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Physiochemical, Sensory and Nutritional Properties of corn-fenugreek Flour Composite biscuits

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Abstract: Raw, soaked and germinated fenugreek flour were prepared. Gelatinized corn flour (GCF) was partially replaced by raw fenugreek (RF), soaked fenugreek (SF) and germinated fenugreek (GF) flours in ratios of 10 and 20 %, then used for manufacture of biscuit. Chemical composition of raw materials and biscuits was determined. Rheological properties of doughs, baking quality (diameter, height and spread ratio), color attributes and sensory characteristics of prepared biscuits were evaluated. These biscuits were applied on hypercholesterolemic rats. These rats were divided into seven groups .Group 1 was fed on corn flour biscuit and considered as control group .Groups2,3 were fed on row fenugreek (10&20%)respectively ,groups 4,5 were fed on biscuits containing soaked fenugreek (10&20%) respectively, and groups 6,7were fed on biscuits containing germinated fenugreek (10&20%) respectively. The results revealed that fenugreek flour is considered as a good source for protein, fat, fiber and minerals (Ca, P, Fe and Zn). Max. of Viscosity (BU), Break down viscosity (BU)and Setback Viscosity (BU)increased as a result of replacing wheat flour using the prepared fenugreek flours, meanwhile temperature of transition (°C) and temperature of max. viscosity decreased. Baking quality, color attributes and organoleptic evaluation revealed that GCF can be replaced using 10% SF and 20%GF flours to produce acceptable and high nutritional value biscuits. The result also revealed that fenugreek seed (raw, soaked and germinated)significantly reduced serum total cholesterol ,total lipids, LDL cholesterol but serum HDL cholesterol and triglycerides showed non significant changes ,so it can be suggested that fenugreek may be used for lipid lowering purposes.

Key words: Gelatinized corn flour, fenugreek, rheological properties, organoleptic , biscuits, hypercholesterolemic rats, lipid parameters.

INTRODUCTION

Biscuits are the most popular bakery items consumed nearly by all sections of the society in Egypt. Some of the reasons for such wide popularity are low cost in compared with other processed foods (affordable cost), good nutritional quality and availability in different forms, varied taste and longer shelf-life. Bakery products are sometimes used as a vehicle for incorporation of different nutritionally rich ingredients (Gandhi *et al.,* 2001; Sudha *et al.,* 2007).

Fortification with high protein legume flours could provide a good opportunity to improve the nutritional quality of protein consumed by many people. Also, fortification of wheat flour with non-wheat proteins (e.g fenugreek) increases protein quality by improving its amino acid profiles (Stark *et al.,* 1975; Hoover, 1979).

Fenugreek (Trigonella foenum graecum L.; family Leguminoceae) is one such plant whose seeds and leaves are used not only as food but also as an ingredient in traditional medicine. Seeds of fenugreek are used as a condiment and as a supplement to wheat and maize flour for bread making and as a constituent of the daily diet of general population in Indian subcontinent. Its leaves are consumed widely in India as a green leafy vegetable and are a rich source of calcium, iron, β-carotene and other vitamins (Sharma *et al.,* 1996). Seeds of T. foenum graecum contain tannic acid, fixed and volatile oils and a bitter extractive, diosgenin, alkaloids trigonelline, trigocoumarin, trigomethyl coumarin, and steroidal saponin such as gitogenin and traces of trigogenin and vitamin A (Jayaweera 1981, Petit *et al.,* 1995). Some of the therapeutic uses of T. foenum graecum include its use as hypoglycemic, antiulcerogen hypocholesterolemic and antihypertensive agent (Sharma *et al.,* 1996 & Tayyaba *et al.,* 2001).

Recent scientific reports indicate that fenugreek does indeed have therapeutic properties that may be beneficial in treating such diseases as diabetes and hypercholesterolaemia (Singhal *et al*. 1982; Madar, 1984;

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Sharma, 1984, 1986a, b; Valette *et al*. 1984; Bhat *et al*. 1985; Ribes *et al*. 1986; Madar *et al*. 1988). In addition, diets enriched with fenugreek increased both fecal weight and excretion of bile acids and cholesterol.

Fenugreek has been found to contain relatively large quantities of saponins (Valette *et al*. 1984). Saponins are a heterogeneous group of amphiphilic compounds found mainly in plants. They are highly surface-active and have many diverse properties. Most saponins are haemolytic, can bind cholesterol, and form stable foams (Price *et al.* 1987). A crude saponin fraction isolated from fenugreek reduced serum cholesterol in rats (Sharma, 1986).

Autio *et al.,* (1998) reported that the germination–induced very extensive microstructure changes of cell walls in dough's. The larger values of the area of visible cell walls of the germinated than for the native grains suggest that germination induces swelling of cell walls, but the smaller values suggest that germination causes fading of cell walls. However, the microstructure examination of dough section showed that germination caused two types of structural changes in the cell walls: (1) swelling and (2) fading of the blue fluorescence of cell walls. Moreover, doughs made from flours of germinated grains were always softer than dough's made from flours of native grains. Hence, development and consumption of such therapeutic bakery products would help to raise the nutritional status of the population. Information on incorporation of treated and untreated legumes flour in bakery products is scanty.

