Biologically evaluation of pan bread supplemented with vital gluten, lupin, fenugreek and turmeric flour blends

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ABSTRACT: The current study was carried out to utilize each of whole meal wheat flour (control), some legumes (lupin and fenugreek), turmeric and vital gluten flour in blends for preparation of pan bread more nutrients and healthy in order to enhance the dietary fiber and amino acids contents. The biological parameters of rats (non and induced diabetic) fed on such pan bread was also estimated. A significant higher amount of soluble, insoluble and total dietary fiber contents was found in the turmeric, fenugreek and lupin, pan bread compared to that found in control once(whole wheat flour). Normal rats (nondiabetic and fed on basal diet) exhibited an insignificant decrement in blood glucose. However, in the diabetic rats a significantly lowered blood glucose trend was found. The tested pan bread samples were more slightly effective in lowering liver and kidney function in the diabetic rats in a relation to diabetic rats, when compared with the positive control. Finally, it is recommended to utilize whole meal flour to prepare healthy diets to deal with diabetic status and control of some biological parameters.

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Key words: Whole meal wheat, Vital gluten, Fenugreek seeds, Legumes, Turmeric, Diabetes.

INTRODUCTION

Diabetes mellitus (DM), one of the major metabolic disorders, is characterized by high blood glucose levels due to the inability of body cells to utilize glucose properly. By the 2010 year, the total number of people worldwide with DM will be as high as 239 millions. Regions with greatest potential are Asia and Africa, where DM incidence could rise to 2–3-folds of the present incidence (Xue *et al*., 2007). Recently, Vijayalakshmi *et al*. (2009) reported that diabetes is a disease of great concern to many all over the world and is known for its complications that include diabetic nephropathy, neuropathy, and retinopathy**.** In any form of management of diabetes, be it with insulin or drug, diet is a common factor. Some of the foods and their derivatives are recommended for better management of diabetes. In this direction, bitter gourd is one of the vegetables, which is advocated and well practiced in the control of diabetes.

Marques *et al*. (2007) reported that, more wheat –based products, including flour, bread, breakfast cereals, pasta and crackers, are available. It seems that such cereals products possess valuable nutritional and/or physiological properties, which could help promoting the consumption of these products.

Wheat gluten is a readily available protein source that has been extensively used in baked products (Barber and Warthesen, 1982). Hemstad (2005) reported that vital gluten is a unique water-insoluble protein and carbohydrate complex that is extracted from wheat by wet processing.

Legumes play an important role in human nutrition since they are rich sources of protein, calories, certain minerals and vitamins. In African diets legumes are also, the major contributors of protein and calories for economic and cultural reasons (ELMaki *et al*., 2007). Madhusudhan and Tharanathan (1995) found that, legumes have been shown to decrease blood glucose responses compared to other cereal based foods such as whole meal bread and are of very vital benefit in the diets of diabetes and hyperlipidemia patients. Moreover, Wolever *et al*. (2003) reported that the low-fat, high-carbohydrate diets are known to stimulate hepatic triglyceride production in diabetic subjects.

The turmeric is rich in dietary fibers and contains both soluble and insoluble dietary fibers. Dietary fibers are well established to play a beneficial role against various diseases like diabetes, colon cancer, heart disease…. *etc,* as reported by

Vijayalakshmi *et al*. (2009). Turmeric (*Curcuma longa L*.) is a medicinal plant extensively used in Ayurveda, Unani and Siddha medicine as a home remedy for various diseases (Ammon and Wahl, 1991). Turmeric is used as a food additive (spice), preservative and coloring agent in Asian countries, including China and South East Asia. It is also considered as an auspicious and is a part of religious rituals. In old Hindu medicine, it is extensively used for the treatment of sprains and swelling caused by injury (Kamal–Eldin *et al*., 2000). Arun and Nalini (2002) reported that, both turmeric and curcumin decrease blood sugar level in alloxan-induced diabetes rats. Curcumin also decreases advanced glycation end products induced complications in diabetes mellitus.

On the other hand, Patil *et al*.(2009) reported that, during diabetes, a profound alteration in the concentration and composition of lipids occurs. Liver and kidney organs are important for glucose and lipid homeostasis, they participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. Basch *et al*. (2003) reported that, in human studies, fenugreek seeds reduced the area under the plasma glucose curve and increased the number of insulin receptors. Also, fenugreek seeds exert hypoglycemic effects by stimulating glucose-dependent insulin secretion from pancreatic beta cells as well as by inhibiting the activities of alpha-amylase and sucrase, two intestinal enzymes involved in carbohydrate metabolism.

Therefore, the objectives of the current study are to incorporate and utilization of whole wheat flour, vital gluten, fenugreek, lupin and Turmeric rhizome to prepare an edible and healthy. The objectives are extended also to i*nvetro* and *invivo* estimation of such products.

