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Evaluation of the Nutritional Protein Quality of Wheat Biscuit Supplemented by Fenugreek Seed Flour

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Abstract: The effect of partial replacement of wheat flour by different levels (5, 10, 15 or 20%) of soaked or germinated fenugreek seed flours (FSF) on the sensory and chemical characteristics for biscuits produced was studied. Also, these biscuits prepared were evaluated for their amino acid contents and biological properties and compared to the control biscuit (100% wheat flour). Results revealed that biscuits processed from wheat flour supplemented by 5 or 10% of FSF had higher acceptance scores for all sensory characteristics than other blend biscuits. The biscuits containing 15 or 20% of FSF were significantly different ($P<0.05$) and were unacceptable to the panelists as compared with the other samples. Results of chemical analysis indicated that incorporation of FSF into biscuits formula obviously increased protein, fat, fiber, ash and indispensable amino acid (IAAs) contents with increasing FSF levels. The amino acids composition revealed that FSF-biscuits contained most of IAAs at higher concentrations than reference protein pattern of FAO/WHO, with exception of lysine and sulfur amino acids (Meth+cyst). Lysine was the first limiting amino acid in wheat biscuit (36%). Lysine score was elevated and reached to 89% after supplementation with 10% germinated FSF. Rat feeding experiments indicated that supplementation of wheat biscuit with 5 or 10% FSF substantially improved their nutritive quality. Hence rats fed on FSF-biscuit diets exhibited extremely higher values in weight gain, food intake and protein consumption as compared to rats fed on wheat biscuit diet. The biscuit diet containing 10% germinated FSF recorded the highest values of PER, NPR and NPU (1.60, 2.31 and 60%, respectively) between all tested diets. It could be concluded that, using of 10% germinated FSF into wheat biscuit formula resulted in improving their chemical and nutritional quality criteria.

Key words: Biscuit · Fenugreek seeds · Sensory properties · Chemical composition · Biological evaluation

INTRODUCTION

The quality of biscuits depends on quantity and quality of ingredients, especially the flour. It was found that mixing two or more different materials will help to solve the deficiency problem of cereals as low nutritional value by used legumes as food protein source [1]. The use of legume proteins is almost limited to the protein of soybean seeds. Studies should now focus on a search for proteins from other sources, such as fenugreek. It has historically been utilized mainly as whole seed; it is a potential protein source (20-25%) with high nutritive value [2]. Also, fenugreek seed flour has a great potential, due to its high contents of lysine (5–6%), soluble (20%) and insoluble dietary fiber. Addition, it also possesses hypocholesterolemic and hypoglycemic properties [3, 4]. It has been reported that these seeds are a cheap source of good quality protein and may be mixed with cereals as a supplement for some limiting amino acids and hence for improving their protein quality through amino acid balance. Hence, development and consumption of such therapeutic bakery products would help to raise the nutritional status of the population [5]. Information on incorporation of fenugreek seed flour in bakery products is scanty. Therefore, this study was designed to evaluate the effect of replacement wheat flour by 5, 10, 15 or 20% of both soaked and germinated fenugreek seed flours on the sensory and chemical characteristics of produced biscuits. Also, investigating the nutritional protein quality of final products through chemical evaluation of amino acid patterns and rat feeding experiments was another target.

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MATERIALS AND METHODS

Materials: Fenugreek seeds were obtained from Agriculture Research Center, Giza, Egypt. Wheat flour (72% extraction) and other ingredients were obtained from the local market. Ingredients used in processing of biscuits included 65.1% wheat flour or blends, 21.4% sugar, 9.3% shortening (palm oil), 0.93% skimmed milk powder, 1.86% high fructose, 0.37% sodium bicarbonate, 1.02% ammonium bicarbonate, 0.02% vanilla and required amount of water.

Methods

Experimental Treatments

Preparation of Fenugreek Seed Flour (FSF): Fenugreek seeds were cleaned and freed of broken seeds, dust and other foreign materials and divided into two equal parts: the first part was soaked, while the second part was germinated as following:

Soaking: The seeds were soaked in tap water for 12 h at 37 °C. with a seeds: water ratio of 1:5 (w/v). The unimbibed water was discarded and the soaked seeds were rinsed twice by distilled water.