Therefore, this study was designed to evaluate the effect of adding raw, soaked and germinated fenugreek flours at ratios of 10 and 20% on the chemical, rheological properties of dough, baking quality, color attributes, sensory characteristics, and to determine the effect of these biscuits on hypercholesterolemic rats.

MATERIALS AND METHODS

I-Materials:

The grains of white corn (Pioneer 30 K8) were purchased from the Corn Breeding Section, Field Crops Department, Agric. Res. Center, Giza, Egypt. Fenugreek seed, shortening, egg, sugar, salt and sodium bicarbonate were obtained from local market, Cairo.

II-Methods:

1-Processing:

Gelatinized corn flour:

Laboratory process for preparing dry corn masa according to Vidal-Quintanar *et al.,* (2001) with some modification as follows: The whole white corn grains (1kg) were soaked in 1% calcium hydroxide solution (1:3 w/w ratio) and then cooked for 95 min on an electronic stove adjusted to 95º C by the traditional cooking method (Fig.1). The nixtamal was steeped over night (15 h) at 24±1º C; Washing followed with excess (5L) tap water followed by decantation using a sieve. The washing process was repeated three times. The wet masa was spread on aluminum foil in a layer of 2.5 cm thickness and. dried in convection oven adjusted to 85 º C for about 6 hours, with occasional mixing. The dry masa was electrically ground (Brabender mill (Junior) to pass a 60 mesh screen (0.0028 in sieve opening), and a minimum of 0.102±.0.06 cm of free space between the shaft and the stationary body of the mill. The masa prepared from grains were packed in Polyethylene bags from the Technopack Co., Cairo, Egypt, and the bags were stored in a refrigerator (4°C) until used.

Soaking:

Fenugreek seeds were first cleaned and freed from broken seeds, dust and other foreign materials and then soaked in tap water for 12 h at 37 °C. A seed to water ratio of 1:5 (w/v) was used. The unimbibed water was discarded. The soaked seeds were rinsed twice in distilled water and then dried at 55-60 ° C.

Germination:

The soaked seeds were germinated in sterile Petri dishes lined with wet filter paper for 48 h at 37 °C with frequent watering. The sprouts were rinsed in distilled water and dried at 55-60 °C. The dried samples of raw, soaked and germinated seeds were ground to obtain fine powder (60 meshes) and then stored in plastic containers for further use.

Preparation of flour mixtures:

Gelatinized corn flour was well blended with fenugreek flour (raw, soaked, and germinated) at different ratios of 10 and 20%, and then prepared mixtures were used to manufacture biscuit.

2-Rheological properties:

Rheological properties of doughs were evaluated using an amylograph (Brabender amylograph ; Duisburg Nr. 940053, type 680022) as described in (A.A.C.C., 2000).

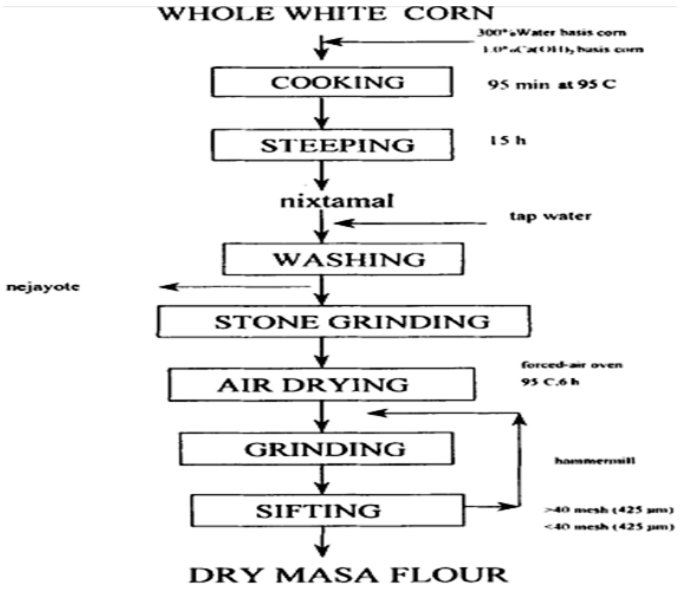

Fig. 1: Method for Preparing Lab-Made Corn Masa Flours

3-preparation and Evaluation of Biscuits:

Biscuits were prepared by mixing 100 g of Gelatinized corn flour and fenugreek flours, 30 of sugar, 15g of shortening, 0.93g of salt, 1.11g of sodium bicarbonate, required of water and 14.66 ml of dextrose solution (5.93%) according to the method described in (A.A.C.C.,2000).

Weight, volume, specific volume, diameter, thickness and spread ratio of biscuits were recorded. Organoleptic characteristics of biscuits were evaluated with some modifications, according to Hussein *et al* (2008) by 10 trained panelists. The tested characteristics were color (10), flavor (10), taste (10), texture (10), appearance (10) and overall acceptability (10).

4-chemical Evaluation:

Gross Chemical Composition:

RF, SF, GF, Gelatinized corn flour, and prepared biscuits were chemically analyzed for moisture, crude protein; fat, crude fiber and ash according to methods described in A.O.A.C. (1990). Total carbohydrates were calculated by difference (100-fat, protein, ash, and fibers on dry weight basis).