MATERIALS AND METHODS

Materials:-

Wheat grains (*Triticum aestivum*, Skha 69 variety) were obtained from the Wheat Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. The tested legumes lupin, *lupinus albus*, Giza 1variety and fenugreek seeds, *Trigonella foenung raecum*, Giza 30 variety were obtained from Legumes Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.Turmeric rhizome (*Curcuma longa*) was obtained from the local market at Giza, Egypt. Commercials wheat gluten which was purchased from Crestar Co. 7 Rue Du Marechal, Jaffre, BP 109, France, was obtained from Cairo Unvi., Faculty of Agricultural, Special Baking Technology Unit. Bread improver was obtained from Saint Paul Company Milk and Chocolate, Badr Industrial City, Cairo, Egypt. Commercial compressed yeast: The compressed Bakers yeast was purchased from the local market at Giza, Egypt. Alloxan (the diabetes mellitus inducer drug for rats) was obtained from Sigma Company, USA. Blood glucose, albumin, total protein, alkaline phosphatase, glutamic-oxalacetic transaminase(GOT), glutamic-pyruvic transaminase (GPT), creatinine, urea and uric acid kits were obtained from Biodignostic Company, 29 El Tahreer street, Dokki, Giza, Egypt.

Methods:-

-Preparation of raw materials

-Germination and cooking of legumes

Germination of fenugreek seeds were carried out according to the method of Marero *et al*. (1988). fenugreek seeds were cooked individually by boiling with sufficient amounts of water, till they became tender and well cooked. Lupin debittering processes, were carried out in the laboratory by washing and soaking the lupin in tap water for 24 h at room temperature, followed by germinating of whole seeds at 30 ^{o} C in the dark for 3 days, followed by warming using boiled water for 30 min and submerging the lupin seeds in a running tap water at room temperature for 4 days (Trugo *et al*., 1993).

Drying and milling of materials

All such materials (legumes) were dried at 55^{o} C for 12 h, in an air forced oven. Wheat grains, turmeric, dried germinated fenugreek and lupin seeds were milled with a laboratory mill (MLW, Type: Sk1, watt100, West Germany).

Preparation of blends

The optimum gluten scheme amount was 30%. Exactly 5, 5 and 1% of fenugreek, lupin and turmeric flour, respectively, were substituted (except the control blend) instead of a resemble amount of whole meal wheat flour to achieve the healthy impact.

Baking procedures:

A straight dough bread making process was performed according to Wang *et al*., (2002). Basic dough formula of 500g flour basis was consisted of salt (5g), compressed yeast (10g), sugar (5g), bread improver (0.2g), oil (5g) and the required amount of water to reach 500 BU of consistency. The doughs were optimally mixed, fermented for 10min, and then dough pieces (450g) were divided, hand –moulded and sheeted. The dough was proofed for 55 min in a fermentation cabinet under controlled temperatures (30^{o}C) and a relative humidity (78%) for 50 min and

then baked for 40 min at 180 ° C in a baking oven. The pan bread attributes were evaluated after cooling for 1hr at room temperature.

Methods of analysis

Determination of amino acids

All amino acids content, except tryptophan, of the cooked flour of each of lupin, fenugreek, as well as the flour of whole wheat meal, gluten and turmeric were determined using HPLC- PICO- TAG method according to the method described by Cohen *et al*. (1989). Tryptophan was calorimetrically determined in A.O.AC. (2000).

Determination of total dietary fiber (TDF)

Total dietary fiber (TDF) was determined in according to the method described by Prosky *et al*. (1984) and the modification by Vadivel and Janardhanan (2001).

Determination of soluble and insoluble dietary fiber

Soluble and insoluble dietary were determined according to the method described by Asp *et al*. (1983).

Computation of protein efficiency ratio (PER).

Protein efficiency ratio of pan bread was calculated using the equation suggested by Alsmeyer *et al*. (1974) as follows:

PER = - 1.816 + 0.435 (methionine) + 0.78 (leucine) + 0.211 (histidine) – 0.944 (tyrosine).

Biological assay:

The experimental study was conducted on 45 adult male albino rats, 180-200 g weighed. Animals were housed at the animal house, Crops Technology Department, Food Technology Research Institute (FTRI). Before and during the experiment rats were fed on a basal diet containing 20% casein, 10% corn oil, 5% cellulose, 4% salt mixture and 1% vitamin and completed to 100% with corn starch (AOAC, 2000). After randomization to various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental condition of temperature, relative humidity (55%), and dark/light cycle.

The experimental design:

All the animals were randomly divided in nine groups (five rats for each one group) and namely negative control (the normal group which fed on a basal diet), positive (the diabetic group fed on basal diet), WWF (the diabetic group fed on whole meal wheat pan bread diet), WWG (the diabetic group fed on whole meal and gluten pan bread), WWGT (the diabetic fed on whole meal, gluten and turmeric pan bread), WWGL (the diabetic fed on whole meal, gluten and lupin pan bread), WWGF (the diabetic fed on whole meal, gluten and fenugreek pan bread), WWGLT (the diabetic fed on whole meal, gluten, lupin and turmeric pan bread),WWGFT (the diabetic fed on whole meal, gluten, fenugreek and turmeric pan bread) and WWGFLT (the diabetic fed on whole meal, gluten, fenugreek, lupin and turmeric pan bread). During the experiment, rats were separately kept in well aerated cages and the diet as well as water were *ad libitum* supplied.