Germination: The soaked seeds were germinated in sterile Petri dishes lined with wet filter papers for 48 h at 37°C, with frequent watering. The sprouts were rinsed by distilled water. The soaked and germinated seeds were dried in an oven at 55-60°C for 10-12 h. The dried seeds were ground to particles passing through 20 mesh sieve which is similar to the size of wheat flour as described by Shalini and Sudesh [6].

Preparation of Wheat Flour-fenugreek Seed Flour Blends: Wheat flour was supplemented by 5, 10, 15 or 20% of both soaked and germinated FSF. The flour mixtures were individually blended and homogenized, packed in polyethylene bags, tightly closed and stored at room temperature until utilized.

Processing of Biscuits: Fat and sucrose were firstly creamed by using the mechanical mixer for 10 min. Sodium and ammonium bicarbonate were dissolved in a part of water and added to the prepared creamed mixture, then high fructose was added. As creaming process was continued, flour, skimmed milk powder and vanilla were added and stirred well together. The full prepared dough was laminated, sheeted, extruded, molded and formed to the required form. The formed biscuits were baked at 230°C for 7 min. as described by A.A.C.C. [7]. After cooling for 30 min. biscuits were packaged in cellophane and sensory evaluated.

Analytical Methods

Sensory Evaluation of Biscuits: The organoleptic characteristics of control biscuit (wheat flour) and FSF-biscuits were evaluated by using a taste panel, consisting of 20 judges. The panelists were asked to evaluation of color, appearance, taste, flavor, texture and overall acceptability. The ratings were on a 9-point hedonic scale, ranging from 9as like extremely to 1 as dislike extremely as outlined by Austin and Ram [8].

Chemical Analysis: Proximate composition of tested samples was estimated according to A.O.A.C. [9].

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Amino Acids Composition: It was determined using HPLC-Pico-Tag method according to Millipore Cooperative [10]. Amino acid score = amino acid (g/100 g) of test protein / amino acid (g/100 g) of reference pattern x 100. The reference protein pattern was of FAO/WHO [11].The lowest score obtained will be considered as the first limiting amino acid and subsequently second limiting amino acids.

Rats Feeding Bio-evaluation: White weanling male albino rats, weighing an average 70 g, were kept under healthy laboratory conditions and fed milk protein standard for three days as adaptation period. The biological evaluation was carried out at the animal house of Sciences Faculty, Al-Azhar University, Cairo, Egypt. Bio-assays include protein efficiency ratio (PER), net protein ratio (NPR) and net protein utilization (NPU) as the following:

Protein Efficiency Ratio (PER): PER values of all biscuit diets were evaluated according to A.O.A.C. [9]. Weanling male albino rats, of single strain, 20-23 days of age were used (six rats for each diet).Animal were kept in individual cages. A weekly record of food consumption and body weight were maintained. Average four weeks weight gain and protein intake per rat for each group was determined. Protein efficiency ratio (PER) for each group was calculated according to the following equation: PER = weight gain (g) / protein consumed (g)

Net Protein Ratio (NPR): According to A.O.A.C. [9]. A group of rats (each group composed of six rats) was fed on a protein free diet, whereas the other group received the test diets as indicated under PER evaluation. Weights gain of the same rats used in PER was determined at 14 day of age. The weight loss of rats fed on a protein free diet was added to the weight gain of the test groups. Protein intake was determined. NPR values were calculated as follows:

$$NPR = \frac{\text{weight gain (g)+average weight loss of non-protein group (g)}}{\text{protein consumed (g)}}$$

Net Protein Utilization (NPU): The same animals used in PER and NPR, were sacrificed and incisions were made in the skull, thoracic and body cavities of each rat. The animal were dried (the whole rat's body) in a hot oven at 70°C until constant weight and grind. The total carcass nitrogen of each rat was determined by using macro-kjeldahl method as described by A.O.A.C. [9].

