

Reeves P., Nielsen F. and Fahmy G. (1993): Purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad Hoc writing committee on the reformulation of the AIN- 76 a rodent diet. J Nut; 123: 1939 – 1951

Rochforts S. and Panozzo J. (2007): Phytochemicals for health, the role of pulses. J.Agric Food Chem., 55 (20):7981-7994

Rockel, A. (2001): Diabetic therapy of diabetic glomerulosclerosis. Vita. Min. Spur.; 16 (3) 144-148.

Snedecor, G. W. and Cochran, W. G. (1967): Statistical Methods (6th ed). Ames, Iowa State University Press

Stephenson, T. J.; Setchell, K. D.; Kendall, C. W.; Jenkins, D. J.; Anderson, J. W. and Fanti, P. (2005): Effect of soy protein-rich diet on renal function in young adults with insulin-dependent diabetes mellitus.Clin-Nephrol. Jul; 64(1): 1-11.

Tabacco, A.; Searcy, R. L. and Reardon, J. E. (1979): Standard methods of clinical chemistry. Edited selig son, D., Chin – Chen., 25: 336

Teixeira, S.R.; Tappenden, K.A.; Carson, L.; Jones, R.; Prabhudesai, M; Marshall, W.P and Erdman, J.W. (2004): Isolated soy protein consumption reduces urinary albumin excretion and improves the serum lipid profile in men with type 2 diabetes mellitus and nephropathy.J. Nutr.; 134(8): 1874-1880.

Trinder, P., (1969): Determination of blood glucose using 4-Aminophenazone as oxygen acceptor. J. Clin. Path.; 22(2): 246.

Villegas R., Gao Y., Yang G., Li H., ElasyT., Zheng W. and Shu X.O.(2008): Legume and soy food intake and the incidence of type 2 diabetes in the Shanghai Women's Health Study Am. J. Clin. Nutr., 87(1): 162-167.

WHO, (2005): Guidelines for diabetes management. World Health Organiziation.The national institute of diabetes and endocrinology.

Wilkinson, J. H., (1976): The principles and practice of diagnostic enzymology. Year Book Medical Publishers

Xu, H.; Tan, S.M. and Li, S.Q. (2001): Effects of soybean fibers on blood sugar, lipid levels and hepatic-nephritic histomorphology in mice with diabetes mellitus. Biomedical-and-Environmental-Sciences.; 4(3): 256-261.

تأثير البسكويت المدعم بدقيق فول الصويا كعامل خافض لمستوى الجلوكوز فى الفئران المصابة بمرض البول السكرى

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تهدف هذه الدراسة إلى اختبار تاثير البسكويت المدعم بدقيق فول الصويا كذلك بسكويت المدارس على بعض المعاملات البيولوجية و الكميائية مثل الطعام المتناول، نسبة الزيادة فى وزن الجسم ، جلوكوز الدم،و كذلك وظائف الكلى و الكبد و حالة الحديد فى فئران التجارب الطبيعية والمصابة بارتفاع في مستوى السكر في الدم.تم تدعيم البسكويت بنسبة 10%، 15% و 20% دقيق فول الصويا وبعد إجراء عملية الخبز تم تقييم هذه الأنواع من المخبوزات تقييما حسيا لإختيار أفضل التركيزات ليتم استخدامها فى التجربة البيولوجية. أجريت التجربة على 60فأر البينو قسمت هذه الفئران الى مجموعتين رئيسيتين (كلا تضم 30 فأر) المجموعة الأولى فئران طبيعية و المجموعه الثانية مصابة بمرض السكر. كلا المجموعتين تم تقسيمهما الى سته مجموعات فرعية تم تغذيتها على غذاء أساسي مضاف إليه 2.5% أو 5% بروتين من البسكويت غير المدعم ، البسكويت المدعم بدقيق فول الصويا10% وكذلك من بسكويت المدارس لمدة خمسة أسابيع. أظهرت النتائج أن المجاميع التي تغذت على غذاء أساسي يحتوي على البسكويت المدعم بدقيق فول الصويا اظهرت تحسن في مستوى السكر ووظائف الكبد والكرياتينين واليوريا نيتروجين و في مستوى الحديد بالدم. ظهرتحسن في مستوى الحديد الكلي بالمجاميع الطبيعية التي تغذت على غذاء أساسي يحتوي على 5% بروتين من بسكويت المدارس ، أما المجاميع الطبيعية التي تغذت على غذاء أساسي يحتوي على 5% بروتين من البسكويت المدعم ب10% دقيق فول الصويا أو من بسكويت المدارس فقد تحسن بها مستوى الحديد الكلي المرتبط. و لهذا يوصى باستعمال دقيق فول الصويا فى الاغذية الخاصة بمرضى السكر و كذلك عمل برامج توعية غذائية لهم لاظهار اهميتة فى التحكم فى مستوى الجلوكوز بالدم لديهم .كذلك يوصى بتدعيم بسكوت المدارس بدقيق فول الصويا لانه غنى غذائيا و يفيد الاطفال المرضى بمرض السكر و الاصحاء على حد السواء.