

# Evaluation of Technological and Antihyperglycemic Effects of Pan Bread Enriched with Okra Pod Waste on Diabetic Male Rats

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## ABSTRACT

Vegetable processing waste is considered a cheap source of bioactive substances that are applicable as additives in the processing of some functional foods. So, this study aimed to use okra pod waste with substitution ratios of 5, 10 and 15 % of wheat flour in pan bread manufacture. The physicochemical, rheological and sensory properties of ready-made pan bread were studied. Also, the impact of feeding diabetic rats pan bread containing okra pod waste on some biological parameters and histological variations of pancreas, kidney and liver was evaluated. The obtained results revealed that okra pod waste powder showed a higher content of protein, fat, ash and crude fiber than wheat flour. Antioxidant activity of okra pod waste was 88.74%. In comparison to the control sample made from only wheat flour, the farinograph and extensigraph results of wheat flour combined with okra pod waste indicated a decrease in dough development, degree of softening, elasticity, and extensibility, but an increase in water absorption and stability time. Pan bread containing 15% okra waste had the highest loaves weight (873.33 g), and the lowest specific volume (1.81 cm<sup>3</sup>/g) compared with control bread (810.67 g and 2.28 cm<sup>3</sup>/g, respectively). When the ratio of okra waste powder in pan bread was increased, there was a substantial rise in the content of moisture, fat, protein, fiber, ash and Antioxidant activity. No significant differences were found in all sensory evaluation scores between pan bread containing 5% okra pod waste and the control. Whereas, pan bread containing 15% okra pod waste obtained the lowest scores but it was satisfactory. Feeding diabetic rats on pan bread containing 5, 10 and 15% of okra pod waste led to a significant reduction in glucose, HbA1c rates and increased insulin levels in blood samples. Also, it caused a significant amelioration in the lipid profile, kidney and liver functions, also pancreas, kidney and liver tissue sections of diabetic rats. In conclusion, pan bread containing okra pod waste can improve the glucose level and protection against diabetes diseases and their complications. Future studies are needed to find a new sources of bioactive compounds from food factory waste and use them in the processing of functional food products.

**Keywords:** Okra pod waste, Pan bread, Rheological properties, Sensory evaluation, Diabetes, Histopathology.

## INTRODUCTION

According to Kumar *et al.* (2020), diabetes mellitus (DM) is a chronic condition resulting from faulty

carbohydrate metabolism that causes blood glucose levels to rise over an extended period of time and low blood insulin rates or insensitivity of object tissues to insulin (Doostkam *et al.*, 2022). Hyperglycemia patients frequently experience polydipsia, polyuria, and polyphagia. These symptoms can also result in a number of consequences, including kidney failure, neuropathy, leg amputation, and vision loss (Laulloo *et al.*, 2021). International Diabetes Federation (2021) forecasted that diabetic adults will be about 537 million in 2021, 643 million in 2030 and 783 million in 2045. The number of deaths associated with DM was 6.7 million in 2021. Fahmy and Abd-Elmaksoud (2020) stated that Egypt is among the top 10 countries in the world for the number of diabetes patients, according to the International Diabetes Federation.

Insulin-dependent diabetes (type 1 diabetes) is a condition in which the patient's body cannot produce insulin due to autoimmune destruction of  $\beta$ -cells (American Diabetes Association, 2010). While, type 2 diabetes (non-insulin dependent diabetes), present more than 90% of diabetic patients, occurs as a result of inadequate release or poor response of the body's tissues to insulin, causing pancreatic  $\beta$ -cells to secrete a higher amount of insulin, leading to their significant damage (Ahmed & Ashiq, 2018 and Doostkam *et al.*, 2022). There are several synthetic hypoglycemic drugs used to manage diabetes, but these synthetic drugs are often expensive and show undesirable side effects (Laulloo *et al.*, 2021). While, a several phytochemicals responsible for hypoglycemia are present in more vegetables, fruits and its wastes.

One of the most essential vegetables in the Malvaceae family and a staple for centuries has been okra (*Abelmoschus esculentus*) (Petropoulos *et al.*, 2018). Okra requires less water and is drought-tolerant so it is grown in the tropics and the climatic temperate zone of the world (Habtamu *et al.*, 2018). In 2020, global okra fruit production reached 10.5 million tonnes on an area of 2.53 million hectares. Recently, India, Nigeria, Sudan, Mali, Coast Ivory, Egypt, Iraq and Pakistan have been the main countries where okra is grown (Fabianová *et al.*, 2022). In Egypt, okra is one of the most crops produced and consumed, its production

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was reported at 56,733 tonnes in 2016 (Central Agency for Public Mobilization and Statistics, 2019) and increased to 82,355 tonnes in 2021 (Food and Agricultural Organization Statistics, 2021). Mature okra pod commonly used in cooking, freezing, drying or canning processes. During prepare processes result several tons of okra pod wastes such as peduncle and septum.

The okra plant and its derived products were used for therapeutic purposes as anticancer, antidiabetic, antioxidant, to ease constipation and immunomodulatory potentials (Elkhalifa *et al.*, 2021 and Al-Shawi *et al.*, 2021). The okra plant pod, seeds, buds, flowers, fresh leaves and stems have several uses or functions. In the last few years, okra stem fibers were used to manufacture different types of shoe brushes, cleaning brush, painting brushes and ropes; they were also used in clothing fiber production (Gogoi *et al.*, 2017). Fully mature pod and stem rich with fibers are recycled in the paper manufacturing. When making jaggery, mucilage from the okra's stem and roots is utilized to clear sugarcane liquid. Esan *et al.* (2021) confirmed that the fiber of okra pod, peel and seed assistances to organize blood sugars via regulating the sugars level is absorbed in the gastric tract (Das *et al.*, 2019). Also, okra seed flour used in fortification of cereal flour to increase the content of protein, ash, oil and fiber (Adelakun *et al.*, 2009).