Statistical Analysis: The original sensory panel data and other results were statistically analyzed using analysis of variance (ANOVA) and least significance difference (LSD) at a significance of probability 5 % [12].

RESULTS

Sensory Characteristics of Biscuits Containing FSF: Biscuits supplemented by different levels of soaked and germinated FSF were sensory evaluated and compared with control biscuit (100% wheat flour) as shown in Table 1. Data indicated that there were no significant differences among control sample and biscuit samples containing 5 or 10% FSF in all sensory characteristics. But, the biscuits containing 15 or 20% FSF were significantly different ($P<0.05$) in all properties and were unacceptable to the panelists as compared to the other samples. Therefore, it excluded from this study. Whereas overall acceptability scores for those samples were ranged from 4.69 to 5.72, against 8.37 of control biscuit.

Chemical Composition of Biscuits Containing FSF: The chemical composition of biscuits made from wheat flour blends with 5 or 10% soaked and germinated FSF is tabulated in Table 2. The results indicated that protein, fat, crude fiber and ash contents were obviously increased ($P<0.05$) by increasing FSF levels as compared with control biscuit. While, a slight decrease in carbohydrate content of these samples was noticed and

Table 1: Sensory characteristics* of biscuits supplemented by soaked and germinated fenugreek seed flours

	Organoleptic properties					
Substitution level (%)	Color	Appearance	Flavor	Taste	Texture	Overall acceptability
Control	8.00[a]	8.12[a]	8.50[a]	8.89[a]	8.22[a]	8.37[a]
W: SF						
95: 5	7.72[a]	7.89[a]	7.95[a]	7.98[a]	7.61[a]	7.79[a]
90: 10	7.50[a]	7.68[a]	7.62[a]	7.54[a]	7.45[a]	7.63[a]
85: 15	6.32[b]	6.35[b]	5.23[b]	4.32[b]	5.82[b]	5.58[b]
80: 20	5.18[c]	5.68[c]	4.51[c]	3.21[c]	4.58[c]	4.69[c]
W: GF						
95: 5	7.89[a]	7.97[a]	7.98[a]	8.12[a]	7.69[a]	7.86[a]
90: 10	7.66[a]	7.73[a]	7.71[a]	7.67[a]	7.53[a]	7.69[a]
85: 15	6.45[b]	6.42[b]	5.34[b]	4.57[b]	5.90[b]	5.72[b]
80: 20	5.31[c]	5.55[c]	4.78[c]	3.29[c]	4.67[c]	4.76[c]

*Scores were: 9 = like extremely to 1 = dislike extremely
W: wheat flour - SF: soaked fenugreek - GF: germinated fenugreek
[a], [b]and[c] means in the same column with different superscripts are different significantly (p● 0.05)

Table 2: Chemical composition*(on dry weight basis) of biscuits supplemented by soaked and germinated fenugreek seed flours

	Components (%)				
Substitution level (%)	Protein	Fat	Crude Fiber	Ash	Carbohydrate
Control	9.66[c]	20.11[b]	1.51[c]	1.75[c]	66.97[a]
W: SF					
95: 5	10.41[b]	20.85[a]	1.75[b]	1.88[b]	65.11[a]
90: 10	11.85[a]	20.98[a]	1.96[a]	1.98[a]	63.23[a]
W: GF					
95: 5	10.52[b]	20.84[a]	1.79[b]	1.93[a]	64.92[a]
90: 10	11.93[a]	20.89[a]	2.11[a]	2.09[a]	62.98[b]

*Means in the same column with different superscripts are different significantly (p● 0.05)
W: wheat flour - SF: soaked fenugreek - GF: germinated fenugreek

Table 3: Amino acids content* (g/ 100 g) of biscuits supplemented by soaked and germinated fenugreek seed flours