5-Color:

Color of different samples was measured by using a Spectro-Colorimeter (Tristimulus Color Machine) with CIF lab color scale (Hunter, Lab Scan XE, Germany).

6. Sensory Evaluation of Biscuits:

The biscuits were evaluated for surface colour, taste, odour, texture, and distribution of cell on a 9-point hedonic scale by a panel of 6 judges according to the method of Hussein *et al* (2010)

Feeding experiments:

Experimental animal:

Forty-eight male albino rats of (Sprague Dowley Sp.) within average weight of 100 - 120g were purchased from the Animal House of the National Research Center. All animals were housed individually in stainless steel cages and fed standard rat diet AIN93M (Reeves *et al.,* 1993) for two days for adaptation. The animal room was adjusted to a temperature of 22- 24C, relative humidity of 55±10%, a light-dark cycle of 12h.

Experimental design and diets:

The present study was divided into two phases. During the first phase (4 weeks), all the animals were rendered hypercholesterolemic by receiving a diet containing 20% saturated fat, 1% cholesterol, and 0.25% cholic acid (hypercholesterolemic diet), as described by (Anderson *et al.,* 1994). The composition of the hypercholesterolemic diet is shown in Table (1). At the end of this period fasting blood samples were obtained from all rats, and total cholesterol were immediately determined in the serum, the cholesterol level was 221.0 ±1 mg/100 ml. The animals were then submitted to the second phase of the experiment (6 weeks), during

which one group received biscuit diets without fenugreek and considered as control group(diet 1), while the other six groups received biscuit diets containing different types of fenugreek (raw 10, 20% as diet 2and3,socked 10,20% as diet4and5and germinated10,20% as diet 6and 7) Table(2)

This Stage Was Extended for 6 Weeks:

During the experiment, some biological parameters (food intake, feed efficiency and body weight changes) were determined. At the end of this period, the animals were fasted for approximately 16 h and killed by decapitation. Blood samples were collected (5–7 ml), hemoglobin was determined in the whole blood. Serum was separated by centrifugation at 3000 rpm for 20 minutes and the serum was aliquoted and stored at -20 °C until used for analysis.

Serum analyses:

Total serum cholesterol was determined according to (Richmond, 1973), serum high-density lipoprotein (HDL) Cholesterol by (Lopes-Virella, 1977), serum low-density lipoprotein (LDL) Cholesterol by (Assmann *et al*., 1984), total lipids by (Draven and Schmite, 1964), triglycerides by (Fossati & Prinicip 1982), glucose level according to the methods of (Trinder, 1969) blood hemoglobin was determined according to the method used by Betke and Savelsberg, 1950. Malondialdehyde (MDA) level of the plasma was measured by the following procedure according to the method of (Satoh, 1978). Vitamin C was measured by (Jagota and Dani, 1982), Vitamin E by (Desai and Machlin, 1985) and serum iron concentration were measured accoding to (Dreux, 1977).

7-Statistical analysis:

Data of organoleptic evaluation of biscuits were subjected to analysis of variance and least significant difference (L.S.D) at 0.05 level according to the method described by McClave & Benson (1991).

Data of biochemical parameters were performed by two-way analysis of variance and test significant differences tests (ANOVA). Duncan's multiple range tests was also used to test the significant differences between the mean values (Armitage and Berry, 1987).

RESULTS AND DISCUSSION

Chemical Composition of Raw Materials and Their Blends:

Data presented in Table (3) show gross chemical composition of gelatinized corn flour (GCF), raw fenugreek (RF), soaked fenugreek (SF), germinated fenugreek (GF) and the prepared biscuits. It is clear that fenugreek flour is a good potential source for crude protein, fat, crude fibers, and total ash. Moisture, crude protein, fat, crude fiber, and ash contents increased with increasing the RF, SF and GF level in GCF, whereas total carbohydrates contents decreased in biscuits fortified with RF, SF and GF compared to the control.

Pasting Properties of Dough:

The obtained data showed that, the replacement GCF with RF, SF and GF tend to reduce temperature of transition from 72 to 67, 67 and 57°C respectively. Data in Table (4) indicated also that the presence of fenugreek flour increased the corn flour viscosity, i.e. from 951 to 1740, 3040 and 3580 BU for RF, SF and GF added. On the contrary, the viscosity at 50°C was lower in corn flour that supplemented with RF, SF and GF. The breakdown and setback viscosity of this formula were also markedly high. All parameters of viscosity were increased in formula. All corn samples supplemented with RF, SF and GF at different levels increased in viscosity during cooling.

Baking Quality of Biscuits:

Baking quality of biscuits, such as diameter, height and spread ratio, were affected by the increase in the level of raw, soaked and germinated of fenugreek flour (Table 5). The changes in diameter and height are reflected in spread ratio which decreased consistently from 5.64 to 4.46, 4.42 and 4.34 in RF, SF and GF respectively. These results indicated that the addition of RF, SF and GF adversely affected the diameter and height and thus, spread ratio of the supplemented biscuits.

