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EFFECT OF FORTIFIED BREAD WITH DIFFERENT LEVELS OF GERMINATED FENUGREEK SEEDS FLOUR ON feeding DIABETIC RATS

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ABSTRACT

Effect of blending germinated fenugreek seeds flour from 1 to 5% with wheat flour (82%) on the rheological and organoleptic qualities of bread were investigated. The farinograph and extensograph results indicated that water absorption, mixing time, arrival time, dough weakening and extensibility were non-significantly increased with increasing levels of fenugreek flour. On the other hand, stability, resistance to extension and energy show non-significant decrease when compared with wheat flour (control). However, bread prepared from the wheat-fenugreek blends at 1, 3 and 5% levels were found organoleptically acceptable at all levels. Effect of fortified bread with levels of fenugreek on blood glucose levels of diabetic rats was determined at various times during 10 h after feeding. The glucose levels of diabetic rats were significantly decreased with increasing fenugreek level and was decreased after 2 h from feeding. The body weight, liver weight and spleen weight of diabetic rats were significantly decreased, but kidney weight was significantly increasing after 21 days of feeding of diabetics than that of control rats. Experimental bread was improved body parameters with increasing fenugreek levels.

INTRODUCTION

Fenugreek (*Trigonella foenum graecum*) is becoming popular around the world, its extract used to flavor cheese in Switzerland, artificial maple syrup and bitter-run in Germany, roosted seeds as coffee-substitute in Africa, seeds powder mixed with flour as fortification to make flat-bread in Egypt, as an anti-diabetic herb in Israel, whole seeds and dried plant used as insect and pest repellent in grain storage and oil used in perfumery in France (Rajagopalan, 1998).

Balady bread prepared from wheat-fenugreek blends at 5, 10 and 20% levels were found organoleptically acceptable, especially at 5% level of fenugreek flour (Sahar *et al.*, 2006). Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipids and protein (Scheen, 1997).

The fenugreek seeds are rich in dietary fiber, which may be the main reason that can lower blood sugar levels in diabetics (Ribes *et al.*, 1986). Fenugreek can help lower cholesterol and blood sugar levels in persons with moderate atherosclerosis and non-insulin-dependent diabetics (Bordia *et al.*, 1997).

Randomized and uncontrolled studies have confirmed fenugreek helps stabilize blood sugar control in patients with insulin-dependent and non-insulin-dependent diabetes (Sharma *et al.*, 1990; Madar *et al.*, 1988 and Raghuram *et al.*, 1994). It helps lower elevated cholesterol and triglyceride levels in the blood (Sharma *et al.*, 1991), including in those with diabetes

(Sharma *et al.*, 1996). According to several controlled studies. The German Commission E monograph recommends a daily intake of 6 grams (Blumenthal, *et al.*, 1998). The typical range of intake for diabetes or cholesterol lowering is 5-30 grams with each meal or 15-90 grams all at once with one meal. The objective of this investigation was to study the effect of fortified bread with different levels of germinated fenugreek seeds flour on diabetic rats.

MATERIALS AND METHODS

Materials:

Fenugreek seeds and wheat flour (82% extraction) were obtained from local marked at Karol-Sheikh Governorate.

Methods:

Preparation of germinated fenugreek flour:

Fenugreek seeds were first cleaned and soaked in tap water for 12 hour at room temperature. The seeds to water ratio of 1:5 (w/v) was used. The soaked seeds were rinsed in tap water, transferred to moistened cotton layers, and allowed to germinate in the dark place at 25^{o}C for 3 days. During germination, the cotton layers were kept always moist. After germination, the fenugreek seeds were dried at 55-60%. Germinated seeds were ground to a fine powder in an electric grinder and then stored in plastic containers for further use.

Preparation of bread:

Germinated fenugreek flour was blended separately with wheat flour (82%) at different levels 0, 1, 3 and 5% as illustrated in Table (A). The technological procedure that used for preparing bread was carried out in home (Salaam *et al.*, 1995).

Table (A): Formula used in making wheat-fenugreek bread.

