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INFLUENCE OF FENUGREEK SEEDS FLOUR ON THE RHEOLOGICAL CHARACTERISTICS OF WHEAT FLOUR AND BISCUIT QUALITY

Eid A. El-Naggar*

Food Sci. and Technol. Dept., Al-Azhar Univ., Assuit, Egypt

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ABSTRACT: Nowadays, demand for health-oriented products such as functional foods which has high fiber products is increasing. So, this work studied the effect of partial replacement of wheat flour (72% extraction) by different levels (5%,10%, 15% and 20%) of raw, soaked and germinated fenugreek seed flour (*Trigonella foenum graecum*). The chemical and rheological characteristics for produced dough blends, and on the sensory, physical and chemical characteristics for produced biscuits were studied. Results indicated that incorporation of fenugreek seed flour (FSF) obviously ($P \geq 0.05$) increased in protein, fat, crude fiber, and ash with increasing FSF supplementing levels. The mineral contents of treated fenugreek seed flours almost was higher than the control samples. Germination process of fenugreek seed flour caused a decrease in the most of minerals compared with the soaking process. The replacement of wheat flour with fenugreek seed flour caused a decrease ($P \geq 0.05$) in extensibility of the dough as measured by the extensograph and an increase ($P \geq 0.05$) in resistance to extension, proportional number and dough energy tests. Farinograph results showed that all additions of fenugreek seed flour increased water absorption, dough development time and mixing tolerance index, while decreased dough stability and weakening values in the case of higher replacing with fenugreek seed flour levels. The effects on dough development time, water absorption, and mixing tolerance indices were intensified with GF (germinated fenugreek seed flour) samples under 5% or 10% levels. Generally, the addition of germinated fenugreek flour to wheat flour at ratio of 5 or 10 levels had a less deleterious effect on the chemical and rheological properties of dough than that of RF(raw fenugreek seed flour) and/or SF(soaked fenugreek seed flour). The sensory evaluation results of supplemented biscuit showed that a maximum of 10% FSF can be incorporated to made acceptable quality biscuits. While, biscuits containing 15 or 20% FSF were unacceptable to the panelists as compared with the control biscuit. Results of chemical analyses indicated that incorporation of FSF into biscuits formula obviously ($p \geq 0.05$) increased each of protein, fat, crude fiber, ash and indispensable amino acid (IAAs) contents with increasing FSF supplementing levels. The amino acids composition revealed that supplemented biscuits with FSF contained the most of IAAs and will cover a highly percentages of reference protein pattern of FAO/WHO (1973). Lysine was the first limiting amino acid in control biscuit samples. Supplemented biscuits with germinated FSF had higher lysine contents than other treatments. It could be concluded that, incorporation up to a 10% level of germinated FSF into wheat biscuit formula resulted in improving their chemical, sensory quality characteristics and complementing the deficiency in lysine amino acid.

Key word: Biscuit, fenugreek seed, sensory evaluation, chemical composition, rheological properties.

INTRODUCTION

Biscuits are convenient food product, becoming very popular among both rural and urban populations of Egypt. The quality of biscuits depends on quantity and quality of ingredients, especially the flour (**Manohar and Rao, 1997**). The enrichment of protein may be achieved through incorporation of protein-rich non-wheat flour (**Sharma and Chauhan, 2000**).

***Corresponding author: Tel. :** +201006398936
E-mail address: eid.elnaggar72@gmail.com

Studies should now focus on a search for protein from other sources, such as fenugreek. Fenugreek is an annual herb belonging to the legume family; it is widely grown in Egypt (**El-Nasri and Tinay, 2000**). It becomes a good source of protein and its supplementation in wheat flour would help to improve the nutritional quality of the products (**Hooda and Jood, 2005; Nassar *et al.,* 2008**). It was found that mixing two or more different materials will help to solve the deficiency problem of cereals as low nutritional value by using legumes as food protein source (**Rennan *et al.,* 2008; Masood and Batool, 2010; Feyzi *et al.*, 2015**).

Among them fenugreek seed flour has a great potential, due to its high and good quality protein (20 – 25%), lysine (5 – 6%), soluble (43%) and insoluble dietary fiber (57%) (**Billaudece and Adrain, 2001; Shalini and Sudesh, 2003**). **Hooda and Jood (2005)**. **Afzal *et al.* (2016)** who found that raw fenugreek seeds contained high amount of dietary fiber (46.5%), followed by 42.12% in soaked seeds and 32.5% in germinated seeds. Soaking reduced the level of total soluble sugar, reducing sugar, non-reducing sugars, and dietary fiber and improved the protein, starch digestibility and availability of minerals. Germinated fenugreek seeds had significantly higher contents of total protein (29%) and total lysine (6.48 mg/100, protein) compared to untreated fenugreek seeds. **Hefny (2017)** reported that the addition of 10% of FSF to wheat flour increased protein content, fiber, calcium and iron. This indicates that FSF (fenugreek seed flour) can be incorporated to prepare acceptable biscuits, and may also be mixed with cereals as a supplement for some limiting amino acids and hence for improving their protein quality through amino acid balance.

In vitro protein digestibility and availability of Fe, Ca and Zn were also increased appreciably due to reduction after in anti-nutrient content of phytic acid after 48 hr., of germination (**Billaudece and Adrian, 2001**). On the other side, **Ali *et al.* (2005)** found that the chemical component of wheat flour (72% extraction) was 11.20% moisture, 12.38% protein, 0.54% fiber, 0.50% ash and 0.97% fat. Hence, development and consumption of such therapeutic bakery products would help to raise the nutritional status of the population (**Mathur and Choudhry, 2009**). In addition; it possesses hypocholesterolemic and hypoglycemic properties (**Hegazy and Ibrahium, 2009**). However, the acceptance of protein fortified biscuits depends on their nutrition and organoleptic quality and cost of production (**Kasaye and Jha, 2015**). Some investigation regarding the galactomannan of fenugreek had been carried out by some researchers. The structure of galactomannan of fenugreek seeds has galactose and mannose residues in the ratios of 1 :1 or in few cases of
1 : 2. Fenugreek, with high galactomannan content, swells in cold water. The most important property of galactomannan is the high water binding capacity and formation of very viscous solutions (**Toufeili *et al.,* 1999**). Rheology can be related to product functionality: many rheological tests have been used to predict final product quality such as mixing behavior, dough stability, dough development time, dough extensibility and dough resistance to extension. This is based on the structural engineering analysis of materials (**Pagani, 2006; Pandey and Wasthi, 2015; Wani and Kumar, 2016**).

Roberts *et al.* (2012) and Khorshidian *et al.* (2016), studied the correlation between chemical structure and rheological properties of gluten. They concluded that, cysteine content, degree of amidation and content of hydrophobic side chain amino acids have significantly effects on rheological properties. Rheological quality tends to decrease with increasing contents of low molecular weight protein fractions. Therefore, this study was designed to evaluate the effect of replacement wheat flour by 5, 10, 15 or 20% of raw, soaked and/or germinated fenugreek seed flours on the chemical and rheological characteristics for produced dough blend and on the sensory, physical and chemical characteristics of produced biscuits. Also, investigating the chemical evaluation of amino acid patterns of final products was another target.