		Substitution level (%)			
		SF		GF	
Amino acids (g/100 g)	Control (wheat biscuit)	5%	10%	5%	10%
Leucine	5.63	7.32	7.78	7.69	7.83
Isoleucine	2.50	3.09	3.48	3.45	3.57
Lysine	2.08	4.11	4.53	4.55	5.14
Methionine+cystine	1.64	1.76	1.89	1.86	2.07
Phenylalanine	4.87	4.99	5.21	5.19	5.24
Tyrosine	1.69	1.82	1.98	1.97	2.06
Theronine	2.02	3.13	3.22	3.18	3.24
Treptophan	1.01	1.21	1.23	1.22	1.36
Valine	2.89	3.35	3.61	3.65	3.83
Total IAAs	24.33^{c}	30.78^{b}	32.99^{a}	32.81^{a}	34.34^{a}
Aspartic	4.14	6.97	7.09	7.07	7.44
Serine	4.71	4.72	4.79	4.73	4.81
Glutamic	31.22	29.58	28.04	28.01	27.40
Proline	6.17	6.32	6.44	6.43	6.80
Glycine	1.93	2.21	2.46	2.55	2.78
Alanine	3.07	3.37	3.62	3.61	3.86
Histidine	2.09	2.21	2.49	2.48	2.68
Arginine	9.32	9.36	9.52	9.49	9.64
Total DAAs	62.65^{b}	64.74^{a}	65.75^{a}	65.77^{a}	65.41^{a}
Total amino acids	86.98^{c}	95.52^{b}	98.74^{a}	98.58^{a}	99.75^{a}

*Means in the same raw with different superscripts are different significantly (p● 0.05)

W: wheat flour - SF: soaked fenugreek - GF: germinated fenugreek

Table 4: Amino acids score of biscuits supplemented by soaked and germinated FSF, compared to reference protein pattern of FAO/WHO

		Substitution level (%)				
		SF		GF		
Amino acids (g/100 g)	Control (wheat biscuit)	5%	10%	5%	10%	FAO/WHO
Leucine	85	111	118	116	118	6.61
Isoleucine	89	110	124	123	128	2.80
Lysine	36	71	79	78	89	5.80
Methionine+cystine	48	52	56	55	61	3.40
Phenylalanine+tyrosine	104	108	114	114	116	6.30
Theronine	84	130	134	132	135	2.40
Treptophan	92	110	112	115	124	1.10
Valine	83	96	103	104	109	3.50

reached to 63.23 and 62.98% at level 10% of biscuits containing soaked and germinated FSF, respectively, against 66.97% of control.

Nutritional Protein Quality of Biscuits: It was evaluated according to its content of amino acids and comparison with reference protein pattern of FAO/WHO. Table 3 indicated that the amount of total indispensable amino acids (IAAs) in biscuits containing 5 and 10% soaked and germinated FSF exceeded ($P<0.05$) that of control biscuit, being ranged from 30.78 to 34.34 against 24.33 g/100g protein for control. Also, incorporation 5 and 10% FSF into wheat flour resulted in increase ($P<0.05$) of total dispensable amino acids (DAAs) content for biscuits from 62.65 for control to 65.77 g/100g protein for biscuit containing 5% germinated FSF.

Table 4 indicates that IAAs content of different biscuit samples in comparison with the reference protein pattern of FAO/WHO. From Table 4, it could be observed that FSF-biscuits contained most of IAAs at higher concentrations than reference protein pattern of FAO/WHO (higher than 100), with exception of lysine and sulfur amino acids (Meth+cyst). Since, the amino acid score of these three amino acids was lower than 100.

Table 5: Criteria of protein efficiency ratio* (PER) of biscuits supplemented by soaked and germinated fenugreek seed flours.

	Criteria (g)					
Substitution level (%)	Initial body wt.	Final body wt.	Gain in body wt.	Food intake	Protein consumed	PER
Control	71.2[a]	91.7[b]	20.5[b]	224[c]	20.62[b]	0.99
W: SF						
95: 5	72.5[a]	118.5[a]	46.0[a]	309[a]	31.23[a]	1.47
90: 10	71.6[a]	119.3[a]	47.7[a]	290[b]	32.04[a]	1.49
W: GF						
95: 5	70.5[a]	117.5[a]	47.0[a]	295[b]	30.06[a]	1.56
90: 10	71.3[a]	121.4[a]	50.1[a]	290[b]	31.31[a]	1.60

*Means in the same column with different superscripts are different significantly (p● 0.05)

Table 6: Criteria of net protein ratio* (NPR) of biscuits supplemented by soaked and germinated fenugreek seed flours.

