Original Research Article

Effect of Feeding Diabetic Rats on Breadsticks Fortified with Psyllium Seed Powder

ABSTRACT

This study aimed to investigate the effect of breadsticks produced from wheat flour (72% extract) andPsyllium SeedsPowder (PSP) on chemical, and sensory properties. Effect of feeding diabetic rats with breadsticks on blood glucose, biochemical parameters, organs weight, lipid profile, kidney, and liver functionswere also assessed. Breadsticks were prepared by substituting wheat flour by 3, 6, and 9% PSP. Results revealed that PSP contained high values 15.82% of crude protein, 4.03%ash, 5.56%ether extract, 26%crude fiber, 6.50%soluble fiber and 20.02% insoluble fiber. Biological results showed a significant improvement *($p<0.05$)* in values of blood glucose, lipid profile(HDL- LDL- VLDL, triglycerides and total cholesterol in serum for rats fed onbreadsticksupplemented with PSP at extent 3,6,and9% comparing to positivecontrol group. Also, feeding diabetic rats on PSP clarified a remarkable improvement in ALT, AST, urea, uric acid, and creatinine comparing to normal controlrats. It could be concluded that breadsticks prepared using PSP might help for the prevention and treatment of diabetic rats, also, could be beneficial for people with diabetes. This research recommendsusingPSP with wheat flour on a commercial scale for preparation of backing products to treatmentdiabeticpatients in Egypt.

Key words:*Diabetes, PlantagoOvateSeeds, Breadstick, Sensory Evaluation*.

1. INTRODUCTION

Type 2 Diabetes mellitus is considered as a metabolic disorder which is characterized by β-cell dysfunction and high blood glucose level in the context of the relative insulin insufficiency[1].Among the metabolic conditions that type 2 diabetes is accompanied by oxidative stress (excess free radicals and a malfunction in the body's antioxidant defense system), poor lipid and lipoprotein metabolism, hypertension, vascular endothelial dysfunction, and subclinical inflammation[2]. Metabolic diseases caused microvascular and macro vascular consequences[3]. According toModi,[4]the main modalities of treatment for type 2 diabetes are dietary and activity changes, insulin replacement therapy, insulin secretion, insulin sensitizers, and alpha-glucosidase inhibitors. To regulate their blood sugar and avoid complications from diabetes, patients must take numerous medicine combinations. But some negative effects of these medications include hypoglycemia, gastrointestinal distress, and lactic acid poisoning. Therefore, it is preferable for patients to utilize a plant medicine that is effective at reducing the symptoms of diabetes[5].

PlantagoOvata (Psyllium) belongs to the Plantaginaceae family. The plant's husk and seeds have significant therapeutic and economic value. Psyllium seeds are used in traditional medicine for a variety of medical purposes[6]. Psyllium has a characteristic fiber got from psyllium seed. It is a highly fanned arabinoxylan polysaccharide with an extremely high water retention and gelling limit. The soluble base extractable polysaccharides of psyllium's gel-framing part are constituted of arabinose, xylose, and traces of other sugars[7].

Psyllium seeds are categorized as a mucilaginous fiber because of their exceptional capacity to gel in water. The *PlantagoPsyllium* is a significant medicinal plant that contains a variety of chemicals, including flavonoids, alkaloids, terpenoids, phenolic compounds (derivatives of caffeine), and vitamin C.Plantago plants contain significant levels of phenols, and it's possible that these phenolic substances are what give the plant its strong antioxidant properties. Although phenolic chemicals, particularly flavonoids, have antioxidant properties, they cannot completely prevent all mutations brought on by mutagenic agents [8].Psyllium is a viable choice for usage in the nutraceutical business since it includes nutritional antioxidants, flavonoids,

polyunsaturated fatty acids (PUFAs), including essential fatty acids (Omega-3 and Omega-6 fatty acids), sulfur-containing amino acids, and metabolites with bioactivities. Psyllium may help people with diabetes better control their blood sugar and cholesterol levels, according to several studies. In investigations on both animals and people, psyllium consumption has been demonstrated to enhance glucose and insulin responses[9].Due to the binding of bile acids in the intestinal lumen, psyllium has a hypolipidemic effect that decreases serum cholesterol levels and lowers the risk of coronary heart disease[10]. This work confirms the great importance of applied science in bakeries foods[11-21].The goal of the current study was to determine whether psyllium seeds have any anti-diabetic effects in diabetic rats that have been fed a high-fat diet with streptozotocin (STZ).