MATERIALS AND METHODS

Materials

Fenugreek seeds

A fenugreek seeds *(Trigonella foanum graecum,* Giza–30 cultivar) was obtained from Crops Institute, Agric. Res. Cent., Giza Governorate, Egypt. Biscuit ingredients wheat flour (72% extraction rate) was obtained from Assuit Mills Co., Assuit Governorate, Egypt. Baking powder, sugar, sodium chloride, sodium bicarbonate, ammonium bicarbonate and oil, were purchased from local market, Assuit, Egypt.

Chemical and reagents

All chemicals and reagents were obtained from Sigma chemical co. (St. Lewis, Mo).

Methods

Technological Processes

Preparation of fenugreek seed flour (FSF)

Fenugreek seeds was cleaned and freed of broken seeds, dust and other foreign material and divided into three portions: the first portion was raw; the second portion was soaked, while the third portion was germinated as follows:

Soaking

A fenugreek seeds was soaked in the tap water for 12 hr., at 37°C. Fenugreek seeds to water ratio was 1: 5 (*W/V*). Seeds which did not imbided water were discarded. The soaked seeds were rinsed twice in distilled water and then dried at 55 – 60°C for 4 hr. (**Hooda and Jood, 2005**).

Germination

The soaking fenugreek seeds were germinated at 37°C, for 48 hr., with frequent watering. The sprouts were rinsed in distilled water and dried at 55 – 60°C (**Shalaby *et al.*, 2012**). The dried samples of raw, soaked and germinated seeds were ground to fine powder in electric grinder to particles passing through 20 mesh sieves and then stored in plastic containers for further use.

Preparation of blends

Wheat flour was supplemented by 5, 10, 15 and/or 20% of raw, soaked and germinated fenugreek seed flour. The flour mixtures were individually blended and homogenized, packed in polyethylene bags, tightly closed and stored at room temperature until utilized.

Preparation of biscuit samples

Biscuit samples were prepared according to **Manohar and Rao (1997)** with some modifications, as follows: Biscuit samples were prepared from supplemented wheat flours with different levels of fenugreek seed flour (5%, 10%, 15% and 20% (100g of composite flour), oil (20g of sunflower oil) and sugar (30 g sugar powder) and were mixed for 5 min. with addition of water (16 – 19 ml), sodium bicarbonate (1.00 g) and baking powder (0.30 g). Sugar and oil were creamed in a mixer (N. 50) with a flat beater for 2 min. at 61 rpm. Water containing sodium bicarbonate, ammonium bicarbonate and sodium chloride were added to the cream and mixed for 5 min. at 125 rpm to obtain a homogeneous cream. Flour containing baking powder was added slowly to obtain the biscuits dough. The dough was sheeted to a thickness of about 3 mm. by using rolling machine. The sheeted dough was cut into round shape using a 45 mm diameter cutter. The baking process was carried out in electrically heated oven at 220° C for 12-15 min. The biscuits were left to cool at room temperature for 30 min., peaked in polyethylene bag and stored for analysis and evaluation.

Evaluation of Biscuit

Physical characteristics

Width of biscuits was measured by laying six biscuits edge to edge with the help of a scale rotating them go and re-measuring the width of six biscuits in cm and then taking the average value.

Thickness (T) or height of biscuit was measured by stacking six biscuit on top of one another and taking average thickness of six biscuits in cm. Spread ratio was calculated by dividing the average value of width (W) by average value of thickness (T) of biscuit. Spread ratio was calculated according to (**AACC, 2000**), by dividing the average value of W by T of biscuits.

$$\text{Spread ratio} = \frac{\text{Width (W)}}{\text{Thickness (T)}}$$

Sensory characteristics of biscuits

The organoleptic characteristics of biscuits were determined, by a taste panel, consisting of 10 judges. The panelists were asked to evaluate the products for appearance, colour, texture, flavour and overall acceptability. The rating were on a 9- point hedonic scale, ranging from 9 (like extremely) to 1 (dislike extremely), for each organoleptic characteristics (**Hooda and Jood, 2005**).

Chemical Analyses

Proximate composition of samples was determined according to **AOAC (1995)**. Amino acids was determined using automatic amino acid analyzer according to the method of **FAO/ WHO (1973)**. Acid hydrolysis of the samples were performed in the presence of 6 M HCL at 110°C for 24 hr., under nitrogen atmosphere. Tryptophan was chemically determined by the method of **Miller (1967)**. 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 (1973)**. The lowest score obtained will be considered as the first limiting amino acid subsequently second limiting amino acids.

A total soluble sugar was determined according to a colorimetric method (**Dubois *et al.*, 1956**). Reducing sugars was estimated by a modified neocuprione method described by **Dygert *et al.* (1965)**. Non-reducing sugars were determined by the difference. The total amounts of Ca, Fe and Zn in the digested samples were determined by atomic absorption spectrophotometry (**AOAC, 1995**).

Phytic acid was determined by the method of **Uppstrom and Svensson (1980)**. Total polyphenols were extracted and estimated as tannic acid equivalent, according to the method of **Price *et al.* (1978)**.

Rheological properties

Blends of 0%, 5%, 10% and 20% were prepared by substituting the flour with raw, soaked and germinated FSF. The effect of different FSF on dough rheology was determined by: First; Farinograph characterstics (Barabender, Farinograph^R^, Nr. 184543, type, 810104, at Egyptian Baking Technology Center, Giza, Cairo, Egypt) (water absorption, arrival time, dough stability, dough tolerance index, dough weakening and dough development time were carried out according to the method of the **AACC (2000)**. Second; Extensograph characteristics (Barabender, Duisberg^R^, Nr. 184519, type, 860000, at Egyptian Baking Technology Center, Giza, Cairo, Egypt) (Extensibility mm), (resistance to extension BU), (dough strength energy cm^2) and proportional number were carried out according to the method of the **AACC (2000)**.

Statistical Analysis

The experimental data was subjected to analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system **SAS (2000)**. The least significant difference (LSD) test was used to determine the differences among means at the level of 0.05.Mean values of three determinations were calculated.

RESULTS AND DISCUSSION

Chemical Composition of Wheat Flour Supplemented with Raw, Soaked and Germinated Fenugreek Seed Flours

The chemical composition of WF, FSF and composite flour made from wheat and different treated FSF are shown in Table 1. Results showed that, wheat flour (WF) (72% extraction rate) was approximate analyzed for moisture, protein, fat, fiber, ash and the total nitrogen free (TNF) extract were 16.86 , 8.89, 1.27, 0.25, 0.55 and 72.09%, respectively. Whereas, soaked fenugreek seed flour (SFF) was 4.23, 29.93, 5.86, 10.47, 5.05 and 44.46%, respectively. While, germinated fenugreek seed flour (GFF) was 4.92, 30.23, 5.61, 10.81, 5.32 and 43.11%, respectively. Meanwhile, raw fenugreek seed flour (RFF) was 4.16, 29.85, 1.91, 10.26, 5.62 and 45.20%, respectively. From these results it could be stated that, the protein, crude fiber and ash content of composite flours were increased significantly ($P \leq 0.05$) with increasing the supplementing of WF with FSF. Protein content values ranged from 10.51% for sample (75 WF: 5RF) to 16.65% for sample (80WF: 20 GF). The highest crude fiber content was recorded in (80WF:20GF) having a value of 3.30% while sample (95WF:5RF) recording the lowest value of 1.20%. (90WF:10RF) recorded the highest ash content of 1.91% and the lowest being (95 WF : 5RF) with 0.53% ash content. These results agree well with those reported by **Sayed *et al.* (2000)**. The increase in ash, protein and crude fiber content of blend flours related to high ash, protein and crude fiber contents of FSF than WF. The increasing of FSF was proportion in composite flour resulted in decreasing in nitrogen free extract (NFE) content of flour blend which may be related to the lower content of NFE in FSF than in WF (**Hefny, 2017**).