Induced diabetic animals:

Rats were diabetic induced by a single intraperitioneal injection of alloxan monohydrate (150 mg/kg rat). Alloxan was first individually amount calculated for each animal according to the weight and the proper amount was solubilized with saline just prior to injection (Ahmed *et al*., 2005) and the control group was saline injection only. Three days after alloxan injection, rats with plasma glucose levels of >140mg/dl were included in the study. Fasting blood glucose estimation was done at zero, 15, 30 and 45 day of the study. Urea, uric acid, creatinine, GOT, GPT, total protein, albumin and alkaline phosphatase estimation, as well as measurements were immediately carried out after the successful injection and at 45 day of the study start. At the end of the feeding period (after 45 days) rats were anaesthetized using diethyl ether and sacrificed. Insulin in plasma was also measured at the experiment end.

Biochemical analysis:

The blood samples were collected in tubes and centrifuged at 500 xg to obtain serum. It was kept in a deep-freezer until biological analysis was performed and subjected to the following biochemical analysis: fasting blood sugar (according to Trinder, 1969) in the separated serum samples. Serum insulin level (Temple *et al*., 1992, at National Institute of the Diabetic and Endocrine Discos). Serum glutamic oxaloacetic transaminase (GOT) and glutamic- pyruvic transaminase (GPT) activities were calorimetrically measured according to the method described by Reitman and Frankel (1957). The protein content was determined using the method of Gornall *et al*. (1949). The alkaline phosphatase was determined using the method of Belfield and

Goldbery (1971). The albumin content was determined using the method of Doumes (1971).

Statistical analysis:

Data analysis was performed using SAS (1987), software. All data were expressed as mean of three replicates. Analysis of variance was used to test for differences between the groups. Least Significant Differences (LSD) test was used to determine significant differences ranking among the mean values at $P< 0.05$.

RESULTS AND DISCUSSION

Dietary fiber content of the tested materials

Whole meal wheat, cooked lupin, cooked fenugreek, turmeric and vital gluten flour were analyzed for their total dietary fiber (TDF) contents and their soluble (SDF) as well as insoluble (IDF) fractions, the results are given in Table (1).

It is clear from data showed in Table (1) that fenugreek, lupin and turmeric flour were rich sources of dietary fibers and possessed the significant differences for TDF content (37.210, 23.150 and 20.20% on dry weight basis, respectively), of which the IDF represented the major fraction except turmeric flour (20.47 and 21.50 of TDF, respectively). While the highest amount of soluble form of dietary fiber (SDF) was found in turmeric flour (19.80 %). Such results suggested that fenugreek, lupin and turmeric could be considered a good and inexpensive source of fiber content to be a suitable tool for flour and baked products enrichment. The fenugreek powder addion to the experimental diet in the form of dietary fiber, resulting in a higher fiber content in the experimental diet than that found in the control diet. The dietary fiber of fenugreek seeds is dispersed throughout the seed coat and is also found in the endosperm as reported by Madar and Stark (2002). The results of dietary fiber content of lupin flour (21.5 % and 2.45 % IDF and SDF, respectively) are in agreement with the results of Mohamed and Rayas-Durate (1995) who pointed out that lupin cotyledons contained 21.5 and 2.2% insoluble and soluble fiber, respectively. Therefore, while the hull contained 86.2 and 1% insoluble and soluble fiber, respectively, lupin could be used in existing or new products. Consequently, lupin can also be used in bread making, biscuits, pasta products, and a variety of other food products.

On the other hand, data presented in Table (1) showed that vital gluten contained the lowest amounts of total, soluble and insoluble dietary fiber compared to that found in whole meal wheat flour. Kahlon and Woodruff (2002) reported that the gluten is contained 1.90% of total dietary fiber 1.4 % insoluble dietary fiber and 0.5% of soluble dietary fiber (as dry matter basis).

Table 1: Dietary fiber content of the tested materials (on dry weight basis).

Sample	Dietary fiber %		
	Insoluble(IDF)	Soluble(SDF)	Total(TDF)
Whole cooked lupin flour	21.500[a]	2.450[c]	23.150[b]
Whole cooked fenugreek flour	20.475[b]	17.035[b]	37.210[a]
Whole wheat flour	12.172[c]	1.337 [e]	13.109[d]
Turmeric flour	0.900[d]	19.800[a]	20.200[c]
Vital gluten	0.515[e]	1.440[d]	1.755[e]

-Each value (an average of three replicates) within the same column, followed by the same letter is not significantly different at < 0.05.

Amino acids content of the tested materials and pan bread.

The nutritive value of dietary protein is determined by the pattern and quantity of essential amino acids present of the tested materials. The presence of one or more specified of the essential amino acids in adequate amounts would increase the nutritive value of protein. The amino acid contents (g /100g protein) of the tested materials were determined and the results are shown in Table (2).

From the data in Table (2) it could be noticed that turmeric flour had lower quantities of isoleucine and lysine. Moreover, cooked lupin flour contained lower amounts of total sulfur amino acids while cooked fenugreek flour contained lower amounts of valine. On the other hand, gluten had lower quantities of lysine and threonine in relative

to the other tested materials. Whole meal wheat flour also, had lower amounts of lysine compared to the other tested materials. However, a significant variation was existed in the contents of some amino acids, particularly for valine and threonine.

The valine contents was varied from 2.72 gm / 100gm protein in turmeric flour to 4.50 gm /100 gm protein in gluten. Among the tested legumes, lysine, sulfur amino acids, serine, proline, glycine, arginine and glutamic acid were found to be rich in fenugreek, while isoleucine and lysine were found in appreciable amounts in turmeric among the different tested materials. Whole meal wheat and gluten were higher in total sulfur amino acids contents (methionine and cystine) than the other tested materials.