2. MATERIALS and METHODS

2.1. MATERIALS

Psyllium seed(*PlantagoOvata*), wheat flour72% extraction, and other ingredients, including salt(Nacl), sugar(sucrose), dry yeast(*Saccharomyces cerevisiae*), and fat werepurchased from the market in Menoufia, Egypt. Analytical grade chemicals and solvents from the Sigma Company were employed throughout the study, rats were purchased from animal house of food technology research Institute.

2.2. METHODS

2.2.1. Breadsticks Making:

Making breadsticks with psyllium seed powder using the listed ingredients in Table 1. Psyllium seed powder was used in replace of wheat flour at differentextents (3, 6, and 9 %). The control dough was made with only wheat flour (72 % extraction).

Table (1): The Basic (Control) FormulaUsed inThe Preparation of Breadsticks

Ingredients(g)	Blend 1	Blend 2	Blend 3	Blend 4
Wheat flour 72%	100	97	94	91
Psyllium Seeds%	-	3	6	9
Sugar(g)	8	8	8	8
Fat (oil) (g)	10	10	10	10
Salt(g)	0.5	0.5	0.5	0.5

Dry yeast(g)	0.6	0.6	0.6	0.6

Dough preparation.Eachblend of flour was combined with warm water, oil, sugar, fat, salt, and dry yeast. The materials were then well mixed by hand. The dough was allowed to ferment for 30 minutes at (30 ± 2ºC). After cutting the dough into pieces, it was given a 10 minute torest. After shaping the fermented pieces into their final form, they fermented for half an hour at 30ºC and 90% relative humidity. The fermented dough baked for 30 minutes at 170ºC[22].

2.2.2. Chemical Analysis:

Psyllium Seed flour was analyzed for crude protein, ash, crude ether extract and crude fiber according to the methods of[23]. Available carbohydrates were calculated by difference.

% Available carbohydrates = 100 – (protein + ash + ether extract + crude fiber)

Calorie value were calculated agreeing to the Atwater system[24].

Calorie value (kcal/100g) = (% carbohydrate × 4) + (% protein×4) + (% fat× 9).

2.2.3. Determination of Soluble and Insoluble Dietary Fiber:

Soluble and insoluble fiber were determined according to the method described by[25]

2.2.4. Sensory Evaluation:

Twentypanelists conducted an organoleptic examination of the investigated breadsticks with and without Psyllium Seed flour to assess flavor, texture, color, taste, and acceptability in general. According to De Renzo, [26]evaluations were done using scores ranging from 1 to 10, with excellent (10-9), very good (8-6), fair (5-4) and not acceptable (3-2) unacceptable.

2.3. Rats Feeding:

The current experiment in the animal house of Food Technology Research Institute, Agric, Res., Center, Giza, used a total of thirty adult male albino rats that were weighted (145-150g). Under normal, healthy circumstances for 10 days, rats were fed a typical diet (basal diet), as stated in Table 2. The rats were fed a consistent diet and given free access to tap water.

Table 2.Composition of The Basal Diet.

Ingredient	g/100g diet	Ingredient	g/100g diet

Corn starch	60	**Cellulose**	5
Corn oil	10	**Salt mixture**	4
Casein	20	**Vitamin mixture**	1

(Lane-Peter and Pearson, 1971) [27]

2.3.1. Design of Experiment:

Following ten days of only feedingon the basal diet as anadaptation period,a negative control group (G1) of six rats was kept and given the basal diet throughout the experimental. According to Dawson et al,[28]24 rats were given the intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg body weight after fasting overnight. Blood samples from each rat were collected for the measurement of serum glucose after four days of injection (zero time) to ensure the development of diabetes. Diabetic rats were defined as having blood glucose levels of 250–550 mg/100 ml.

2.3.2. Hyperglycemia Experiment of Psyllium Seeds:

G1: Fed on the 50% breadsticks without PSP+50% Basaldiet. (Negative control).