Table 1. Chemical composition of wheat flour supplemented with raw, soaked and germinated fenugreek seed flours

Sample	Moisture (%)	Protein (%)	Ash (%)	Crude fiber (%)	Fat (%)	NFE (%)	Mineral (mg/100g)		
							Ca	Fe	Zn
WF	16.88	8.89	0.55	0.25	1.27	72.09	55.8	7.14	1.70
RF	4.16	29.85	5.62	10.26	1.91	45.2	120	35.2	3.83
(95WF:5RF)	17.11	10.51	0.53	1.20	1.65	69.88	74.2	9.5	1.72
(90WF:10RF)	16.47	10.62	1.91	1.85	1.95	66.20	92.6	11.85	1.74
(85WF:15RF)	15.74	11.49	1.75	2.95	1.97	65.10	95.7	12.50	1.88
(80WF:20RF)	16.24	13.50	1.50	3.20	2.05	63.50	99.15	13.90	2.05
LSD 5%	0.65	0.95	0.45	0.54	0.15	0.70	21.1	3.5	0.85
SFF	4.23	29.93	5.05	10.47	5.86	44.46	110	30.7	3.55
(95WF:5SF)	19.52	10.56	0.74	1.30	1.61	66.27	75.33	9.63	1.74
(90WF:10SF)	18.05	11.77	1.80	1.80	1.90	64.60	94.86	12.14	1.75
(85WF:15SF)	17.61	13.85	1.75	2.50	1.99	62.30	97.10	12.95	1.84
(80WF:20SF)	17.45	15.65	1.45	3.25	2.10	60.10	100.25	14.50	2.00
LSD 5%	0.67	0.75	0.41	0.55	0.20	0.60	20.2	2.85	1.15
GFF	4.92	30.23	5.32	10.81	5.61	43.11	105	35.4	3.50
(95WF:5GF)	19.02	12.60	0.78	1.35	1.58	64.67	71.44	9.85	1.55
(90WF:10GF)	19.76	14.89	1.66	1.85	1.84	60.00	90.65	11.49	1.60
(85WF:15GF)	19.78	1557	1.60	2.60	1.95	58.50	90.0	12.14	1.75
(80WF:20GF)	19.60	16.65	1.30	3.30	2.00	57.15	101.6	13.25	1.90
LSD 5%	0.70	070	0.51	0.61	0.18	0.54	10.5	1.92	1.20

Means in the same row were calculated by different significantly ($P \leq 0.05$) of all treatments
NFE: The nitrogen free extract was calculated by difference; RF: raw fenugreek seeds flour. WF = wheat flour
SF: Soaked fenugreek seeds flour; GF: Germinated fenugreek seeds flour.

Additionally, the ash content represents the total amount of minerals present in the composite flour, thereupon, the mineral contents of different treated FSF almost higher than the control wheat flour sample. Soaking and germination processes of FSF had a slight effect ($P \geq 0.05$) on the percentage of mineral composition. Germination process of FSF caused more decrease ($P \geq 0.05$) in most of the minerals higher than the soaking process. These changes in components may be due to consumption in respiration during germination (**Sharma and Chauhan, 2000; Wani and Kumar, 2016**).

Rheological Characteristics of Wheat Flour Dough Supplemented with Raw, Soaked and Germinated Fenugreek Seed Flour

The effect of supplementation with FSF (fenugreek seed flour) on the rheological characteristics of dough was examined by using farinograph and extensograph apparatus on the fermentation period (135 min).

Farinograph tests

The effect of replacing wheat flour by different levels of RF (raw), SF (soaked) and/or

GF (germinated fenugreek seeds) at levels of 5 ; 10 ; 15 and 20% on Farinograph tests is presented in Table 2 and Fig. 1 of farinograph curves. Results indicated that water absorption, mixing time, dough development increased with increasing the levels of RF, SF and/or GF comparing with control wheat flour dough. Water absorption of WF started with 54.80% and reached to 60.40% after the addition up to 20% of RF, SF and /or GF. The increase in the water absorption in the cases of 5% and 10% of RF, SF and GF were marginal. Results indicated insignificance ($P \leq 0.05$) differences in water absorption at the different levels of SF, but the replacing by RF and/or GF at 15 and 20% lead to increase ($P \geq 0.05$) the water absorption. The increasing water absorption is mainly caused by the greater number of hydroxyl groups. These results show a direct proportion between the protein content of supplementing material and water absorption. This is due to more protein content and more water absorption. The obtained results are in accordance with those of **Jyothsna and Rao (1997) and Develi and Karababa (2005)**.

Development (mixing) time is the time from the first addition of water to the time the dough reaches the point of greatest torque. During this phase of mixing, the water hydrates the flour components and the dough is developed. Results declared that replacing WF by different levels of RF, SF and GF had significant ($P \geq 0.05$) changes compared with control wheat flour sample (1.5 min). Dough mixing studies showed that inclusion of RF, SF and GF blends increased both of farinograph mixing time and dough stability when substituted by wheat flour. Also, results indicated insignificant ($P \leq 0.05$) changes in the stability time under 5% and 10% replacement levels of SF and/or GF. While, replacing FSF by 15 or 20% lead to decrease ($P \geq 0.05$) the stability time, which started with 3.5 min of the control to (2.5, 3.5 and 3.6 min) at levels of and 20% for RF, SF and/or GF, respectively. Similar results were obtained by **Manohar and Rao (1997) and Develi and Karababa (2005)**. According to the weakening of the dough resulted, given in Table 2 and Fig. 1 of Farinograph curves, declared that, addition of FSF at 5% and 10% levels resulted in a decrease ($P \geq 0.05$) in the dough weakening parameter values. Weakening values relatively decreased by increasing the supplementing level, up to 10% in the case of RF and SF incorporation, and reached up to 15% for GF incorporation. Moreover, the highest ($P \geq 0.05$) increase (more weakening) were observed at 15 and 20% levels for SF which reached to 100 and 110 BU, respectively, while it was 100 BU for the control wheat sample. But, results indicated that slightly significant ($P \leq 0.05$) difference were recorded in dough weakening values under 5 and 10 levels for SF (soaked fenugreek flour dough) and GF (germinated fenugreek flour dough). Therefore, the increment in the dough weakening at 15 and 20% may be explained by the small amount of gluten in the blend. Therefore, the point of contact would be few and weak leading to increase the weakening value. With respect to the tolerance index, it was noticed that, the addition of FSF obviously caused significant ($P \geq 0.05$) differences compared with control wheat flour sample (Table 2). While meaning that tolerance index proved a positive proportional relation with added percentages of FSF. These findings could be related to the breakdown of gluten network and its small amount. These findings are similar with those obtained by **Manohar and Haridas (2002)**.