Glutamic acid was found to be the major non-essential amino acids in the tested samples, while maximum amount of proline was found in gluten. Total essential amino acids contents were the highest in gluten. On the other hand, legumes have been reported to contain adequate amounts of lysine, but are deficient in S-containing amino acids (methionine and cystine). However, Roccia *et al*. (2009) reported that the wheat flour is deficient in lysine. A common practice in breadmaking is to incorporate proteins in the product formulation to increase their nutritional value. Legume proteins are the major component of the diet of food producing animals and are increasingly important in human nutrition. Finally, the same data presented in Table (2) showed that the fenugreek flour contained highest amounts of tryptophan when compared to turmeric flour.

Data presented in Table (3) showed the mathematically amino acids content of 100gm of the tested pan bread. It could be concluded from such data that each mixture seemed to contain the same amount, with a slightly changes, of all the estimated amino acids. It was due to the variation of the ingredients in each mixture to prepare a complementary mixture containing the required amino acids with an adequate amount.

Data presented in Table (3) showed the amino acids content of manufactured pan bread of the suggested blends. The highest essential amino acids content was noticed in the tested blends pan bread, than that found in the control pan bread (prepared from whole meal wheat flour). Results in Table (3) showed that the amino acid contents of isoleucine, leucine, lysine and valine in WWG, WWGL, WWGT, WWGF, WWGFT, WWGLT and WWGFLT pan bread blends were higher than that found in pan bread from whole meal wheat flour. On the other hand, tryptophan content was higher in pan bread originated from whole meal wheat flour, vital gluten, turmeric and fenugreek flour than that found in all the other pan bread blends.

Table 2: Amino acids content of the tested materials (calculated as g/100g protein).

Amino acid	Whole meal wheat	Gluten	Lupin	Fenugreek	Turmeric
Isoleucine	3.28	3.40	5.15	3.64	1.12
Leucine	6.35	6.20	2.01	1.93	3.23
Lysine	0.44	0.31	3.4	4.90	1.35
Methionine	0.42	0.15	0.82	0.77	1.72
Cystine	4.06	3.51	0.77	0.83	0.038
Phenylalanine	1.58	4.07	2.91	0.389	3.070
Tyrosine	2.85	3.49	3.95	5.280	6.340
Threonine	2.78	0.00	2.78	3.57	4.67
Valine	4.14	4.50	3.01	1.27	2.72
Tryptophan	0.72	0.75	0.60	1.25	0.35
Total essential amino acids	26.62	27.38	25.40	23.82	24.61
Aspartic acid	4.83	2.04	14.50	4.00	12.99
Serine	2.60	5.58	1.91	8.00	3.03
Glutamic	26.78	25.75	29.80	37.55	21.85
Proline	1.48	15.22	1.01	1.58	1.90
Glycine	4.88	3.32	4.02	6.12	3.67
Alanine	18.66	8.71	3.80	2.71	8.52
Arginine	3.09	9.44	6.095	9.66	7.66
Histidine	6.12	1.42	10.80	1.54	5.89
Total amino acids	95.06	97.86	97.33	94.98	90.12

Data presented in Table (3) showed also, that the calculated PER of manufactured pan bread could be divided into two groups. The first group included WWG, WWGT, WWGF and WWGL mixtures which had PER values less than 1.10. The second group included WWF, WWGFT, WWGLT and WWGFLT mixtures which had PER values more than 1.10. This variation in PER could be attributed to the variation of essential amino acids in the tested pan bread. However, WWGLT pan bread had the highest PER value when compared with other manufactured pan bread. Such results are due to its high amounts of lysine, phenylanlanine and threonine. These results agreed with those of Doxastakis *et al*., (2002) who reported that the lupin flours can be considered as an excellent choice for improving the nutritional value of bread.

Table 3: Amino acids content of the pan bread (calculated as g/100g sample).

Amino acid	WWF	WWG	WWGT	WWGF	WWGL	WWGFT	WWGLT	WWGFLT
Isoleucine	0.434	1.068	1.064	1.105	1.144	1.101	1.139	1.176
Leucine	0.841	1.984	1.978	1.972	1.980	1.967	1.974	1.963
Lysine	0.058	0.109	0.110	0.184	0.170	0.185	0.171	0.273
Methionine	0.055	0.071	0.0722	0.081	0.083	0.0818	0.084	0.093
Cystine	0.537	1.165	1.160	1.152	0.839	1.146	1.147	1.133
Phenylalanine	0.209	1.062	1.062	1.057	1.106	1.057	1.106	1.102
Tyrosine	0.377	1.049	1.050	1.089	1.080	1.115	1.106	1.171
Threonine	0.336	0.235	0.234	0.275	0.270	0.275	0.270	0.310
Valine	0.548	1.396	1.385	1.389	1.426	1.385	1.422	1.415
Tryptophan	0.095	0.234	0.233	0.544	0.240	0.248	0.241	0.257
Total essential amino acids	3.527	8.578	8.639	6.611	8.942	8.818	8.918	9.123
Aspartic acid	0.639	0.906	0.909	2.810	1.149	0.941	1.152	1.185
Serine	0.344	1.497	1.496	1.800	1.516	1.607	1.515	1.625
Glutamic	3.548	8.284	8.250	6.620	8.673	8.690	8.653	9.079
Proline	0.196	3.566	3.566	5.760	3.575	3.580	3.574	3.589
Glycine	0.646	1.199	1.198	1.264	0.950	1.260	1.239	1.305
Alanine	2.472	3.692	3.672	3.611	2.453	3.593	3.622	3.541
Arginine	0.409	2.412	2.407	2.546	3.847	2.547	2.507	2.642
Histidine	0.810	0.886	0.881	0.869	1.050	0.866	1.046	1.030
Total amino acids	12.59	30.853	30.796	31.745	32.07	31.691	32.016	32.91
PER	1.321	1.043	1.049	1.088	1.049	1.116	1.954	1.134