Color characteristics:

The fortified flours blends showed a difference in color in relation to their control (100% GCF). The slight improvement in color was interpreted as an intense color and it was dependant on the fortification level. Table 6 shows Hunter values of whiteness (L), redness (a) and Yellowness (b) measured for crust colors. All fortified samples had slightly lower L values for crust than the control and therefore a slightly darker crumb color.

Sensory Characteristics of Biscuits:

The effects of RF, SF and GF supplementation on the sensory characteristics of biscuits are presented in Table (7). With the increase in the level of fenugreek (RF, SF and GF) in the formulation, the sensory scores for Shape, Surface colour, odor, taste, texture and Distribution of cell of biscuits decreased sharply. Replacement of GCF with 10% and 20% fenugreek flour (RF, SF and GF) impaired the taste of biscuits (control samples) had 7.7 score, which decreased significantly from 6.5 to 5.4 for RF, 7.2 to 6.5 for SF and 7.2 to 7.0 for GF.

The texture and Distribution of cell score for control was 7.0 and 7.9 on a 10-point hedonic scale. Biscuits made from blends containing 10% level of RF and 20% level of SF flours did not differ significantly ($p<0.05$) from the control. At 20% levels of substitution, the texture and Distribution of cell was decreased.

Biological evaluation:

The present data in table (8) indicated that all the studied diet showed significant decrease in body weight gain BWG of the rats as compared with control group, but this decrease was non significant in rats fed soaked and germinated fenugreek (10%). Furthermore data indicated that fenugreek biscuit diets significantly decreased food intake of the rats except the diets containing raw fenugreek (10%, 20%). Data given in the table revealed that the diets containing soaked and germinated fenugreek (10%) showed significant increased in feed efficiency of rats as compared with the control group.

Biochemical measurements:

It can be noticed from the results recorded in table (9) that all the studied diets showed non significant change in hemoglobin and glucose levels of rat as compared with control group. However data in the same table illustrated that all the studied diets significantly increased the iron level as compared with control group but this increase was non significant in rats fed on diets containing raw fenugreek (10%).

Results of the effect of the different types and levels of fenugreek biscuit diets on the lipid profile of the hypercholesterolemic rats are presented in table (10). These results showed that all the studied diets decreased the levels of total lipids, total cholesterol and low density lipoprotein (LDL) of the rats fed on fenugreek biscuit diets. With respect high density lipoprotein (HDL) there is no significant differences between all tested group and control group except the biscuit diet containing 10% raw fenugreek. Data represented in the same table revealed significant decrease in the level of Triglyceride (TG) of the rats feeding on raw (20%) and soaked (10%) fenugreek biscuit diet.

Serum levels of vitamin E and vitamin C did not change generally during the experimental time (Table 11), while the levels of serum Malondialdehyde of the rats fed the diets containing soaked and germinated fenugreek (10, 20%) decreased as compared with control group. With regard to the rats fed the diets containing raw fenugreek (10, 20 %) showed no change in the level of serum Malondialdehyde comparing with control group.

Discussion:

The increase in protein, fat, ash and fibers of fenugreek supplemented biscuits can be attributed to the high content of those ingredients in fenugreek. These results are confirmed with the results of Eissa *et al.,* (2007) ; Hooda & Jood (2003) who reported higher protein content of biscuits prepared from blends of wheat-raw and germinated leguminous flour. This was also consistent with findings of Sharma & Chauhan (2000) who also reported higher protein content of breads prepared from blends of wheat-fenugreek flours.

The increase in viscosity upon cooling has been reported to be due to the starch retrogradation i.e., the recrystallization or reassociation of gelatinized starch (Appelquvist and Debet, 1997 and Kamel, 2001). Possible explanations based on the interactions between amylose and fenugreek polysaccharide (FP) or segregation of components leading to an increase in the effective concentration of biopolymers are given. The results in the present study are consistent with these findings. That is, FP may interact with the leached-out amylose outside the starch granule to produce a more complex matrix of amylose and galactomannan surrounding the gelatinized granules, resulting in higher viscosity (Alloncle & Doublier, 1991). In other words, when starch and fenugreek are added together, the enhancement on the viscosity is much greater than when the two ingredients are added individually. FP enhanced the peak, trough, final viscosity and viscoelasticity of corn starch soups and increased retrogradation. The thickening ability of FP and/or possible molecular interactions between the galactomannans and amylose and/or amylopectin components can explain the effects on the gelatinization and retrogradation behaviour of starch and viscoelasticity of the soups (Ravindran and Matia-Merino, 2009).

Cookies having higher spread ratios are considered most desirable (Kirssel & Prentice, 1979, Eissa *et al*, 2007). Other research workers also reported that the height of supplemented biscuits increased, whereas diameter and spread ratio of biscuits decreased with the increasing level of rice bran-fenugreek blends, fenugreek flour and different bran blends (Sharma & Chauhan, 2002; Dhingra & Jood, 2004 Hooda & Jood,

2005; Sudha *et al.,* 2007). Reduced spread ratios of raw, soaked and germinated fenugreek fortified biscuits were attributed to the fact that composite flours apparently form aggregates with increased numbers of hydrophilic sites available for competing for the limited free water in cookie dough (Hooda & Jood, 2005). Rapid partitioning of free water of these hydrophilic sites occurs during dough mixing and increases dough viscosity, thereby limiting cookie spread and top grain formation during baking

L values are declined in all fortified samples using legumes flour compared to control. It was confirmed that the RF, SF and GF flour biscuits were getting darker, redder (a-values) and with higher browning index (BI) than with germinated legumes flour and control samples. The results showed that the a-values (redness) are getting higher in the fortified biscuit samples with enhanced levels of RF, SF and GF flour from 10% to20% (Table 6). These results are consistent with those obtained by Barron & Espinoza, (1993), Ahmed (1999), Kenny *et al.* (2000) and Eissa *et al.* (2007).