Components	Quantity (g)			
	100% W	99% W ± 1%F	97 W ± 3%F	95% W ± 5%F
Wheat flour	100	99	97	95
Fenugreek	0	1	3	5
Salt	3	3	3	3
Yeast	5	5	5	5
Water	100	100	100	100

W= Wheat flour. F= Fenugreek.

Experimental animals:

Male Wistar albino rats (25 in number) with body weight ranging from 210 to 230 g, brought from experimental animals house of Food Technology Research Institute, Agricultural Research Center, Giza, Egypt were used as experimental animals. The rats were distributed into five groups according to similar weights by five animals in each group:

Group 1: Normal rats treated with wheat bread.
Group 2: Diabetic rats treated with wheat bread.
Group 3: Diabetic rats treated with bread contains 1% fenugreek flour.
Group 4: Diabetic rats treated with bread contains 3% fenugreek flour.
Group 5: Diabetic rats treated with bread contains 5% fenugreek flour.

Induction of diabetes:

Diabetes was induced by a single interaperitoneal injection of alloxan monohydrate subcutaneously (15 mg/100 g body weight) freshly prepared in 0.154 M sodium acetate buffer (pH 4.5) after an overnight fast. The normal rats were injected with citrate buffer alone. The rats were given 5% glucose water for 24 hours following alloxan injection to prevent initial drug induced hypoglycemic mortality. After 72 hours of administration of injection, fasting blood glucose levels were determined in these alloxan injected rats in the blood. Rats with blood glucose level above 225 mg/dl were distributed into the four diabetic groups (Sochor *et al.*, 1985).

All the animals were housed facility at a room temperature 25^{o}C and relative humidity 55%. The animals feed, used in experiment was procured from the experimental prepared bread for 21 days. Water was boiled, filtered and the pH was made slightly acidic to prevent microbial group. Normal groups 1 and diabetes group 2 received non-fenugreek bread. While the experimental groups 3, 4 and 5 received, bread mixed with fenugreek at 1, 3 and 5% respectively. Each group received one diet in the day for 30 min and recovered.

Blood glucose was measured at 0, 2, 4, 6, 8 and 10 h after diet recovered. Blood was with drawn from the tail vein every seven days and stored at -20^{o}C until used for glucose determination.

After 21 days, rats of each group were weighed and sacrificed by cervical dislocation. The liver, kidneys and spleen were rapidly excised, washed with normal saline, blotted dry and weighed.

Analytical methods:

The characteristics of dough prepared from wheat flour and fenugreek seeds flour (germinated) mixed at 0, 1, 3 and 5% levels were measured by means of farinograph and extensograph according to the method described in the A.A.C.C. (1995). Organoleptic properties of the bread was evaluated by the method described by Abd El-Rahmin (1992). Samples were served to panel of judges. The panelists were asked to evaluate loaf rising, crust quality, crust color, crumb uniformity and odor (on a 1 to 10 hedonic scale), as well as crumb color and taste (on a 1 to 25 hedonic scale). A score of 1 being (dislike extremely), 10 and 25 being (like extremely).

Blood glucose in the serum was separated by centrifugation and determined by using RA-1000 autoanalyzer.

Statistical analysis

All data were expressed as means $\pm$ SE. Significant differences among the groups were determined by one-way analysis of variance using the SPSS statistical analysis program. Statistical significance was considered at $P<0.05$ Steel and Torrie (1980).

RESULTS AND DISCUSSION

Dough rheological properties:

Rheological properties of dough prepared from wheat and fenugreek mixtures are shown in Table (1). It was observed that water absorption,

mixing time, arrival time, dough weakening and extensibility increased with increasing fenugreek flour concentration ranged from 1 to 5. The increases in such measurements were 0.7, 2.4 and 3.9%; 0.4, 0.6 and 1.0 min; 0.3, 0.8 and 1.5 min; 2, 7 and 10 mm and 1, 3 and 5 mm, respectively. While, stability, resistance to extension and energy decreased with increasing fenugreek flour concentration the decrease were about 0.3, 0.8 and 1.0 min; 1, 4 and 5 BU and 2, 5 and 8 (cm^2), respectively. Dough stability had been attributed to protein poor in sulfhydryl groups, which normally caused a softening or degradation action of the dough (Ortega *et al.*, 1995 and Sahar *et al.*, 2006).