Extensograph tests

The effect of replacing wheat flour with different levels of raw FSF, soaked FSF or germinated FSF on the extensograph tests is represented in Table 3 and Fig. 2 of extensograph curves. Concerning the resistance to extension, results indicated an increase ($P \geq 0.05$) in the resistance to extension which recorded 260, 280, 283, and 285 BU at levels of 5, 10, 15 and 20% addition of raw FSF; respectively comparing with control wheat flour samples (160 BU). This may likely be due to the presence of galactomanans which used in the food industry for their ability to thickening and stabilizing properties (**Sudha *et al.*, 2007; Wani and Kumar, 2016**). On the other hand, no significant ($P \leq 0.05$) differences were observed between the resistance to extension at level of 5% and 10% from of the soaked or germinated fenugreek seeds flour. Regarding to extensibility results, a fluctuant reduction in extensibility values was observed upon increasing the levels of RF, SF and GF. The dough extensibility started with 140 mm. in control sample, and then

Table 2. Farinograph parameters of wheat flour dough supplemented with raw, soaked and germinated fenugreek seed flour

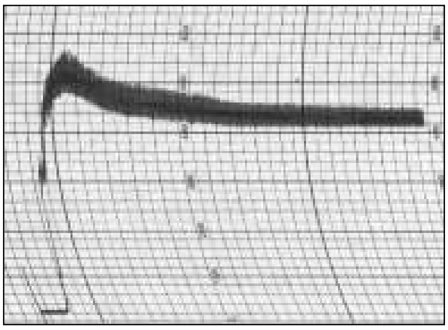
Farinograph data		Water absorption (%)	Dough development time (min)	Dough stability (min)	Tolerance index (BU)	Weakening (BU)
Control		56.00	1.50	3.50	50	100
WF: RF	5	57.00	1.60	2.60	80	90
	10	58.00	1.80	3.80	85	95
	15	58.80	2.50	2.50	100	100
	20	59.60	3.00	2.50	130	110
LSD 5%		0.585	0.821	0.950	15.17	1.99
WF: SF	5	54.80	1.60	4.00	70	95
	10	57.50	1.80	4.00	75	100
	15	55.00	2.50	3.50	80	110
	20	56.00	3.00	3.50	85	115
LSD 5%		0.675	0.950	0.980	13.45	1.85
WF: GF	5	56.80	1.50	4.50	65	85
	10	57.50	1.60	4.50	75	90
	15	59.30	2.00	3.60	80	95
	20	60.40	2.50	3.60	80	100
LSD 5%		0.570	1.10	1.24	11.98	1.77

Means in the same column were expressed by different significantly ($P \leq 0.05$).

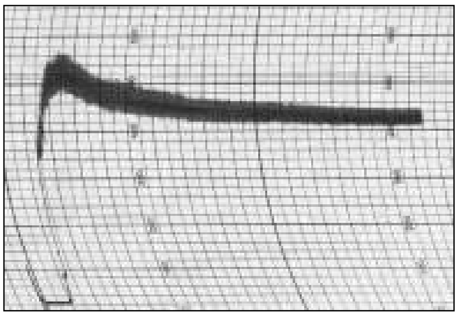
Table 3. Extensograph parameters of wheat flour dough supplemented with raw, soaked and germinated fenugreek seed flour at different levels

Extensograph data		Dough extensibility (E) mm	Dough resistance to extension (R) BU	Proportional number R/E	Dough energy $(cm)^2$
Control		140	160	1.14	30
WF: RF	5	120	260	2.16	33
	10	120	280	2.33	36
	15	130	283	2.17	40
	20	125	285	2.28	45
LSD 5%		1.93	2.50	--	4.73
WF: SF	5	130	175	1.34	32
	10	130	190	1.46	37
	15	130	210	1.61	38
	20	125	220	1.76	40
LSD 5%		1.83	2.37	--	4.25
WF: GF	5	130	180	1.38	35
	10	134	190	1.41	37
	15	140	200	1.42	40
	20	140	210	1.50	41
LSD 5%		**1.59**	**2.85**	**--**	**4.022**

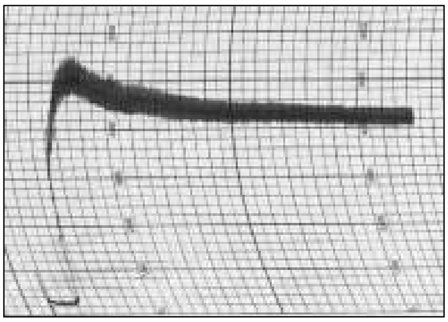
Means in the same column were calculated by different significantly ($P \leq 0.05$).



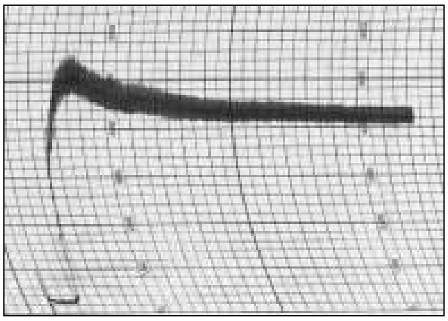
Control



95% WF +5% RF



90% WF+ 10% RF



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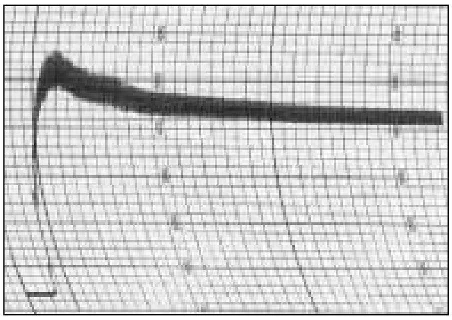
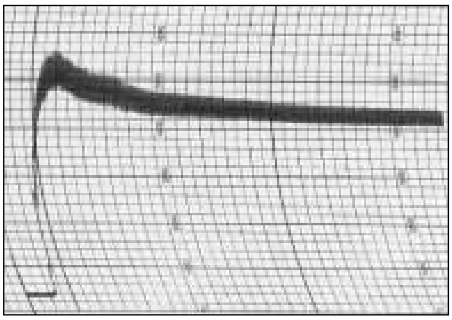
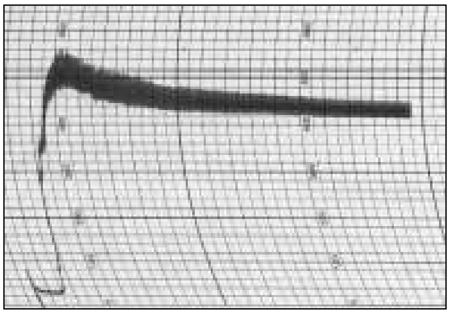


90% WF+ 10% SF



95%WF +5% GF



90% WF+ 10% GF

Fig. 1. Farinogragh curves of WF(72%) dough supplemented with raw, soaked and germinated fenugreek seed flour at 5% and 10% (Control wheat flour, 72%)