The GF flour-supplemented biscuits performed better than the other RF and SF flours. Thus, might be due to the bitter taste of fenugreek flour. Similar observation was also reported with supplementation of raw and germinated legumes flour-supplemented biscuits (Eissa *et al*, 2007) and rice bran-fenugreek blends flour (Sharma & Chauhan, 2002). Organoleptic evaluation revealed that GCF can be replaced using 10% SF and 20%GF flours to produce acceptable and high nutritional value biscuits.

Biological assessment of different diets of Fenugreek on body weight gain and food intake of the rats showed significant decrease as compared with the control groups (Table 8). These results given are in good agreement with those reported by (Takumi *et al*., 2009), who found that fenugreek seeds have bitter taste, and this effect reduce the appetite of rats. Furthermore Handa *et al*., (2005) concluded that fenugreek seed extract reduced the body weight gain induced by a high fat diet in obese mice. This effect is due to the presence of 4-hyroxyisoleucine in fenugreek extract which decrease plasma triglyceride gain, consequently fenugreek seed extract is expected to prevent the obesity induced by a high fat diet.

In addition (Mathern *et al*., 2009) found that 8grm of fenugreek fiber significantly increased satiety and reduced energy intake at lunch, suggesting it may have short-term beneficial effects in obese subjects. Also these results are supported by (Chevassus *et al*., 2009) who reported that the administration of fenugreek extract decrease dietary fat consumption in healthy over weight subjects.

Contrast findings were given by (Petit *et al*., 1995) who reported that fenugreek seed extract containing steroid saponins increased food intake and the motivation to eat in normal rats which results in a prograsive weight gain in these animals.

However (Choudhary *et al*., 2001) reported that the diet containing powered fenugreek seed showed no significant effect on body weight of mice.

Data concerning the effect of fenugreek diets on hemoglobin and glucose levels showed non significant change as compared with the control group Table (9). Some authors (Mowla *et al.,* 2009 and Xue *et al.,* 2007) proved that fenugreek seeds possess anti-diabetic activity in experimental diabetic animals. These literature were not in agreement with our results which might be due to the fact that our study was done on hypercholesterolemic rats, these effects may be more prominent in hyperglycemic rats.

However data in the same table illustrated that all the studied diets significantly increased the serum iron level as compared with control group.

Table 1: Composition hyperlipidemic diet per 100 g.

Ingredients of the diet	%
Casein (86 % protein)	11.62***
Tallow	10
Palm oil	10
Sucrose	20
Salt mixture*	4
Vitamin mixture**	1
Cholesterol powder	1
Bile salts	0.25
Starch	37.83
Methionine	0.3
Cellulose	4
Total	100

* Vitamin mix. (American Institute of Nutrition, 1999)
**Salt mix. (Bringgs and Williams, 1963)
*** Equal 10 % protein.
Composition hyperlipidemic diet (Anderson *et al*., 1994

Table 2: The constituents of the diets used infeeding hypercholesterolemic experiment rats (g/100g).

Diets Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
biscuits	83.2	82.1	73.2	82.4	82.0	83.2	71.42
Casein	3.65	1.41	-	1.66	-	1.42	-
Fat	-	-	2	-	-	-	2.73
Fiber	2	2	2	2	2	2	2
Vitamin**	1	1	1	1	1	1	1
Salt*	4	4	4	4	4	-	4
Fat	-	-	2	-	-	-	2.73
Starch	6.15	9.49	17.8	8.94	11.0	8.38	18.85

* Vitamin mix. (American Institute of Nutrition, 1999)
**Salt mix. (Bringgs and Williams, 1963)
All diets containing 10% protein

Table 3: proximate composition of raw materials and fenugreek supplemented biscuits (On dry weight basis)

Samples	Moisture	Protein	Fat	Crude fiber	Ash	Total carbohydrate
GCF	9.1	10.62	4.00	4.00	2.50	80.92
RF	6.87	27.20	7.00	7.96	4.28	53.56
SF	8.17	25.08	6.08	8.00	4.35	56.29
GF	7.51	28.55	6.02	8.36	4.89	52.26
Control	3.26	9.51	21.12	2.00	1.92	65.36
GCF:RF						
90:10	4.05	13.00	21.32	2.78	1.80	61.90
80:20	4.62	14.66	21.52	3.07	2.00	58.75
GCF:SF						
90:10	3.80	11.56	21.22	2.55	1.84	62.83
80:20	3.93	13.55	21.32	2.76	1.86	60.51
GCF:GF						
90:10	3.96	12.30	21.05	2.77	1.94	61.94
80:20	4.50	15.00	21.15	2.96	2.15	58.74