Biological estimation of different tested blends.

It was of importance to estimate the impact of different tested diet types on some specific biological parameters (blood glucose, insulin levels, liver and kidney function). The values of blood glucose, GPT and GOT, alkaline phosphatase (ALP), total protein, albumin, serum uric acid, urea and creatinine of each rat group for zero time (initial period) and after adaptation period (where the rats fed on basal diet were nearly the same level.

- Effect of the different pan breads on blood glucose and insulin levels of nondiabetic and diabetic rats.

Blood glucose and insulin levels of the rat groups fed on the tested pan bread are presented in Table (4). The diabetic rats showed a range of 2.24-2.89 folds increment in the blood glucose after injection with alloxan. This increased of blood glucose after injection with alloxan may be due to that the alloxan may either increase the entrance rate of glucose into the blood stream from the liver (increased hepatic glycogenolysis or gluconeogenesis) or decrease the rate of glucose removal from the blood by tissues (decrease the storage and utilization). These influences might be due to the absence of an adequate amount of insulin. The blood glucose concentration was significantly higher in the diabetic rats fed on the basal diet (positive control) than of those diabetic rats fed on the tested pan bread (diabetic) as well as the nondiabetic rats fed on basal diet (negative control).

Table4: Blood glucose level (mg/dl) and insulin level (mu/l) in serum of rat groups, fed on the tested diets.

Groups*	After injection (3days)	Feeding period			Insulin level**
		15 day	30 day	45 day	
Negative control	95.0^{h}	97.0 ^{j}	95.0 ^{j}	93.0 ^{l}	0.525^{b}
Positive control	264.0^{c}	258.0^{a}	252.0 ^{a}	246.0 ^{a}	0.220^{k}
WWF	213.0^{f}	200.0 ^{k}	195.0 ^{c}	183.0^{b}	0.315^{j}
WWG	262.0^{d}	240.5^{b}	202.0^{b}	161.0^{c}	0.365^{h}
WWGL	268.0^{a}	235.0^{c}	191.7^{d}	154.5^{e}	0.390^{g}
WWGF	264.5^{c}	231.0^{d}	184.0^{f}	150.0^{f}	0.420 ^{f}
WWGT	261.0^{d}	235.0^{c}	189.0^{e}	156.0^{d}	0.430^{e}
WWGLT	253.5^{e}	228.0^{e}	189.0^{e}	148.0^{g}	0.480^{d}
WWGFT	261.0^{d}	225.0^{g}	178.0^{g}	143.0^{h}	0.490^{c}
WWGFLT	267.0^{b}	211.0^{h}	160.0^{h}	129.0^{j}	0.530^{a}

*** Previously identified and listed in Materials and Methods of the current study.**
****At the end of experiment.**
-Each value (an average of three replicate) within the same column, followed by the same letter are not significantly different at <0.05.
-Value of blood glucose was 84.0 and 95.0 at zero time and after adaptation peroid, respectively.

There were significant differences in serum blood glucose level in both negative control groups compared with the other diabetic rats groups. The decrease in blood glucose is due to the effect of dietary fiber from the tested materials. These results are agreed with Adam *et al*., (2003) who reported that the dietary fiber intake, especially from whole grain sources, reduced the serum glucose level and lead to reduce the risk of coronary heart disease and diabetic. Such treatments containing fenugreek and turmeric flour reduced the serum blood glucose. These results agreed with Patil *et al*. (2009) who found that the mean fasting blood glucose in the diabetic untreated group (control positive) was 280±8.33 mg/dl after 21 days of diabetes induction. In the normal health group this value was 76±2.59 mg/dl. In comparison with the positive control group, the group which consumed fenugreek extract showed significantly lower mean fasting blood glucose 141.83±9.04 mg/dl (P<0.05) after 21 day of induced of diabetes. These results confirmed with Kumar *et al*. (2005) who reported that the beneficial effect of fenugreek seed mucilage is due to some of the bioactive compounds present in the mucilage, including 4-hydroxy isoleucine. 4-Hydroxy isoleucine is a novel amino acid known to facilitate insulin secretion. The effect of spent turmeric in rats may be mainly due to the high amount of dietary fiber, which would facilitate a slower absorption of glucose in the gastrointestinal tract. Results in Table (4) revealed that, within the diabetic rat groups, the whole meal possessed lower serum glucose content than that of positive control groups, respectively.