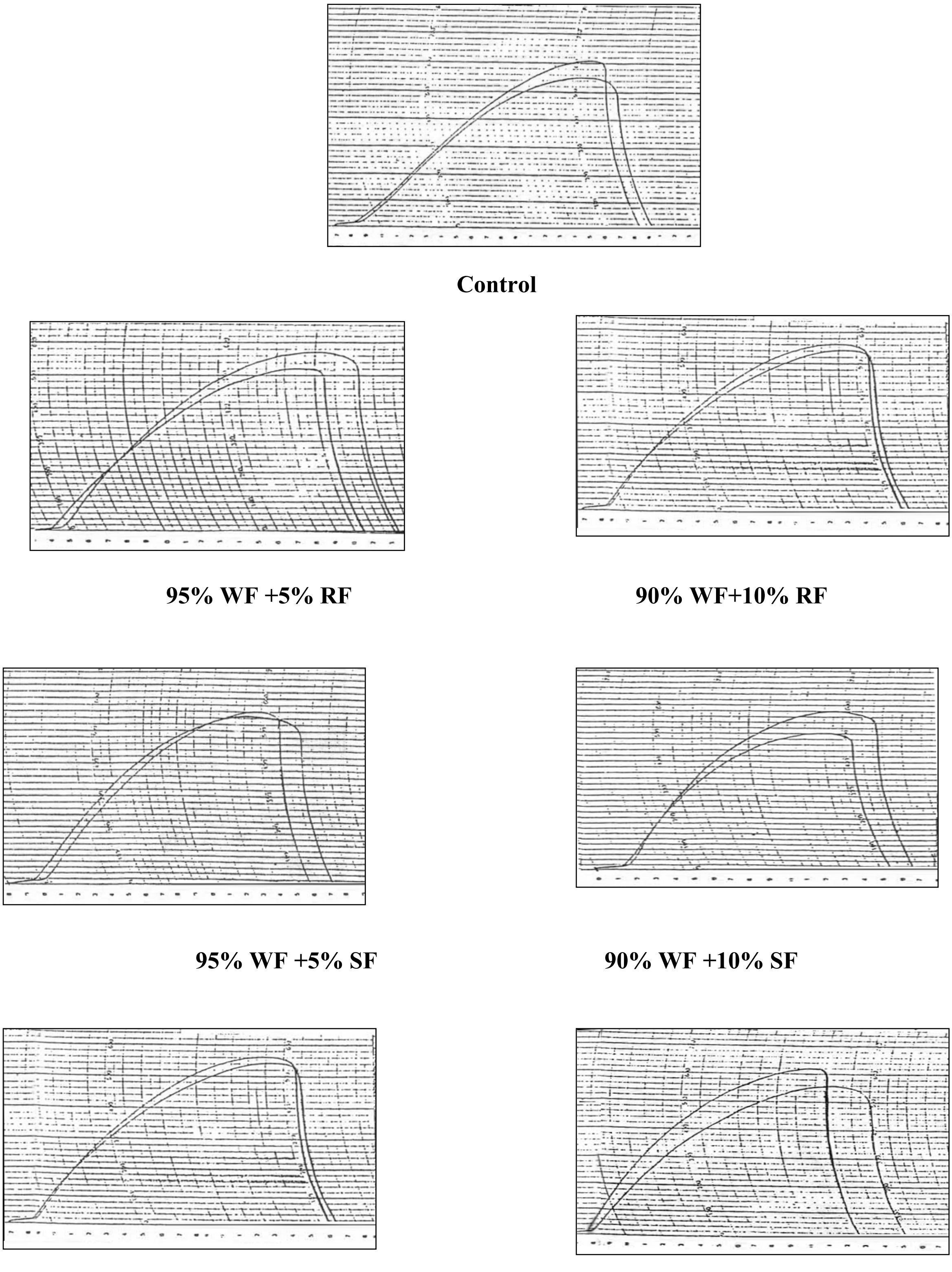


Fig. 2. Extensogragh curves of WF (72%) dough supplemented with raw, soaked and germinated fenugreek seed flour at 5% and 10% (Control wheat flour, 72%)

reduced to 120 mm in case of 5 and 10% levels of RF. These findings may be attributed to the deficiency of gliadine in fenugreek protein. Concerning the dough energy, the results indicated an increase ($P \geq 0.05$) in dough energy with increasing the levels of RF, SF and GF, which may be attributed to the high resistance to extension. Herein, area under the curve increased with the increase in the level of fenugreek seed flour in the blends. With respect to the addition of different levels of treated FSF an increase ($P \geq 0.05$) in the proportional number was observed, which could be related to the increase of resistance to extension and the reduction in extensibility. The proportional number increased with the increasing level of RF seed flour indicating the dough becoming harder in the presence of RF blends at a ratio of 15% or 20% level. The extent of increase in GF or SF blends was marginal. These findings are similar with those obtained by **Weegels *et al.* (1996), Toufeili *et al.* (1999), Manohar and Haridas (2002), Ammar *et al.* (2009) and Hussein *et al.* (2012)**.

Organoleptic Properties of Wheat Biscuits Supplemented with Raw, Soaked and Germinated Fenugreek Seed Flours at Different Levels

Biscuits formulations were prepared using wheat flours supplemented with different levels (5%, 10%, 15% and 20% of RF, SF and GF seed flour. The effects of fenugreek seed flour supplementation on the sensory evaluation of biscuits are presented in Table 4. With increasing level of RF, SF or GF in the formulation, the sensory scores for colour, texture and flavour of biscuits significantly ($p \geq 0.05$) decreased. Results indicated that there were no significant ($p \leq 0.05$) differences among control wheat biscuits sample and biscuit samples containing 5 or 10% fenugreek seed flour in all sensory properties. While, the biscuits containing 15 or 20% of FSF were significantly different ($p \geq 0.05$) in all sensory properties and were unacceptable to the panelists as compared to the control wheat biscuits sample. Therefore, it excluded from the overall acceptability rating. Fenugreek seed flour could be incorporated up to 10% level in the formulation of biscuits without affecting their sensory quality. The overall acceptability scores for biscuit samples containing 5 or 10% FSF were ranged from 7 to 8.50 against 9 of control wheat biscuit. Whereas, overall acceptability scores for biscuit samples containing 15 or 20% FSF were ranged from 4.66 to 6, against 9 of control wheat biscuit. These results goes compained with the results obtained by **Sharma and Chauhan (2000), Gamlath and Ravindran (2008), Elizabeth *et al.* (2012) and Laura *et al.* (2013)**. It was found that best supplementing levels with GF followed with SF then RF seeds flour were 5% and 10% levels. Therefore, the substitution of WF with different ratios (5 and 10%) of germinated fenugreek seed flour gave biscuits more acceptable for appearance, texture, flavour, taste and overall acceptability than that of biscuits made from the other ratios (15 and 20%) of GF, SF, OR RF (fenugreek seed flour). These results are in agreement with those obtained by **Hefny (2017)**.