G2: Fed on the 50%breadsticks without PSP+50% Basaldiet. (Positive diabetic control).

G3: Fed on the basal diet with 50% blend2+50% breadstick containing 3% PSP.

G4: Fed on the basal diet with 50% blend3+50% breadstick containing 6% PSP.

G5: Fed on the basal diet with 50% blend4+50% breadstick containing 9% PSP.

Each animal had ethyl ether anesthesia at the end of the experiment, which lasted six weeks. Blood was drawn from the venous plexus's eye, centrifuged for 10 minutes at 3000 rpm to extract the serum, and then frozen at 20°C in dry, clean plastic until analysis.

2.4. Biochemical Analysis:-

Kits relate to serum glucose, total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C), triglyceride (TG), serum alanine amino-transferase (ALT), serum aspartate amino-transferase (AST), Albumin, urea, creatinine, uric acid, purchased from SPINREACT Co, SPAIN 2021.

2.5. StatisticalEvaluation

The obtained results were analyzed statistically using Analysis of Variance (One way ANOVA) using SPSS 26.0.

3. RESULTS

3.1. ProximateComposition of PsylliumSeeds Powder.

The chemical composition of psyllium seeds shown in Table 3. The results obtained were based on dry weight. From the tabular data, it can be seen that the crude protein content of psyllium seeds powderwas 15.82 %. In addition to psyllium seeds powder contains5.56% ether extract, 26.00% crude fiber content, 4.03%ash, 47.52% available carbohydrates, 74.59% total carbohydrates and 303.4 (kcal/100g) Caloric value.El-Hadidy,[14]reported that psyllium seeds powder comprise 16.40%crude protein,5.40%fat, 3.70%ash,48%carbohydrates,26.50%crude fiber, 6%soluble fiber, and 20.55%insoluble fiber.

Table (3): Chemical Composition of PsylliumSeeds Powder Raw Materials (g/100g on Dry Weight Basis)

Contents	Psyllium Seeds Powder
Moisture	86.34±2.52
Crude protein %	15.82±1.22
Crude ether extract %	5.56±0.72
Ash %	4.03±0.82
Total carbohydrates %	74.59±3.32
Available carbohydrates %	47.52±2.62
Crude fiber %	26.00±1.23
Soluble fiber %	6.50±0.62
Insoluble fiber %	20.02±0.92
Caloric value (kcal/100g)	303.4±3.78

3.2. Effect of FeedingBreadsticks with Different Levels of Psyllium Seeds Powder on Body Weight

Data in Table 4 revealed that initial body weight at the beginning of the experiment ranged from 145 to 150**g** of all groupsof animals. While, the most effective of supplementation of psyllium seeds Powder in rats fed breadsticks on change in body weight of rats. Psyllium seeds powder supplementation a significantly increased

(p≤0.05) final body weight gain (%) compared with rats fed with standard diet after 6 weeks of the experimental period (p≤0.05).

Table 4. Effect of Feeding Breadsticks Different PsylliumSeeds Powder on Body Weight Gain of Diabetic Rats:

Group of rats	Initial weight (g)	Final weight (g)	Change in body weight (%)
G1	148.43^{a}±1.87	170.36^{a}±1.95	14.77^{b}±1.20
G2	149.43^{a}±1.95	139.48^{d}±1.85	-6.66^{c}±1.15
G3	146.95^{a}±1.96	166.86^{c}±2.16	13.55^{b}±1.30
G4	147.10^{a}±1.35	167.35^{c}±1.49	15.02^{b}±1.40
G5	147.43^{a}±1.68	169.57^{b}±1.51	22.14^{a}±1.35

Each value is an average of five determinations.

Values followed by the same letter in column are not significantly different at (p ≤ 0.05).

3.3. Effect of FeedingBreadstickswith Psyllium Seeds Powder on Organs Weight:

The results in Table 5 indicate that there is a significantly decrease(p≤0.05) in the weight of the organs of diabetic rats fed breadsticks with psyllium seeds powder compared to the control, which indicates that the diet of breadsticks psyllium seeds powder has an effective role on the organs of the body.