Kim *et al*., (2006) reported that alloxan induces “chemical diabetes” in a wide variety of animal species by damaging the insulin secreting pancreatic β-cell, resulting in a decrease in endogenous insulin release. Therefore, Table (4) concerned the plasma insulin of the different groups at the end of the present study. It was found that the insulin level was significantly higher in diabetic rats fed on the WWGFLT and the negative control, while it was significantly lower in the positive control. The results showed that serum glucose level was increased, whereas serum insulin was decreased in the diabetic rats groups. It could finally concluded that the whole meal diet and the other blends containing fenugreek and turmeric flour could be used as a serum glucose controller via the lower sugar content either be decrease the glucose level or increase the insulin level in the serum. Such treatments contained fenugreek and turmeric flour may potentate the insulin secretion by 4- hydroxyisolcucine in fenugreek seed and this effect of turmeric could be done by antioxidant and dietary fiber influence. Also, demonstrated that the in vitro amino acid 4-hydroxyisolcucine in fenugreek seeds, increased glucose-induced insulin release in human and rat pancreatic islet cells. This amino acid appeared to act only on pancreatic beta cells, since the levels of somatostatin and glucagon were not altered. Chattopadhyay *et al*., (2004) reported that, both turmeric and curcumin decrease blood sugar level in alloxan-induced diabetes in rat. Curcumin also decreases advanced glycation end products

induced complications in diabetes mellitus. Srinivasan (2005) mentioned that, daily intake of curcumin (coloring principle of turmeric) not only reduced the fasting sugar level, but also lowered the dosage of insulin needed for normoglyaemia. It was observed that the rhizome of turmeric showed blood glucose lowering activity in alloxan diabetic rats.

Vijayalakshmi *et al*., (2009) reported that the role of spent turmeric in the management of diabetes could be mainly due to the presence of dietary fibers. Both soluble and insoluble dietary fibers contribute to the management of diabetes. Both of them not only serve in the slow absorption of glucose (in the gastrointestinal tract) but also are fermented by the microflora present in the colon, which release short chain fatty acids. In recent years, beneficial effect of short-chain fatty acids and that of butyric acid are receiving much attention in the improvement of disease conditions like cancer and diabetes.

Table 5. GOT, GPT (U/L) and alkaline phosphatase (IU/L) in rat groups fed on the tested diets.

Groups*	GOT (U/L)		GPT (U/L)		Alkaline phosphatase (IU/L)	
	After injection (3days)	45 day	After injection (3days)	45 day	After injection (3days)	45 day
Negative control	29.75^{h}	25.00^{i}	38.75^{k}	30.00^{h}	25.50^{h}	20.50^{h}
Positive control	78.25^{a}	57.75^{a}	48.50^{g}	46.95^{a}	48.75^{a}	40.45^{a}
WWF	70.25^{f}	56.25^{b}	44.75^{i}	32.75^{b}	39.50^{g}	31.50^{b}
WWG	65.50^{g}	41.25^{c}	47.50^{h}	31.25^{g}	39.50^{g}	31.25^{c}
WWGL	70.75^{e}	41.00^{d}	51.50^{e}	32.50^{c}	41.53^{f}	30.95^{d}
WWGF	73.25^{d}	39.75^{f}	54.50^{d}	31.75^{d}	45.58^{d}	31.25^{c}
WWGT	65.50^{g}	41.00^{d}	49.50^{f}	31.75^{d}	47.50^{b}	31.25^{c}
WWGLT	75.25^{c}	40.00^{e}	56.0^{b}	31.50^{e}	45.50^{d}	30.65^{f}
WWGFT	76.50^{b}	39.25^{g}	55.50^{c}	32.50^{c}	44.50^{e}	30.75^{e}
WWGFLT	78.25^{a}	38.50^{h}	58.25^{a}	32.50^{c}	46.25^{c}	29.50^{g}

*** Previously identified and listed in Materials and Methods of the current study.**
-Each value (an average of three replicates) within the same column, followed by the same letter are not significantly different at <0.05.
Value of GOT was 25.0 and 30.0 at zero time and after adaptation, respectively.
Value of GPT was 40.0 and 39.0 at zero time and after adaptation, respectively.
Value of Alkaline phosphatase was 25.0 and 23.0 at zero time and after adaptation, respectively.

- Effect of the tested pan bread on liver functions of nondiabetic and diabetic rats

- Effect of the tested pan bread on GOT, GPT and ALP

The results in Table (5) showed that serum GOT, GPT and ALP activities were significantly increased in diabetic rats after injection with alloxan when compared with the nondiabetic rat. These results agreement with Eidi *et al*., (2007) who reported that the serum GOT and GPT levels increased in diabetic control rats when compared with nondiabetic control rats. There was a decrement of liver function, serum GOT, GPT and ALP as a result of feeding the rats on the tested pan bread in the 45 days in *vivo* experimental assay, as shown in Table (5). Data presented in Table shows that there were significant changes in impact of all the tested diets under investigation on both of serum GOT, GPT and ALP tests either in diabetic control or healthy (normal rats). Serum GOT was the lowest in all diabetic rats fed on pan bread prepared from fenugreek, lupin and turmeric and pan bread from fenugreek and turmeric. GPT was lower in diabetic rats fed on WWG, WWGLT, WWGF and WWGT pan

bread blends compared with values of the other groups. There was an increment in liver function, serum GOT and GPT, as a result of feeding the rats on the basal diet (positive control). The decrement in GOT and GPT may be due to the decreased in serum glucose level which inhibited the glyconeogenesis (conversion of amino acids into sugars). These results are in agreement with Abbas (2008).