Physical Characteristics of Wheat Biscuits Supplemented by Raw, Soaked and Germinated Fenugreek Seed Flour

Biscuits were prepared using 0%, 5%, 10%, 15% and 20% of RF, SF and GF seeds flour. Flour samples in the blends were evaluated for physical properties. Physical characteristics of biscuit (Width, Thickness and Spread ratio) were affected significantly ($p \geq 0.05$) with the increase in the level of FSF as shown in Table 5. Substitution of WF with 5 and 10% of FSF caused decreasing in width of biscuits before baking and increasing in the width after baking .The reason for the decreasing in width may due to the biscuit cell structure being unable to retain gas during proofing (**Feyzi *et al.*, 2015; Hefny 2017**). Results indicated that, the average width of control biscuit before baking was 23.36 cm, whereas after baking was 24.66 cm. While that supplemented biscuits varied from 23.22 to 23.27 cm. before baking and 24.23 to 24.40 cm. after baking (raw FSF), 23.18 to 23.21 cm. before baking, while 24.23 – 24.32 cm. after baking (soaked FSF) and 23.30 – 23.35 cm. before baking, whereas 24.28 – 24.44 cm. after baking (germinated FSF) under 5 – 10 % levels. The thickness of the biscuits was increased with increment the supplementation of WF with FSF. The average thickness of control biscuit was 1.50 cm. and for other supplemented levels, it

Table 4. Organoleptic properties of wheat biscuits supplemented with raw, soaked and germinated fenugreek seed flours

Treatment		Colour	Appearance	Texture	Flavour	Taste	Overall acceptability
Control		9.00	8.50	8.15	9.00	8.00	9.00
WF: RF	(95WF:5RF)	8.43	8.42	8.36	8.89	8.50	8.52
	(90WF:10RF)	8.20	8.30	8.40	8.30	8.30	8.30
	(85WF:15RF)	6.76	6.00	5.00	5.66	5.99	5.00
	(80WF:20RF)	6.00	6.00	4.00	5.00	4.86	4.66
WF: SF	(95WF:5SF)	8.90	8.97	8.86	8.50	8.75	8.79
	(90WF:10SF)	8.86	8.85	8.50	8.86	8.32	8.67
	(85WF:15SF)	7.22	7.35	5.30	6.00	5.46	6.00
	(80WF:20SF)	5.12	5.30	4.00	4.22	4.12	4.34
WF: GF	(95WF:5GF)	8.99	8.95	8.95	8.90	8.50	8.85
	(90WF:10GF)	8.88	8.86	8.55	8.40	8.76	8.69
	(85WF:15GF)	6.40	6.42	5.90	6.00	5.00	5.75
	(80WF:20GF)	5.22	5.35	4.50	4.70	4.47	4.66
LSD 5 %		2.439	1.89	2.15	2.05	1.90	2.25

Means in the same column were calculated by different significantly ($P \leq 0.05$).

Table 5. Physical characteristics of wheat biscuits supplemented with raw, soaked and germinated fenugreek seed flour

Treatment		Width, W (cm)	Thickness T (cm)	Spread ratio W/T
Control	B	23.36	1.50	15.70
	A	24.66	2.20	11.20
WF: RF	(95WF:5 RF) (B)	23.27	1.56	14.91
	(95WF:5 RF) (A)	24.40	2.31	10.56
	(90WF:10 RF) (B)	23.22	1.66	13.98
	(90WF:10 RF) (A)	24.23	2.35	10.31
LSD 5%		0.405	0.320	---
WF: SF	(95WF:5 SF) (B)	23.21	1.65	14.15
	(95WF:5 SF) (A)	24.32	2.34	10.39
	(90WF:10 SF) (B)	23.18	1.71	13.55
	(90WF:10 SF) (A)	24.19	2.38	10.16
LSD 5%		0.309	0.046	---
WF: GF	(95WF:5 GF) (B)	23.35	1.53	15.26
	(95WF:5 GF) (A)	24.44	2.28	10.71
	(90WF:10 GF) (B)	23.30	1.64	14.20
	(90WF:10GF) (A)	24.28	2.33	10.42
LSD 5%		0. 330	0.489	---

Means in the same column were expressed by different significantly ($P \leq 0.05$).
WF: wheat flour B: before baking, A: after baking

varied from 1.56 to 2.35 cm. (raw FSF), 1.65 – 2.38 (soaked FSF) and 1.53 – 2.33 cm. (germinated FSF) at 5–10% levels. The changes in width and thickness are reflected in spread ratio which was calculated by dividing the width (W) by thickness (T) of biscuit. Spread ratio of control biscuit was 15.70 before baking and 11.20 after baking, which decreased significantly ($p \geq 0.05$) and consistently from 10.31 – 10.56 in RF, 10.16 – 10.39 in SF and 10.42 – 10.71 in GF after baking under 5 – 10 % levels. Similar results were obtained by **Wattrers (1978), Kirssel and Prentice (1979), Chen *et al.* (1996), Hooda and Jood (2005), Ajila *et al.* (2008) and Ammar *et al.* (2009)**.

Chemical Composition of Wheat Biscuits Supplemented with Raw, Soaked and Germinated Fenugreek Seed Flour

Proximate composition of biscuits made from wheat flour supplemented with 5 or 10% of (raw, soaked or germinated) FSF is tabulated in Table 6. Results showed that moisture, protein, fat, crud fiber and ash contents were obviously increased ($p \geq 0.05$) by increasing FSF replacement levels as compared with wheat biscuit of control. While, a slight decrease ($p \geq 0.05$) in NFE content of these samples were observed, and reached to 62.00, 61.90 and 60.60% under 10% replacement level of biscuits containing raw, soaked and germinated fenugreek seed flour, respectively, against 64% of wheat biscuit as control. The minerals content were increased significantly ($p \geq 0.05$) by increasing FSF replacement levels as compared with control wheat biscuit. These results agree with those reported by **Iskander and Davis (1992)**. The increase in protein content of FSF supplemented biscuits might be due to the result of the appreciably higher protein content of fenugreek (**Gandhi *et al.,* 2001; Khorshidian *et al.,* 2016**).

Sugars, Phytic Acid and Polyphenols of Wheat Biscuits Supplemented with Raw, Soaked and Germinated Fenugreek Seed Flour

Total, reducing and non-reducing sugar contents of biscuit made from wheat flour supplemented with FSF are presented in Table 7. The biscuits supplemented by 5% and 10% replacement levels of RF and SF showed a non-significantly ($p \leq 0.05$) decrease in total, reducing and non-reducing sugar contents as compared to wheat biscuit as control. Among the supplemented biscuits, biscuits with SF-supplemented flour exhibited minimum values of total, reducing and non-reducing sugars valued 13.28%, 5.10% and 8.18%, respectively. While, supplemented biscuits with GF-flour exhibited maximum contents of total, reducing and non-reducing sugars. This increase might be due to hydrolysis of seed polysaccharide during germination of fenugreek, leading to more available sugars (**Hooda and Jood, 2005; Khorshidian *et al.,* 2016**).

The results regarding the phytic acid and polyphenols are given in the same table. Wheat biscuits had 223 mg/100 g phytic acid and increased significantly ($p \geq 0.05$) with the rise in the level of RF. The maximum increase of 1.34% and 17.9% in phytic acid over the control was found in biscuit containing RF flour with 5% and 10% replacement level, respectively, while the maximum decrease (15.24%) was found in soaked fenugreek flour, under the control. But, the minimum increase (2.24 %) was found in GF, over the wheat biscuit as control.