Table 5. Effect of FeedingBreadstickswith PsylliumSeeds Powder on Organs Weight:

Rat Groups	Liver (g)	Kidney (g)	Heart (g)	Spleen (g)
G1	6.73^{bc} ±0.42	1.39^{d} ±0.05	0.58^{c} ±0.04	0.69^{c} ±0.07
G2	9.06^{a} ±0.53	1.90^{a} ±0.03	0.77^{a} ±0.03	0.87^{a} ±0.06
G3	7.99^{ab} ±0.56	1.87^{a} ±0.04	0.67^{ab} ±0.07	0.86^{ab} ±0.08
G4	7.45abc ±0.72	1.77^{b}±0.05	0.65^{b} ±0.05	0.78^{b}±0.09
G5	6.79^{bc} ±0.62	1.47^{c} ±0.05	0.61^{b}±0.06	0.61^{cd} ±0.06

Each value is an average of five determinations.

Values followed by the same letter in column are not significantly different at p ≤ 0.05.

3.4. Blood Serum Glucose Levels of Rats Fed on Prepared Breadsticks.

Table 6 and illustrates the blood glucose levels of negative and diabetic groups through the experimental period (6 weeks). Blood glucose levels of diabetic groups (G2) were markedly significantly increased ($p \leq 0.05$)compared that of normal negative control (G1). The results also indicate that, the groups (G3 to G5) feedingonbread sticks contained psyllium seeds powder caused a significantly decrease($p \leq 0.05$)in blood glucose levels compared negative and diabetic rats.

Table 6. Effect ofFeeding onBreadstickswithDifferent PsylliumSeeds Powder on Blood Glucose Level of Diabetic Rats.

Rat groups	After 3 days (mg/dl)	After 2weeks (mg/dl)	After 4 weeks (mg/dl)	After 6 weeks (mg/dl)
G1	$105.33^{b} \pm 1.54$	$105.44^{e} \pm 1.68$	$103.78^{e} \pm 1.17$	$100.78^{e} \pm 1.57$
G2	$304.66^{a} \pm 1.41$	$313.35^{a} \pm 1.87$	$317.45^{a} \pm 1.86$	$319.64^{a} \pm 1.67$
G3	$306.23^{a} \pm 1.57$	$287.47^{b} \pm 1.65$	$239.12^{b} \pm 1.85$	$216.61^{b} \pm 1.35$
G4	$305.12^{a} \pm 1.37$	$273.55^{c} \pm 1.68$	$204.13^{c} \pm 1.55$	$164.42^{c} \pm 2.03$
G5	$305.93^{a} \pm 2.03$	$264.46^{d} \pm 2.03$	$189.15^{d} \pm 1.46$	$147.33^{d} \pm 2.13$

Each value is an average of five determinations.

Values followed by the same letter in column are not significantly different at $p \leq 0.05$.

3.5. Effect of Feeding onBreadsticksEnriched withPsyllium SeedsPowder on Lipids Serum Parameters.

Data in Table 7, also show that treatments with psyllium seeds powder led to a gradual significantly increased ($p \leq 0.05$)in serum HDL-C. Feeding onPsyllium seeds powder treatments of the experiment caused an significantly increased ($p \leq 0.05$)in serum HDL-C. While total cholesterol, LDL-C, VLDL-C and triglycerides significantly decreased ($p \leq 0.05$). The results indicate an increase in the percentage of HDL-C and a decrease in the percentage of TC, LDL-C andTriglycerides in the groups fed on thePsyllium seeds powder, compared to negative and Positive diabetic control.

Table 7. Effect of FeedingBreadsticks withPsylliumSeeds Powder on Lipids Serum Parameters.

Rat groups	TC (mg/dl) (M±SD)	HDL-C (mg/dl) (M±SD)	LDL-C (mg/dl) (M±SD)	VLDL (mg/dl) (M±SD)	Triglycerides (mg/dl) (M±SD)
G1	110.53^{e} ±1.62	67.93^{a} ±1.45	20.77^{e} ±1.34	21.83^{e} ±2.01	109.15^{e} ±1.66
G2	153.51^{a} ±1.62	33.39^{e} ±1.37	84.3^{a} ±1.58	35.82^{a} ±2.14	179.1^{a} ±2.42
G3	133.85^{b} ±1.43	42.20^{d} ±2.56	63.34^{b} ±1.97	28.31^{b} ±2.54	141.55^{b} ±2.83
G4	123.54^{c} ±2.26	46.13^{c} ±2.51	51.59^{d} ±2.73	25.82^{c} ±2.41	129.10^{c} ±2.35
G5	121.86^{d} ±1.45	49.69^{b} ±1.98	48.16^{c} ±2.12	24.01^{d} ±2.61	120.05^{d} ±2.63

Each value is an average of five determinations.