Table (1): Rheological properties of wheat and mixtures flour.

Mixtures	Farinograph					Extensograph			
	Water absorption %	Mixing time (min)	Arrival time (min)	Stability (min)	Dough weakening (mm)	Extensibility (mm)	Resistance to extension (BU)	Proportion (No.)	Energy (cm^2)
100% **W**	55.0	1.0	2.5	6.0	70	125	325	2.6	88
99% W ± 1% F	55.7	1.4	2.8	5.7	72	126	324	2.6	86
97% W ± 3% F	57.4	1.6	3.3	5.2	77	128	321	2.5	83
95% W ± 5% F	58.9	2.0	4.0	5.0	80	130	320	2.5	80

W = Wheat flour (82% ext.)
F = Germinated fenugreek flour
BU = Brabender unit.

Organoleptic qualities:

The organoleptic qualities of wheat-fenugreek bread were scored as shown in Table (2). All the flour mixtures were accepted, but increasing fenugreek flour percent in the flour mixture led to non-significant decrease of all properties. The overall acceptabilities were 94, 90, 88 and 86% for 100% w, 99%w+1% F, 97% w + 3% F and 95% W + 5% F, respectively. Increasing fenugreek percent in the flour mixtures led to slight changes in deterioration of overall acceptability and enhanced the unpleasant odor and bitter taste. It can be concluded that all concentrates can be used in making acceptable bread. These results were in agreement with those of Sahar *et al.* (2006) who reported that 5% from germinated fenugreek flour was highly acceptable.

Effect of experimental feed on plasma glucose level:

The effect of fortified bread with germinated fenugreek seed flour concentrates on plasma glucose levels of experimental animals was determined at various times during 10 h after feeding as shown in Table (3). There was a significant elevation in the blood glucose level by 5 times during experimental period of diabetic rats, when compared to normal rats. Using of experimental bread fortified with fenugreek at levels 1, 3 and 5% caused a significant reduction in the blood glucose of 3.6, 10.4 and 12.1% after 2 h; 14.9, 21.9 and 29.8% after 4 h; 4.7, 15.4 and 25.4% after 6 h; 5.1, 10.3 and 21.4% after 8 h and 5, 12.7 and 20.4% after 10 h, respectively ($P<0.05$). Maximum reduction of 29.8% was observed after 4 h at level of 5% fenugreek. These results were in agreement with Ji *et al.* (2006).

Table (2):Organoleptic properties of prepared bread from wheat germinated fenugreek flour mixtures.

Properties	Score	Mixtures			
		100% W	99% W + 1%F	97 W + 3%F	95% W + 5%F
Loaf rising	10	10±0.22	9±0.23	8±0.31	8±0.15
Crust quality	10	9±0.27	9±0.29	8±0.26	8±0.16
Crust color	10	9±0.31	8±0.18	8±0.24	7±0.21
Crumb uniformity	10	9±0.28	9±0.31	8±0.21	7±0.30
Crumb color	25	24±0.17	24±0.27	23±0.18	22±0.17
Odor	10	9±0.18	8±0.23	8±0.29	7±0.22
Taste	25	24±0.31	23±0.28	22±0.20	21±0.31
Overall acceptability	100	94±0.28	90±0.13	88±0.13	86±0.16

W = Wheat flour (82% ext.)
F = Germinated fenugreek flour
Each value was an average of ten determinations ± SE.

Table (3): Effect of feeding on fortified bread with different germinated fenugreek seeds powder concentrates on plasma glucose levels of diabetic rats (mg dL^{-1}).