These findings confirmed, also, the healthy roles of legumes and whole meal in lowering the GOT and GPT activities as reported by Eidi *et al*., (2007). It was found that, an increase in these enzyme activities reflects active liver damage. Inflammatory hepatocellular disorders result in extremely elevated transaminase levels. Administration of the fenugreek seeds extract (0.25 and 0.5 g/kg body wt) and glibenclamide (600µg/kg) similarly decreased GOT and GPT levels when compared with control diabetic rats. Data presented in (Table 5) showed that alkaline phosphatase activity in nondiabetic rats fed on basal diet and diabetic rats fed on WWGFLT pan bread blend was significantly lower than that found in the other rats groups fed on the suggested blends. The significant highest activity in ALP was noticed in case of diabetic rats fed on basal diet (positive control). There was,also, an improvement in ALP of in all groups relative to diabetic control. These results agreed with Mahmoud *et al*., (2007) who reported that the feeding with legumes to alloxanized diabetic rats was characterized by a significant inhibition in ALP activity.

Table 6. Albumin and total protein concentration and(g/dl) in rat groups fed on the tested diets.

Groups*	Albumin concentration (g/dl)		Total protein (g/dl)	
	After Injection (3days)	45 days	After Injection (3days)	45 day
Negative control	2.31[a]	4.93[a]	4.93[a]	7.53[f]
Positive control	1.71[d]	3.52[g]	3.14[g]	5.94[i]
WWF	1.92[b]	3.54[g]	3.34[f]	6.53[h]
WWG	1.82[c]	4.23[d]	3.35[f]	7.10[g]
WWGL	1.96[b]	4.52[c]	3.47[e]	8.52[c]
WWGF	1.86[c]	4.12[e]	3.67[d]	8.13[e]
WWGT	1.72[d]	4.08[f]	3.85[c]	7.15[g]
WWGLT	1.81[c]	4.73[b]	3.43[e]	8.61[b]
WWGFT	1.93[b]	4.22[d]	3.97[b]	8.22[d]
WWGFLT	1.85[c]	4.97[a]	3.65[d]	8.75[a]

*** Previously identified and listed in Materials and Methods of the current study.**
-Each value (an average of three replicates) within the same column, followed by the same letter are not significantly different at <0.05.
Value of albumin concentration (g/dl) was 1.50 and 2.10 at zero time and after adaptation, respectively.
Value of total protein (g/dl) was 4.52 and 4.90 at zero time and after adaptation, respectively.

- Effect of the tested pan bread on albumin and total protein concentration of nondiabetic and diabetic rats

Data presented in Table (6) showed that a significant lower in total protein and albumin content was found among the rat groups serum after injection with alloxan. In this study, the decrease in serum protein may be due to increasing the excretion of nitrogen in urine and protein synthesis. The present results are in a good agreement with Venkateswarty *et al*., (1993) who proved that there was a marked decrease in the serum protein content of alloxanized diabetic rats when compared with that of the normal control animals. These effect may be due to the increasing in the excretion of nitrogen in urine alloxanized diabetic rats and increased the activity of transaminases. After 45 days feeding, the protein level was significantly lower in the diabetic rats fed on the basal diet (positive

control) than those diabetic rats fed on the tested pan bread, nondiabetic fed on basal diet (negative control). There were significant differences in serum total protein among the diabetic rats fed on the WWGFLT,WWG, WWGT and WWF pan bread.

Table (6) showed that, after feeding the rats on the tested pan bread, it was found that there was a significant increment in serum albumin content in diabetic rats at the experimental feeding period end (45 days). It was also found that there were significant differences in serum albumin in normal rats and diabetic rats at each feeding stage(after and after adaptation) and within the tested period. The present results showed that serum albumin increased in diabetic rats fed on the tested pan bread when compared with diabetic control (positive control) rats. These results agreed with that found by Mahmoud *et al*., (2007) who found that the feeding with legumes to alloxanized diabetic rats was characterized by improvement in protein and albumin.

- Effect of the tested pan bread on kidney function of nondiabeti and diabetic rats

It is of great importance to estimate the renal function which includes the determination of serum uric acid, urea and creatinine to evaluate disorders which may be occurred as a result of alloxan injection (El-Abd *et al*., 2007).

The results in Table (7) indicated that alloxan injection caused a highly increase in serum uric acid, urea and creatinine values. The present results showed that serum urea, uric acid and creatinine increased in diabetic control rats when compared with nondiabetic control rats. This may be due to the protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid, as well as increased activity of xanthine oxidase. These results confirmed by Eidi *et al*., (2007) who showed that serum uric acid, urea, and creatinine levels were increased in diabetic rats. This may due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels. Data presented in Table (7) shows that there were significant changes in impact of all the tested diets under investigation serum uric acid, urea and creatinine amounts either in diabetic or healthy (normal rats). Serum uric acid was significantly decreased in diabetic rats fed on WWG, WWGF, WWGFT,WWGFLT and WWF followed by WWGLT and WWGT at the experimental period (45 days) end compared with that of diabetic (whole meal wheat and positive control) rat groups. Also, results in the same Table showed that, feeding on pan bread samples prepared from the tested materials flour led to a serum significantly decrement in urea amount in the diabetic rats group fed on the WWGFLT and nondiabetic rats group fed on the basal diet or whole meal wheat bread for 45 days compared with that of the positive control. These results agreed with Rubio *et al*., (1998) who mentioned that, there is an inverse correlation between the biological value of foodstuff and blood urea concentration in rats. Because the urea is one of the main end products of protein catabolism in mammals, high plasma values are associated with disturbances in protein metabolism and increased protein degradation, which can finally result in a high loss of N through the urine.