Contrary to this, polyphenolic contents decreased progressively with increasing level of fenugreek flour. Among the supplemented biscuits, GF–supplemented biscuits had contained significantly ($p \geq 0.05$) lower contents of poly phenols under 5% and 10% replacement levels. This might be due to oxidation and decarboxylation of the phenolic acid during germination (**Ammar *et al.,* 2009; Wani and Kumar, 2016**).

Amino Acids Composition of Biscuits Supplemented with Raw, Soaked and Germinated Fenugreek Seed Flour

It was evaluated according to its content of amino acids, as shown in Table 8. The amount of total essential amino acids (EAA) in fenugreek-supplemented biscuits containing 5% and 10% levels of RF, SF and GF increased ($p \geq 0.05$) that of wheat biscuit as control, being ranged from 3.874 to 5.18 against 2.92 g/100 g of biscuit. Also, incorporation 5 and/or 10% levels of FSF into wheat flour resulted increase ($p \geq 0.05$) of total non essential amino acids (non EAA) content, being ranged from 8.93 to 9.82 against 7.76 g/100g of biscuit (**Kasaye and Jha, 2015**).

Table 6. Chemical composition of wheat biscuits supplemented with raw, soaked and germinated fenugreek seed flour

Treatment		Moisture (%)	Fat (%)	Protein (%)	Crude fiber(%)	Ash (%)	NFE	Ca mg/100g	Fe mg/100g	Zn mg/100g
Control		3.33	21.00	9.34	1.98	1.75	64	56.30	4.64	2.41
WF: RF	(95WF:5 RF)	3.37	21.15	9.88	2.13	1.79	63	78.10	7.20	3.49
	(910WF:10 RF)	3.43	21.24	10.15	2.35	1.83	62	79.25	7.50	4.10
WF: SF	(95WF:5 SF)	3.35	21.13	9.70	2.25	1.75	63	71.60	7.10	3.30
	(90WF:10 SF)	3.40	21.22	10.12	2.46	1.82	61.90	72.50	7.40	3.50
WF:GF	(95WF:5 GF)	3.39	21.10	10.20	2.34	1.82	62.10	65.10	7.25	3.40
	(90WF:10 GF)	3.45	21.12	10.80	2.66	1.89	60.60	67.20	7.45	3.65
LSD 5%		0.449	3.506	1.778	0.167	0.160	1.28	0.88	0.54	0.51

Means in the same column were calculated by different significantly (P≤0.05)
NFE: The nitrogen free extract was calculated by difference.

Table 7. Sugars, phytic acid and polyphenols of wheat biscuits supplemented with raw, soaked and germinated fenugreek seed flour

Treatment		Sugar			Phytic acid (mg/100g) of biscuits	polyphenols (mg/100g) of biscuits
		Total (%)	Reducing (%)	Non reducing (%)		
Control		13.32	5.00	8.32	223	286
WF:RF	(95WF:5 RF)	13.40	5.15	8.25	226	280
	(90WF:10 RF)	13.45	5.19	8.26	263	273
WF:SF	(95WF:5 SF)	13.31	5.10	8.21	197	232
	(90WF:10 SF)	13.28	5.10	8.18	189	239
W:GF	(95WF:5 GF)	14.30	5.75	8.55	241	224
	(90WF:10 GF)	15.30	6.12	9.18	228	213
LSD 5%		1.233	0.70	1.75	11.06	15.03

Means in the same column were expressed by different significantly (P≤0.05).
RF: raw fenugreek seed flour; SF: Soaked fenugreek seed flour; GF: Germinated fenugreek seed flour.

Table 8. Amino acids content (g/100g) of wheat biscuits supplemented by raw, soaked and germinated fenugreek seed flour

Amino acid	Control 72 %	RF		SF		GF		LSD 5%
		5 %	10 %	5 %	10 %	5 %	10 %	
Leu.	0.678	1.02	1.11	1.093	1.16	1.10	1.18	
Ile.	0.299	0.354	0.464	0.44	0.52	0.46	0.53	
Lys.	0.251	0.601	0.611	0.59	0.67	0.69	0.78	
Met. + Cys.	0.196	0.200	0.25	0.24	0.28	0.25	0.31	
Phe. + Tyr	0.781	0.875	1.015	0.98	1.074	0.92	1.10	
Thr.	0.244	0.235	0.47	0.44	0.48	0.40	0.49	
Thrp.	0.122	0.172	0.18	0.175	0.18	0.16	0.20	
Val.	0.342	0.417	0.498	0.485	0.54	0.47	0.59	
Total IAA	2.92	3.874	4.59	4.44	4.904	4.45	5.18	
Asp.	0.498	0.95	1.03	1.02	1.06	1.03	1.12	0.087
Ser.	0.589	0.495	0.713	0.69	0.71	0.63	0.72	
Gln.	4.085	4.32	4.41	4.32	4.33	4.30	4.44	
Pro.	0.735	0.85	0.93	0.92	0.99	0.93	1.02	
Gly.	0.231	0.26	0.33	0.32	0.36	0.33	0.42	
Ala.	0.370	0.480	0.497	0.48	0.54	0.50	0.58	
His.	0.253	0.24	0.33	0.32	0.37	0.39	0.432	
Arg.	0.996	1.34	1.40	1.37	1.42	0.98	1.09	
Total Non EAA	7.76	8.93	9.64	9.44	9.78	9.09	9.82	
Total amino acids	10.68	12.804	14.23	13.88	14.68	13.54	15	

Means in the same row were expressed by different significantly ($P \leq 0.05$) of all treatments
IAA: indispensable amino acids; DAA: dispensable amino acids. ; RF: raw fenugreek seed flour.
SF: Soaked fenugreek seed flour; GF: Germinated fenugreek seed flour.

Amino Acids Content (g/100g) of Wheat Biscuits Supplemented with Raw, Soaked and Germinated FSF, Comparing with Reference Protein Pattern of FAO/WHO

Table 9 shows that EAA content of different biscuit samples compared with the reference protein pattern of **FAO/WHO (1973)**. From this Table, it could be observed that fenugreek supplemented biscuits contained the most of IAAs and will cover a highly percentages of reference protein pattern of FAO/ WHO, with exception of lysine and sulphur amino acids (Met.+ Cys.). Since, the amino acid score of this amino acid was lower than others. Also, lysine was the first limiting amino acid in control (wheat) biscuit (4.46%), when biscuits were supplemented by FSF; lysine score elevated and reached to 14.18% in biscuit containing 10% germinated FSF. Among the supplemented biscuits, germinated FSF supplemented biscuits had higher lysine contents than others treatments. This could be contributed to higher lysine contents of germinated fenugreek flour, lysine content improved on germination due to bioconversion (**Shalaby *et al.,* 2012; Feyzi *et al.,* 2015; Wani and Kumar 2016**).