Where: GCF, gelatinized corn flour; RF, raw fenugreek; SF, soaked fenugreek; GF, germinated Fenugreek; Control, from gelatinized corn flour

Table 4: Viscoamylograph parameters

Samples	Temp.of transition (°C)	Max. of Viscosity (BU)	Temp. Of max. viscosity	Break down viscosity (BU)	Setback Viscosity (BU)
Control	72	9.51	82.5	410	1050
GCF:RF					
90:10	67	1350	84	460	1450
80:20	67	1740	76.5	480	1800
GCF:SF					
90:10	69	2720	87	510	1560
80:20	67	3040	73.5	580	1780
GCF:GF					
90:10	66	3460	81	430	1600
80:20	57	3580	84	460	1900

Table 5: Baking quality of biscuits prepared from different formulas

Samples	Diameter (cm)	Height(cm)	Spread ratio (diam./ht.)
Control	6.2	1.10	564
GCF:RF			
90:10	5.8	1.20	4.83
80:20	5.8	1.30	4.46
GCF:SF			
90:10	5.2	1.11	4.48
80:20	5.4	1.22	4.42
GCF:GF			
90:10	5.2	1.18	4.41
80:20	5.3	1.22	4.34

Table 6: Color characteristics of biscuits supplemented with raw, soaked and germinated fenugreek flours.

Samples	L	a	b	ΔE
Control	74.12	2.74	25.51	76.75
GCF:RF				
90:10	70.11	5.35	30.65	75.01
80:20	64.17	7.67	34.50	71.57
GCF:SF				
90:10	70.51	5.65	29.31	74.88
80:20	60.68	10.31	36.79	70.02
GCF:GF				
90:10	62.94	7.36	30.26	68.52
80:20	57.28	10.15	29.54	63.54

Table 7: Statistical analysis of sensory properties of biscuits prepared from different formulas

Samples	Shape	Surface colour	Taste	Odour	Texture	Distribution of cell
Control	7.9^{A}	8.0^{A}	7.7^{AB}	8.2^{A}	7.0^{A}	7.9^{AB}
GCF:RF						
90:10	6.5^{BC}	8.3^{A}	6.5^{BCD}	7.7^{A}	7.0^{A}	7.9^{AB}
80:20	6.4^{BC}	7.4^{ABCD}	5.4^{D}	7.5^{A}	7.6^{A}	7.3^{AB}
GCF:SF						
90:10	7.0^{ABC}	7.6^{ABC}	7.2^{ABC}	7.6^{A}	6.6^{A}	8.1^{A}
80:20	6.8^{AB}	7.3^{ABCD}	6.5^{BCD}	6.9^{A}	6.7^{A}	7.8^{A}
GCF:GF						
90:10	7.7^{AB}	8.0^{AB}	7.2^{ABC}	7.0^{A}	7.7^{A}	7.3^{AB}
80:20	7.1^{ABC}	7.3^{ABCD}	7.0^{ABC}	6.4^{A}	7.6^{A}	6.9^{BC}
LSD (0.05)	1.03	1.82	1.1	0.92	0.96	0.90

Table 8: Nutritional parameters of hypercholesterolemic rats fed different types and levels of fenugreek biscuit diets for 4weeks.

Parameters Groups	Body weight gain (g)	T. Food intake (g)	Food efficiency ratio
Control Buscui	83.0 ± 9.919^{a}	1391.4 ± 36.005^{ab}	0.068 ± 0.012^{b}
Row fenugreek10 %	65.0 ± 3.224^{bc}	1342.8 ± 23.672^{b}	0.049 ± 0.003^{bc}
Row fenugreek 20 %	45.5 ± 2.513^{d}	1418.4 ± 19.835^{a}	0.036 ± 0.005^{c}
Soaked fenugreek10 %	75.4 ± 6.555^{ab}	805.9 ± 16.18^{c}	0.093 ± 0.007^{a}
Soaked fenugreek 20 %	52.8 ± 4.118^{cd}	783.8 ± 17.75^{c}	0.068 ± 0.004^{b}
Germinated fenugreek 10 %	71.5 ± 3.373^{ab}	800.7 ± 17.37^{c}	0.089 ± 0.003^{a}
Germinated fenugreek 20 %	38.7 ± 6.053^{d}	800.8 ± 19.09^{c}	0.048 ± 0.007^{bc}

Statistical analysis was carried out for each column
Same letters means nonsignificant difference; different letters means the significance among the tested groups.

Table 9: Biochemical parameters of hypercholesterolemic rats fed different types and levels of fenugreek biscuit diets for 4weeks.