However, data in Table (7) showed that the serum creatinine was significantly decreased in diabetic rats fed on WWGFLT and WWGFT followed by WWGLT for the experimental period (45 days) compared with that of diabetic (whole meal wheat, positive control) rat groups. These results agreed with Yadav *et al*., (2004) who showed the presence of lipid deposits in the kidney of diabetic human and experimental animals and they have proposed that these deposits may play an important role in the pathogenesis of diabetic kidney disease.

From aforementioned results in Table (7) it could be concluded that fenugreek flour decreased the serum uric acid, urea, and creatinine levels in diabetic rats. These results are concurrent with Eidi *et al*., (2007) who reported that the fenugreek seeds significantly decreased serum urea, uric acid, and creatinine when compared with control diabetic rats. Elevation of the serum urea and creatinine, as significant renal function markers, are related to renal dysfunction in diabetic hyperglycemia. However, Tharanathan and Mahadevamma (2003) showed that a high fiber diet seemed to lower the urinary phenol and cresol concentrations in humans. It has been suggested that the presence of undigested carbohydrates stimulates rapid growth of colonic bacteria, which can act as 'nitrogen sinks' for using remaining protein and protein metabolites for their metabolism and growth. kumar *et al*., (2005) also, mentioned that the water extract of fenugreek seeds is used in the management of diabetes and is known to improve kidney function during diabetes.

Finally, it is recommended to utilize whole meal flour, fenugreek, lupin and turmeric flour to prepare healthy diets to deal with diabetic status and control of some biological parameters.

Table 7. Uric acid, urea and creatinine (mg/dl) in rats groups fed on the tested diets.

Groups*	Uric acid (mg/dl)		Urea (mg/dl)		Creatinine level (mg/dl)	
	After injection (3days)	45 day	After injection (3days)	45 day	After injection (3days)	45 day
Negative control	3.47h	3.01h	35.23j	20.25j	0.76e	0.62g
Positive control	6.13a	5.70a	54.29a	40.14a	1.52b	1.4a
WWF	5.18d	3.91d	45.16i	34.18c	1.46c	1.38b
WWG	4.27g	3.14g	50.30f	32.51d	1.53b	1.37b
WWGL	5.87c	4.80b	51.32c	32.32e	1.51b	1.32d
WWGF	4.56f	3.16g	50.22h	31.48f	1.55a	1.30d
WWGT	5.19d	4.09c	50.28g	35.51b	1.50b	1.35c
WWGLT	4.83e	4.09c	50.78d	31.22g	1.42d	1.25e
WWGFT	5.92b	3.29f	50.63e	30.49h	1.48c	1.22e
WWGFLT	4.58f	3.82e	53.45b	26.21i	1.44d	1.19f

*** Previously identified and listed in Materials and Methods of the current study.**
-Each value (an average of three replicates) within the same column, followed by the same letter are not significantly different at <0.05.
Value of uric acid (mg/dl) was 3.90 and 3.70 at zero time and after adaptation, respectively.
Value of urea (mg/dl)was 37.10 and 36.3 at zero time and after adaptation, respectively.
Value of creatinine(mg/dl) was 0.85 and 0.80 at zero time and after adaptation, respectively.

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المستخلص العربي

التقييم البيولوجي لخبز القالب المدعم بالجلوتين ودقيق الحلبة والترمس و الكركم

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الهدف من الدراسة هو الاستفادة من حبة القمح الكاملة و الجلوتين وبعض البقوليات و الكركم لتجهيز خبز القالب وتقييمه من حيث احتوائه على الألياف الغذائية و الأحماض الامينية الأساسية و بعض صفاته البيولوجية. وجد ارتفاع معنوي في الألياف الغذائية الذائبة والغير ذائبة والكلية في كلا من الحلبة والترمس والكركم بالمقارنة بدقيق القمح الحبة الكاملة. قد ارتفعت بدرجة معنوية نتيجة عملية اصابة الفئران بالسكر. وكانت الفئران الطبيعية (التي تم تغذيتها علي الوجبة الاساسية دون احداث اصابة بمرض السكرى لها) قد أعطت انخفاض غير معنوي في مستوي السكر الدم بينما حدث انخفاض معنوي في حالة الفئران المصابة بمرض السكر فى وظائف الكبد والكلى بعد تغذيتها بالخبز المختبر .

ولذلك فانه ينصح باستخدام دقيق الحبة الكاملة مع الجلوتين و البقوليات والكركم في تجهيز وجبات صحية تعمل علي تنظيم بعض الاختبارات الحيوية فى الحالات المصابة بمرض السكرى او العادية.

الكلمات الدالة: حبة القمح الكامل – الجلوتين- بذور الحلبة- البقوليات- الكركم-السكرى .

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