Table 9. Amino acids score of wheat biscuits supplemented with raw, soaked and germinated fenugreek seed flour, compared to reference protein pattern of FAO/WHO

Amino acids (g/100g) of biscuits	Control 72 %	RF		SF		GF		FAO/ WHO
		5 %	10 %	5 %	10 %	5 %	10 %	
Le.	9.68	14.57	15.85	15.61	16.57	15.71	16.85	7.00
Ile.	7.47	8.85	11.60	11.00	13.00	11.5	13.25	4.00
Lys.	4.56	10.92	11.10	10.72	12.18	12.54	14.18	5.50
Met. + Cys.	5.6	5.71	7.14	6.85	8.00	7.14	8.85	3.50
Phe. + Tyr.	29.68	14	16.91	16.33	17.90	15.33	18.33	6.00
Thr.	6.10	5.87	11.75	11.00	12.00	10.00	12.25	4.00
Trp.	12.20	17.20	18.00	17.5	18.00	16.00	20.00	1.00
Val.	6.84	8.34	9.96	9.70	10.80	9.40	11.80	5.00

RF: raw fenugreek seed flour; SF: Soaked fenugreek seed flour; GF: Germinated fenugreek seed flour.

Conclusion

It may be concluded from this study that fenugreek seeds flour could be incorporated up to a 10% replacement level in the formulation of biscuits without affecting their overall quality. The physical, chemical and sensory characteristics, in general, revealed that biscuits containing 10% germinated fenugreek seeds flour were the best among all the composite FSF biscuits. Hence, utilization of such function foods will not only improve chemical and sensory quality characteristics but may be also help those suffering from degenerative diseases. Additionally it also complemented the deficiency in lysine, hence neutralized the amino acids imbalance due to its high IAAs contents.

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تأثير دقيق بذور الحلبة علي الخواص الريولوجية لدقيق القمح وجودة البسكويت

عيد السيد عبدالعزيز النجار

قسم علوم وتكنولوجيا الأغذية - كلية الزراعة - جامعة الأزهر فرع أسيوط - مصر

يتزايد الطلب في الوقت الحاضر علي المنتجات الصحية الموجهة كالأغذية الوظيفية عالية الألياف، لذا فقد تم دراسة تأثير الاستبدال الجزئي لدقيق القمح (استخلاص ٧٢%) بنسب مختلفة (٥%، ١٠ %، ١٥%، ٢٠%) من دقيق الحلبة صنف جيزة ٣٠ (الخام أو المنقوع أو المنبت) على الخواص الكيميائية والريولوجية لخلطات الدقيق الناتج بواسطة جهازي الأكستنسوجراف والفارينوجراف مع مقارنته بالدقيق الناتج من دقيق القمح استخلاص ٧٢%، وقد دلت نتائج دراسة الاستبدال الجزئي للتركيب الكيميائي لخلطات العجين زيادة المحتوى من البروتين، والدهن، والألياف الخام، والرماد، والليسين، كما تبين زيادة المعادن لدقيق الحلبة المعامل مقارنة بالعينات القياسية، وأن معاملة الإنبات لدقيق الحلبة سببت نقصاً في معظم المعادن المختبرة مقارنة بمعاملة النقع، وأظهرت النتائج المتحصل عليها من جهاز الفارينوجراف زيادة نسبة امتصاص الماء، ومعامل تحمل الخلط، ومدة الخلط، بينما لوحظ نقص في قيم مقياس ضعف العجين، وفترة الثبات بزيادة مستوى الاستبدال بدقيق الحلبة، وأن كلاً من مدة الخلط ونسبة امتصاص الماء ومعامل تحمل الخلط كانت الأفضل في عينات الدقيق المستبدل بدقيق الحلبة المنبت عند مستوى ٥ أو ١٠% من عينات الدقيق المستبدل بدقيق الحلبة الخام أو المنقوع عند نفس المستوى، وقد أظهرت نتائج الدراسة باستخدام جهاز الأكستنسوجراف أن إضافة دقيق الحلبة إلى دقيق القمح ينتج عنه نقص في مطاطية العجين، وأن استخدام دقيق الحلبة الخام له تأثير ريولوجى اقل من استخدام دقيق الحلبة المعامل سواءً بمعاملة النقع أو الإنبات، وأظهرت نتائج الدراسة زيادة مترددة في قيم كل من المرونة والرقم النسبي وقوة العجين بزيادة مستوى الاستبدال بدقيق الحلبة في جميع المعاملات مقارنة بالعينة القياسية، حيث أمكن تحسين الخواص الكيمائية و الريولوجية للعجين باستخدام دقيق الحلبة المعامل بالنقع أو الإنبات، كما أظهرت نتائج التقييم الطبيعي زيادة سمك البسكويت المدعم بالحلبة ونقص في كل من القطر ونسبة الفرد بزيادة مستوي الاستبدال بدقيق الحلبة كما بينت نتائج التقييم الحسي أن البسكويت المصنع من دقيق القمح والمستبدل بمستوي إحلال ٥ ،١٠% من دقيق الحلبة المعامل بمعاملات مختلفة ارتفاع درجات القبول الحسي له أعلي من العينات المستبدلة بمستويات إحلال ١٥ ،٢٠% من دقيق الحلبة، كما تم دراسة التركيب الكيميائي للبسكويت المدعم بالحلبة وتبين زيادة كلا من نسبة البروتين والدهن والألياف الخام والرماد والأحماض الأمينية وانخفاض نسبة المستخلص الخالي من النيتروجين. .وكانت نسبة المحتوي من البروتين (١٠,١٥،١٠,٨٠، ١٠,١٢%) للبسكويت المدعم بدقيق الحلبة الجافة أو المنقوعة أو المنبتة تحت مستوي إحلال ١٠% علي الترتيب،في حين كانت ٩,٣٤%لعينة بسكويت القمح، وزيادة محتوي الأحماض الأمينية الضرورية للبسكويت الناتج بزيادة مستوي الاستبدال من دقيق الحلبة المعاملة. وأظهرت النتائج احتواء البسكويت المدعم بمستويات مختلفة من الحلبة المعاملة علي معظم الأحماض الأمينية الضرورية التي تغطي أو تمثل نسبا مرتفعة من الأحماض الأمينية للبروتين المرجعي الصادر عن منظمتي الصحة العالمية والغذاء والزراعة، وزيادة نقط الليسين Lysine score على النحو التالي (١١,١٠%،١٢,١٨%،١٤,١٨%) للبسكويت المدعم بدقيق الحلبة الجافة أو المنقوعة أو المنبتة تحت مستوي إحلال ١٠ % علي الترتيب .وكان الليسين هو الحامض ألأميني المحدد الأول لعينة البسكويت القياسية ثم الأحماض الأمينية الكبريتية (المثيونين والسيستين) وأن إضافة الحلبة المنبتة حتى مستوي إحلال ١٠% مع دقيق القمح يزيد من محتوي الليسين والأحماض الأمينية الضرورية ، وأثبتت النتائج أنه يمكن إضافة الحلبة الجافة أو المنقوعة أو المنبتة حتي مستوي احلال ١٠% من دقيق القمح لتحسين الخواص الكيميائية والحسية وتعويض نقص الأحماض الأمينية الضرورية في البسكويت الناتج.

المحكمـــــون:

١- أ.د. سامي إبراهيم الصياد أستاذ الصناعات الغذائية – كلية الزراعة – جامعة أسيوط.

٢- أ.د. كمال محفوظ الصاحي أستاذ الصناعات الغذائية المتفرغ – كلية الزراعة – جامعة الزقازيق.