	Criteria (g)			
Substitution level (%)	Gain in body wt.	Average loss in body wt.	Protein consumed	NPR
Control	20.5[b]		20.62[b]	2.06
W: SF				
95: 5	46.0[a]		31.23[a]	2.18
90: 10	47.7[a]	22.1	32.04[a]	2.18
W: GF				
95: 5	47.0[a]		30.06[a]	2.30
90: 10	50.1[a]		31.31[a]	2.31

*Means in the same column with different superscripts are different significantly (p● 0.05).

Table 7: Criteria of net protein utilization* (NPU) of biscuits supplemented by soaked and germinated fenugreek seed flours.

	Nitrogen (g)			
Substitution level (%)	Intake	Rat body	Protein free diet	NPU (%)
Control	3.29[c]	3.32[c]		48.63
W: SF				
95: 5	4.99[b]	4.57[b]		57.11
90: 10	5.12[a]	4.68[b]	1.72	57.81
W: GF				
95: 5	4.89[b]	4.65[b]		59.92
90: 10	5.23[a]	4.85[a]		60.00

*Means in the same column with different superscripts are different significantly (p● 0.05).

Also, lysine was the first limiting amino acid in control biscuit (36%), when biscuits were supplemented by FSF, lysine score elevated and reached to 89% in biscuit containing 10% germinated FSF.

Protein Efficiency Ratio (PER): Table 5 shows wide variation in the average values of food intake, protein consumed and gain in body weight between rat groups. Rats fed on biscuit diets containing FSF exhibited extremely higher ($P<0.05$) values in weights gain, food intake and protein consumption while, the maximum weight gain was for the rats fed on biscuit diets supplemented by 10% soaked or germinated FSF (47.7 and 50.1 g, respectively), also, PER values for both diets were 1.49 and 1.60 compared to 0.99 for control diet as shown in Fig 1.

Net Protein Ratio (NPR): Table 6 and Fig. 2 show that NPR values of biscuit diets containing FSF were gradually increased from 2.18 to 2.31 with increasing FSF levels, as compared with control biscuit diet (2.06).

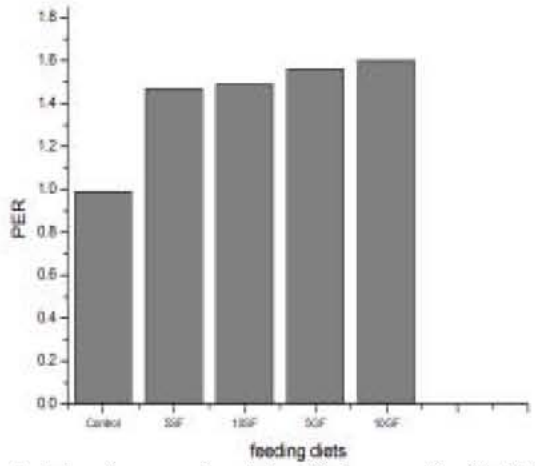

Fig. 1: The changes of protein efficiency ratio (PER) for rats fed on biscuit based diets containing FSF

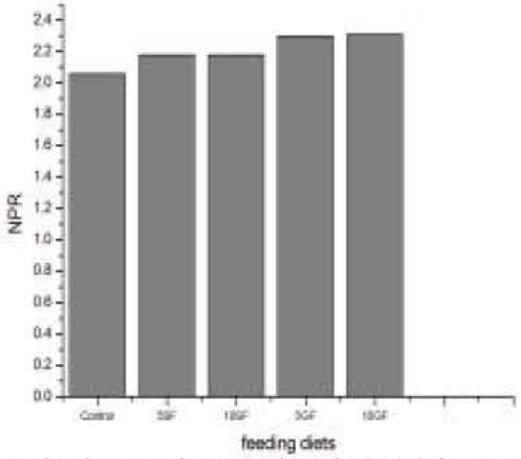

Fig. 2: The changes of net protein ratio (NPR) for rats fed on biscuit based diets containing FSF