Treatment	0 h	2 h	4 h	6 h	8 h	10 h
Normal	93±2.8	131±5.9	125±7.1	116±6.9	110±5.1	112±3.4
Diabetic	465±19.1	470±13.1	483±9.3	512±13.9	543±18.6	560±11.4
99% w ± 1% F	476±14.1	453±9.3	411±6.2[a]	488±7.7	515±13.2	532±14.1
97%w ± 3%F	452.±12.2	421±11.9	377±12.2[a]	433±8.3[a]	487±13.3	489±8.2
95%w±5%F	497±12.3	413±9.2	339±16.3[a]	382±6.3[a]	427±11.3	446±8.7

Values mean concentration of blood glucose ± SE (n=5)
[a] Significantly increased or decreased values compared with 0 h data (P<0.05)

Effect of experimental feed on the changes in general body parameters of diabetic rats:

The changes in body weight and tissue weight in control rats and treated groups are summarized in Table (4).

Table (4):Effect of feeding on fortified bread with fenugreek on general body parameters of diabetic rats after 21 days of induction diabetes.

Body parameters	Normal	Diabetic	99% w + 1% F	97%w + 3%F	95%w+5%F
Body wt. (g)	224±6.1	146±7.3[a]	212±5.6	232±5.2	226±7.7
Liver wt. (g)	5.8±0.9	3.1±0.4[a]	4.9±0.4	5.0±0.6	5.3±0.6
Liver wt./100 g body wt.	2.3±0.2	2.5±0.2	2.3±0.1	2.3±0.1	2.5±0.2
Kidney wt. (g)	1.6±0.2	2.2±0.1	1.7±0.1	1.9±0.1	1.5±0.2
Kidney wt./100 g body wt.	0.7±0.3	1.5±0.4[a]	0.8±0.2	0.8±0.3	0.7±0.4
Spleen wt. (g)	1.8±0.4	1.1±0.5[a]	1.5±0.3	1.6±0.3	1.9±0.1
Spleen wt./100 g body wt.	0.8±0.1	0.8±0.4	0.7±0.3	0.7±0.2	0.8±0.4

Each value is mean ± SE (n=5)
[a] Significant comparisons of each experimental group shown are with the normal value (P<0.05).

The body weights of diabetic rats were significantly decreased after 21 days of diabetes than the control rats. Treatment with fortified bread showed marked gain in weight and it was found most effective. The whole liver and spleen weight showed significant decrease (P<0.05), while whole kidney weight increased significantly. To make a functional comparison of weight gain or loss, tissue weight per 100 g body weight was calculated, while liver

and spleen showed no significant weight difference per 100 g body weight of various experimental groups. Kidney weight per 100 g body weight was significantly increased after 21 days of diabetes. Treatment, especially with increasing levels of fenugreek prevented this increase. These results were consistent with earlier reports of Gupta *et al.* (1999) and Raju *et al.* (2001).

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تأثير التغذية بالخبز المدعم بمستويات مختلفة من طحين بذور الحلبة المنبتة على فئران مصابة بمرض السكر

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درس تأثير خلط طحين بذور الحلبة المنبتة بمستويات (1-5%) بدقيق قمح استخلاص 82% على جودة الخواص الريولوجية والعضوية الحسية للخبز البلدى. أشارت النتائج إلى زيادة غير معنوية لكل من الماء الممتص وزمن الخلط وزمن الوصول وضعف العجينة والانسيابية بزيادة مستوى الحلبة. بينما أدت لتناقص غير معنوى للثبات والمطاطية والطاقة مقارنة بدقيق القمح (الكنترول). كذلك لم تتأثر الخواص العضوية والحسية بزيادة مستوى الحلبة بالخبز. انخفض مستوى جلوكوز الدم بالفئران المصابة بمرض السكر انخفاض معنوى بزيادة مستوى الحلبة بالخبز وسجل أعلى انخفاض بعد مرور ساعتين من التغذية. انخفض وزن الجسم ووزن الكبد والطحال وإزداد وزن الكلى للفئران المصابة مقارنة بالفئران الطبيعية بعد تغذية الفئران المصابة بخبز التجربة والذى أدى إلى تحسن ملحوظ بوزن الجسم والكبد والطحال والكلى خصوصا بزيادة مستوى الحلبة بالخبز.