Parameters Groups	Hemoglobin g/l	Glucose (mg/dl)	Iron mmol/dl
Control Buscuit	13.3 ± 0.407^{abc}	107.4 ± 5.407^{b}	15.3 ± 2.224^{c}
Row fenugreek10 %	11.5 ± 0.641^{cd}	102.3 ± 10.629^{b}	19.4 ± 4.337^{bc}
Row fenugreek 20 %	11.2 ± 0.465^{d}	114.3 ± 3.637^{b}	24.5 ± 2.131^{ab}
Soaked fenugreek10 %	12.7 ± 0.422^{bcd}	110.9 ± 5.279^{b}	29.2 ± 2.913^{a}
Soaked fenugreek 20 %	12.2 ± 0.334^{bcd}	109.9 ± 3.287^{b}	25.2 ± 3.271^{bc}
Germinated fenugreek 10 %	14.9 ± 0.799^{a}	108.4 ± 6.022^{b}	28.5 ± 2.160^{a}
Germinated fenugreek 20 %	13.8 ± 0.976^{ab}	103.3 ± 17.214^{b}	26.8 ± 2.263^{ab}

Statistical analysis was carried out for each column
Same letters means non significant difference; different letters means the significance among the tested groups.

Table 10: Lipid profile of hypercholesterolemic rats feeding on different types and levels of fenugreek biscuit diets for 6 weeks.

Parameters Groups	T Lipid	T Cholesterol	LDL	HDL	Triglyceride
Control Buscuit	511.4 ± 38.97^{a}	93.1 ± 3.506^{a}	53.2 ± 9.738^{a}	65.3 ± 6.434^{b}	92.3 ± 5.058^{c}
Row fenugreek10 %	310.9 ± 27.180^{d}	78.6 ± 3.123^{bc}	32.4 ± 6.631^{b}	86.1 ± 5.489^{a}	98.4 ± 3.136^{bc}

Table 10: Continue

Row fenugreek 20 %	331.3 ± 10.392^{cd}	66.0 ± 6.156^{cd}	26.8 ± 4.044^{b}	79.9 ± 7.135^{ab}	106.1 ± 2.069^{b}
Socked fenugreek10 %	424.9 ± 21.720^{b}	65.8 ± 5.973^{cd}	29.9 ± 5.613^{b}	64.4 ± 6.634^{b}	127.0 ± 2.981^{a}
Socked fenugreek 20 %	437.3 ± 13.674^{ab}	63.1 ± 4.148^{d}	19.5 ± 5.521^{b}	69.3 ± 5.437^{ab}	103.3 ± 3.733^{bc}
Germinated fenugreek 10 %	421.1 ± 34.848^{b}	73.9 ± 3.646^{bcd}	29.6 ± 4.846^{b}	62.6 ± 6.312^{b}	98.9 ± 4.465^{bc}
Germinated fenugreek 20 %	401.4 ± 34.941^{bc}	80.1 ± 1.795^{b}	17.2 ± 3.727^{b}	67.1 ± 7.583^{ab}	93.4 ± 3.022^{c}

Statistical analysis was carried out for each column
Same letters means non significant difference; different letters means the significance among the tested groups.

Table 11: Effect of different types and levels of fenugreek biscuit diets on antioxidant parameters of hypercholesterolemic rats feeding for 6 weeks.

Parameters Groups	Vitamin E mg/dl	Vitamin C mg/dl	Malondialdehyde nmol/ml
Control Biscuit	1.214 ± 0.407^{a}	1.293 ± 0.069^{a}	5.58 ± 0.328^{a}
Row fenugreek10 %	1.236 ± 0.039^{a}	1.217 ± 0.363^{a}	5.74 ± 0.314^{a}
Row fenugreek 20 %	1.163 ± 0.051^{a}	1.267 ± 0.102^{a}	5.77 ± 0.698^{a}
Socked fenugreek10 %	1.111 ± 0.165^{a}	1.145 ± 0.118^{a}	4.42 ± 0.346^{ab}
Socked fenugreek 20 %	1.126 ± 0.134^{a}	1.253 ± 0.078^{a}	3.46 ± 0.588^{b}
Germinated fenugreek 10 %	1.165 ± 0.038^{a}	1.072 ± 0.081^{a}	3.42 ± 0.276^{b}
Germinated fenugreek 20 %	1.149 ± 0.039^{a}	1.224 ± 0.099^{a}	4.55 ± 0.356^{ab}

Statistical analysis was carried out for each
Same letters means non significant difference; different letters means the significance among the tested groups

The results of determined iron were confirmed by (Ibrahim and Hegazy 2009) who reported that adding of Soaked or germinated fenugreek seed flour (FSF) with different levels (5-10, 15 and 20%) into wheat biscuits formula improved bioavalability of iron. Chemical analysis showed evident increase in Fe, Ca and Zn content of FSF-biscuits as compared to wheat biscuits. Phytic acid content of produced biscuits were decreased by ranges between to 20-44% this might be due to phytate leaching or phytat hydrolysis during soaking and germination processes by phytase and phosphatase enzymes.

These data were supported by Jonnalagadda and Seshadri 1994) who found that the addition of fenugreek leaves (100g/meal) to standard cereal meal increased the total iron content of the meal significantly (3.24 mg to 9.12 mg) ($P < 0.001$). This could be attributed to the high iron content of the leaves.