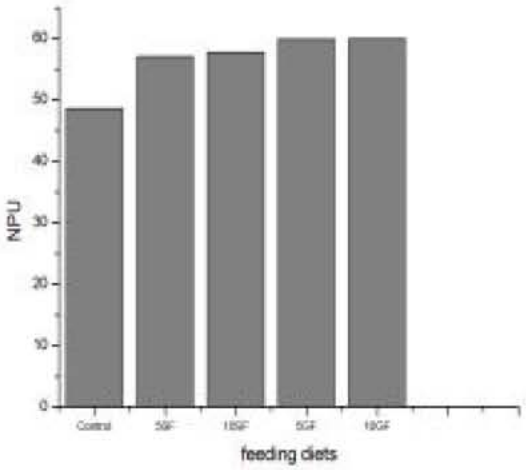

Fig. 3: The changes of net protein utilization (NPU) for rats fed on biscuit based diets containing FSF

Net Protein Utilization (NPU): From Table 7, it could be observed that increasing FSF level in tested biscuit diets resulted in evident increase of NPU values, especially in biscuit diet containing 10% germinated FSF, which recorded the highest value (60%) of NPU between all tested diets as shown in Fig 3.

DISCUSSION

In the recent days there is a trend to use novel sources in the bakery products to decrease the amount of imported wheat by using local and cheap sources, Also fenugreek seed flour have nutritional properties which enhance the nutritional quality of the products which processed by addition of it.

Sensory evaluation of biscuits has revealed that biscuits supplemented by 5 and 10% soaked and germinated FSF had the highest acceptance level for all sensory characteristics, there were no significant differences could be detected among these samples and control biscuit. Whereas, replacement of wheat flour by 15 or 20% FSF was significantly impaired the taste of biscuits, which was attributed to the bitter taste of fenugreek. Chemical analysis showed evident increase in protein, fat, crude fiber and ash contents of substituted biscuits, this was attributed to the higher contents of protein and other ingredients in fenugreek seed flour [6, 13]. On the other hand, a slight decrease in carbohydrate content was noticed in the same samples as compared to control biscuit.

The amino acids composition revealed that wheat biscuit is considered nutritionally poor due to deficiency of most indispensable amino acids (IAAs) especially lysine. Whereas, IAAs content of biscuits containing 5 and 10% soaked and germinated FSF was higher (30.78-34.34 g/100g protein) than control biscuit (24.33 g/100g protein) due to the high amounts of lysine, leucine, isoleucine, theronine and valine in fenugreek seeds [14, 15]. The biscuits made from germinated FSF were higher in total amino acids content than biscuits made from soaked FSF, this might be due to the bioconversion of amino acids during germination [6]. Concerning amino acid score, FSF-biscuits contained most of IAAs at higher concentrations than reference protein pattern of FAO/WHO [11], with exception of lysine and sulfur amino acids. Since, lysine was the first limiting amino acid in wheat biscuit (36%). Lysine score was elevated and reached to 89% after supplementation with 10% germinated FSF. Improvement of lysine score was due to the high lysine contents (4.11-5.14 g/100g protein) in FSF-biscuits formula.

Regarding bio-evaluation of biscuits protein, rat feeding experiments indicated that the rats fed on FSF-biscuit diets exhibited extremely higher ($p<0.05$) values in weights gain, food intake and protein consumption as compared to rats fed on wheat biscuit diet. Hence values of PER, NPR and NPU of those biscuit diets were obviously increased with increasing FSF levels as the result of the quantitative and qualitative improvements in the diet protein after inclusion of FSF [15]. On the other hand, biscuit diet containing 10% germinated FSF recorded the highest values of PER, NPR and NPU between all tested diets.

It could be concluded that, using of 10% germinated FSF into wheat biscuit formula resulted in improving their chemical and nutritional quality criteria. Additionally, it also complemented the deficiency in lysine, leucine, isoleucine, theronine and valine, hence neutralized the amino acids imbalance due to its high IAAs.

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