Values followed by the same letter in column are not significantly different at p ≤ 0.05.

TC=Total cholesterol, HDL-C = High Density Lipoprotein Cholesterol, LDL-C = Low Density Lipoprotein - Cholesterol

3.6. Effect of FeedingBreadsticksSupplemented with Psyllium Seeds Powder on Liver Functions.

Alanine amino transferaseGPT (ALT) and aspartate amino transferaseGOT(AST) enzymes activities are known as cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after the damage of the cell membrane. Therefore, both enzymes are used as indicators for hepatic damage **[29].** Data in Table 8 showed that the liver functions of normal control and diabetic groups through the experimental period (6 weeks). Liver functions of diabetic groups (G2) were significant increased (p≤0.05)compared of normal control rats (G1). The results also indicated that, breadsticks contained psyllium seeds powder caused a significant decrease(p≤0.05)in liver functions of the groups feeding psyllium seeds powder(G3 to G5) comparing with negative control (G1) and positive diabetic control fed on breadsticks contained wheat flour only (G2).

Table 8. Effect of Feeding onBreadsticks Supplemented withPsyllium SeedsPowder on Liver Functions.

Rats groups	AST (GOT) (IU/L) *	ALT (GPT) (IU/L) *	Albumin (mg/dl) *
G1	35.62^{b}±1.78	32.95^{b}±1.66	3.55^{d}±0.31
G2	46.42^{a}±1.94	41.93^{a}±1.78	6.38^{a}±0.25
G3	35.65^{b}±1.61	38.17^{ab}±1.84	4.69^{b}±0.36
G4	34.65^{b}±2.06	36.63^{ab}±1.52	4.10^{b}±0.43
G5	32.32^{b}±1.98	34.93^{b}±1.78	3.95^{c}±0.34

Each value is an average of five determinations.

Values followed by the same letter in column are not significantly different at p ≤ 0.05.

3.7. Effect of FeedingBreadsticksSupplemented with Psyllium Seeds Powderon Kidney Function.

Table 9 shows the effect of bread sticks supplemented with psyllium seeds powder on the kidney function of diabetic rats. The results show that the liver function significantly increased (p≤0.05) in the infected groups(G2,G3,G4, and G5) compared to the control groupG1, while the groups that fed on breadsticks supplemented with psyllium seeds powder improved in the kidney function compared to the control groupG2.

Table 9.Effect of FeedingBreadsticks at Different PsylliumSeeds Powder on Kidney Functions of Diabetic Rats.

Rat groups	Urea (mg/dl)*	Uric acid (mg/dl)*	Creatinine (mg/dl)*
G1	33.57^{b}±2.25	2.82^{ab}±0.23	0.65^{c}±0.09
G2	43.91^{a}±2.43	4.16^{a}±0.38	1.38^{a}±0.12
G3	35.25^{b}±2.63	3.49^{b}±0.27	0.99^{b}±0.13
G4	34.25^{b}±2.43	3.16^{ab}±0.37	0.92^{b}±0.12
G5	31.60^{b}±1.97	3.00^{ab}± 0.16	0.75^{ab}±0.13

Each value is an average of five determinations.

Values followed by the same letter in column are not significantly different at p ≤ 0.05.

3.8. Organoleptic Properties of Breadsticks Supplemented with Psyllium Seeds Powder.

Data in Table 10 shows the organoleptic properties of bread sticks supplemented with 3, 6 and 9% psyllium seeds powder. The obtained data indicated that, no significantly

differences at (p≤0.05)were found between bread sticks produced by using 100% wheat flour and even those 3% psyllium seeds powder except for color, but significantly differences at (p≤0.05) were found in case of others levels of supplementation (6 and9%).However, the results revealed that, the produced bread sticks by using 3% psyllium seeds powder replacement (instead of 100% wheat flour) had properties better than those of 6% and 9%. So, it can be concluded that, the best level of supplemented with psyllium seeds powder 0was 3% followed by 6% for making breadsticks with a very good acceptability. The results are harmony with the results of **EL Hadidy,[14]**which showed that adding psyllium seeds powder at extents 5,10, and 15% to pan bread was suitable for panelists.