With regarde to the hypolipidemic effect of fenugreek diets data represented in table (10) showed that all the studied diets decrease the levels of total lipid, total cholesterol, LDL-cholesterol and there is no significant differences between HDL levels of rats fed tested diets and the control group. The present results are supported by (Moosa *et al* 2006) who reported that administration of fenugreek seed powder of 25gm orally twice daily for 3 weeks and 6 weeks for hypercholesterolmic patients produces significant reduction of serum total cholesterol, triglycride and LDL cholesterol. This study suggests that fenugreek seed powder would be considered as effective agent for lipid lowering purposes. Pipelzadeh *et al*., (2003) confirmed the present results concerned serum total cholesterol and LDL-cholesterol and TG which decreased by feeding rats with fenugreek diets. On the other hand, they contrasted the present findings in serum HDL-cholesterol. They stated that administration of fenugreek seed powder caused a significant increase in serum HDL cholesterol as compared with non treated control group. Therefore this study demonstrated that fenugreek is effective in reducing the features associated with atherosclerosis. Similar results were reported by (Belguith-Hardiches *et al*., 2010) who revealed that administration of fenugreek ethyl acetate extract to rats fed on cholesterol-rich diet significantly lowered the plasma level of total cholesterol (TC), triglycerides (TG) and LDL-cholesterol, while increasing the plasma level of HDL-cholesterol. These lipid effects were correlated to the presence of flavonoids in fenugreek, especially naringenin which was the abundant flavonoid compound in the ethyl acetate extract.

In addition (Prasanna 2000) proved that fenugreek powder given orally before food at 25 and 50gm twice a day may have hypolipidemic effect in hypercholesterolemic patients.

Sowmya, and Rajyalakshmi (1999) confirmed that consumption of germinated fenugreek seed powder resulted in reduction in total cholesterol and LDL levels of hypercholestrmic adults and no significant changes were found in HDL-cholesterol and triglycrides levels in all subjects. This hypocholesterolemic effect occurred through different mechanisms. One such mechanism is increased excretion of fecal bile acids and neutral sterols with depletion of cholesterol stores in the liver. Dietary fenugreek stimulates bile formation in the liver and the conversion of cholesterol into bile salts or the fiber potentially reduces the rate of diffusion towards the absorptive mucosal surface and has been shown to alter glucose, drug and cholesterol absorption or the soluble fiber increases the viscosity of the digest and increases the thickness of the unstirred layer in the small intestine or inhibitions uptake of cholesterol and bile acids. Soluble fiber is an excellent substrate for fermentation by microorganisms in the large bowel. The volatile fatty acids produced by fermentation enter the blood stream and appear to suppress hepatic cholesterol synthesis. Fenugreek seeds contain diosgenin and trigonelline in the form glycosides. These Saponins can form complexes with cholesterol in the intestine reducing its absorption. Another possibility for the lowering effect could be based on amino acid pattern of fenugreek proteins.

Fenugreek given in a dose of 2.5g twice daily for 3 months to healthy individuals did not affect the blood lipids and blood sugar (fasting and postprandial). However, administered in the same daily dose for the same duration to coronary artery disease patients also with non-insulin dependent diabetes, fenugreek decreased significantly the blood lipid (total cholesterol and triglycrides) without affecting the HDL-cholesterol (Bordia *et al*., 1997).

The present results are on the line with (Stark and Madar 1993) who reported that feeding hypercholesterolemic rats with two separate doses (30 or 50 gm) of ethanolic extract from fenugreek seeds contained hypocholesterolemic components which appear to be saponins that interact with bile salts in the digestive tract.

Data in Table (11) showed that the levels of serum malonaldehyde of the rats fed the diets containing different types of fenugreek decreased as compared with control group.

These results refers to the effect of dietary fiber in fenugreek which retards oxidative stress in cardiac tissue of rats, reduces indices of lipid peroxidation i.e. a lower MDA concentration (Diniz *et al*., 2003 Rezor *et al*., 2003 and Jenkins *et al*., 2000).

Annida and Stonely Mainzen Prince (2005) found that supplementation of fenugreek leaf powder at a dose of 1g/kg of body weight significantly lowered lipid peroxidation and significantly increased the antioxidant system in rats.

The antioxidant properties were studied in germinated fenugreek seeds which are considered to be more beneficial than dried seeds. This study reveals significant antioxidant activity in germinated fenugreek seeds which may be due partly to the presence of flavonoids and polyphenols (Dixit *et al*., 2005).

It was also found that simultaneous administration of aqueous extract of fenugreek seed to experimental rats fed on ethanol prevented enzymatic leakage and the rise in lipid peroxidation and enhanced the antioxidant potential. The seeds exhibited appreciable antioxidant property which was comparable with that of reduced glutathione and alpha-tocopherol (Thirunavukarasu *et al*., 2003).

Conclusion:

This present study confirmed that fenugreek flour could be incorporated up to 10% level in the formulation of biscuits without affecting their overall quality. The chemical, physical and sensory properties, in general, revealed that biscuits containing 20% germinated fenugreek flour were the best among all the composite fenugreek flour biscuits. Baking quality, color attributes and organoleptic evaluation revealed that wheat flour can be replaced using 10% SF and 20%GF flours to produce acceptable and high nutritional value biscuits.

The present study confirmed that fenugreek seed (raw, soaked and germinated) significantly reduced serum total cholesterol, total lipids, LDL-cholesterol but serum HDL-cholesterol and triglycerides showed non significant changes. So, it can be suggested that fenugreek may be used for lipid lowering purposes.

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