Table 10. Organoleptic Properties of Breadsticks Produced from Wheat Flour Supplemented with Different Levels of Psyllium Seeds Powder.

Blends	Color	Taste	Flavor	Texture	Overall Acceptability
	10	10	10	10	10
Blend1(100 %Wheat Flour)	9.43^{a} ±0.35	8.97^{a} ±0.37	9.23^{a} ±0.28	8.67^{a} ±0.52	9.16^{a} ±0.46
Blend2 + 3%PSP	7.56^{b} ±0.54	8.36^{b} ±0.26	8.98^{a} ±0.43	8.17^{a} ±0.44	8.72^{a} ±0.36
Blend3+ 6%PSP	7.32^{b} ±0.26	7.78^{b} ±0.53	7.75^{b} ±0.32	7.45^{b} ±0.62	7.42^{b} ±0.28
Blend4+ 9%PSP	6.63^{c} ±0.45	6.83^{c} ±0.46	7.52^{b} ±0.19	7.23^{b} ±0.26	7.37^{b} ±0.34

- Values followed by the same letter in columns are not significantly different at LSD at (p ≤ 0.05).
- Each value was an average of twenty determinations ± standard deviation.

4. DISCUSSION

The results of the present study show that injection with strepotozotocin STZ produced hyperglycemia and hypoinsulinemia in rats .On the other hand, psyllium seeds reversed STZ induced change in glucose and insulin levels. STZ enter β cells via a glucose transporter (GLUT2) using a variety of intracellular toxic mechanisms such as production of oxygen free radicals that causes degeneration of pancreatic β-cells leading to hypoinsulinemia and subsequent hyperglycemia [30].

Also, in agreement with our results Karhunen*et al*, [31] found that psyllium fiber enriched meals improve glucose and insulin level significantly than non- fiber enriched meals. The creation of a thick gel in an aqueous solution is thought to be the cause of psyllium's glucose-lowering effects. This gel might prevent glucose from reaching the small intestine's absorptive epithelium, which would dampen postprandial glucose peaks. Additionally, soluble fiber may postpone stomach emptying, which would reduce the absorption of carbohydrates. Another mechanism that may contribute to the postprandial effect of psyllium is the sequestration of carbohydrates ingested with the meal, thus retarding their access to digestive enzymes [32]. Also, the hypoglycemic activity of psyllium seeds may be due to the inhibition of liver gluconeogenesis. Furthermore, the increased level of serum insulin by increase insulin secretion from the remnant of β-cells and enhancement of peripheral metabolism of glucose. Hypoglycemic activity of psyllium seeds may be due to modulating effects on insulin sensitization and/or insulin secretion and a regulating action on digestion and intestinal absorption.

Also, another mechanism by which psyllium promotes weight loss may be due to increase the energy expenditure in adipose tissue by stimulating lipolysis and thermogenesis by stimulation of adrenaline secretion from adrenal gland. This increase in serum adrenaline leads to activation of hormone-sensitive lipase (HSL), the key enzyme in the regulation of lipid stores. Moreover, HDL-C protects against or reverse atherosclerosis by their ability to serve as acceptor particles for macrophage cholesterol efflux, prevention of endothelial dysfunction, and maintenance of endothelial integrity[33].

Conclusion

The findings suggested that psyllium seeds may be used as a new source of bioactive and functional food as well as an important agent for the treatment of diabetes rats. Comparing diabetic rats to healthy rats, daily administration of psyllium seed powder improved weight loss and decreased serum glucose, TC, TG, LDL and VLDL, urea, and uric acid while increasing HDL and improving liver and kidney function. Finally, it could make some bakery foods like breadsticks using PSP and wheat flour (72%ext) with ahigh quality that are suitable for diabetic patients.

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