Food processing wastes are considered cheap sources of bioactive compounds that can be recycled and used within the food chain as foods and functional additives in various products, whether as an origin of natural antioxidants or dietary fiber. Recently, the demand for functional foods increased, so the development of foods was proceeded by adding one or more nutrient, that can by manned multiple health benefits (Abo Elnaga *et al.*, 2021 and Lau *et al.*, 2021). Pan bread will remain one of the more communal baked products between Egyptian consumers. To produce functional healthy bread to diabetic patients, must be reduced calories during bread prepare by increasing the fiber ratio (El-Hadidy, 2020 and Fahmy & Abd-Elmaksoud, 2020). So, the objective of this research was to utilize okra pod wastes (peduncle and septum) as a source of dietary fiber and antioxidants in pan bread. The influence of this addition on the physicochemical, rheological and sensory properties of pan bread, and the hypoglycemic effect on diabetic rats were also studied.

## MATERIALS AND METHODS

### Ethical statement

This paper was performed following the approval from the institutional animal care and research unit of

Zagazig University (Institutional Review Board Number ZU-IACUC/2/F/295/2023).

### Materials and ingredients

Okra (*Abelmoschus esculentus L.*) pod wastes (peduncle and septum) were collected during okra processing from the Food Industries & Packing Complex – Fipco, 3rd Industrial Zone A1, 10<sup>th</sup> Ramadan City, Egypt, in summer 2022. Wastes were washed first under tap water then dried in oven air (45 ±5°C) for 24 hours. The dehydrated wastes were ground into a powder using a coffee grinder, sieved through a 60-mesh screen, and then placed in a freezer-safe plastic container with a tight seal at -20°C to await additional processing and analysis.

The wheat flour (72% extraction) was gotten from Middle and West Delta Flour Mills Co. Streptozotocin and kits were bought from Sigma – Aldrich (MO, IL, USA). Casein, vitamins, minerals and cellulose were obtained from El-Gomhoria Pharmaceutical Company, Zagazig City, Egypt. Starch and corn oil were bought from public stores.

Forty adult male Wistar strain albino rats weighing 150 ± 10 g were obtained from the Helwan breeding farm located in Cairo, Egypt.

### Formulation and preparation of pan bread:

The straight dough method was performed according to the technique of Omran *et al.* (2020) with some alterations. The formula used to prepare the pan bread was as follows: 1000 g of wheat flour (72% extraction), 30 g yeast, 50 g sugar, 20 g salt, 100 g of full-fat milk powder and 30 g corn oil. This formula served as a control, and then okra pod wastes were used in place of 5, 10, and 15% of wheat flour with same other ingredients. The ingredients were well mixed with required amount of water (obtained by the farinograph test based on the flour weight) then rounded and put it in fermentation room at 37 °C for 60 min. After proofing, the dough samples were rested for 10 min. Pan breads were baked in an electrical oven for 20 min at 220 °C then separated from the pans and cooled for 2 h at room temperature. The resultant pan breads were sliced then packed in polyethylene bags for additional studies.

### Farinograph and exteinsograph tests:

Farinograph and exteinsograph tests were examined according to the procedures outlined by the AACC (2002) with Brabender Farinograph and Exteinsograph apparatus (Barabender OGH Duisburg, Germany) available at the Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.

### **Chemical composition analysis of okra pod waste and pan bread**

The chemical composition analysis of okra pod waste and prepared pan bread was predestined according to the standard methods in AOAC (2005). The caloric value of the cookie samples was estimated according to Krishna and Ranjhan (1980). Lignin content was determined according to Tanaka *et al.* (1985). Cellulose and hemicelluloses contents were measured according to Chahal *et al.* (1979).

### **Physical estimation of pan bread**

#### **Specific volume**

Bread loaves were weighted (g) after 2 hours of cooling at room temperature. Rapeseed replacement was used to calculate the volume (in cm<sup>3</sup>). By dividing (cm<sup>3</sup>/g) in accordance with the procedure outlined by the AACC (2002), a specific volume was obtained.

#### **Crust and crumb color**

The crust and crumb color of the bread loaves (*L*\*, *a*\* and *b*\*) were conducted according to Rao *et al.* (2011) using a Hunterlab color analyzer (Hunterlab color Flex EZ, USA).

#### **Phytochemical analysis**

Dried powder of okra wastes and bread samples were extracted with 70% aqueous ethanol in a ratio of 1:10 w/v. The extraction was left overnight at room temperature with shaking, and the samples were filtered through Whatman paper (No. 1). A rotary evaporator (BÜCHI-water Bath-B-480, Germany) was then used to evaporate the filtrate at 40°C. Using a Thermo-Electon Corporation Heto power dry LL300 freeze dryer (France), extracts were freeze-dried at -58±2°C. After being weighed to calculate yields, the dried extracts were kept at -20 °C until needed again.

Total phenolics were assessed using the Folin Ciocalteu Reagent Method, according to AOAC (1990). Flavonoids were examined according to Zhuang *et al.* (1992). The radical scavenging activity was assessed by the method of Zhang and Hamazu (2004).

#### **Sensory evaluation**

Fifteen panelists from Zagazig University's, Faculty of Agriculture's, Food Science Department conducted a sensory evaluation of pan bread samples, according to El-Bushuty (2020). The overall score means were converted into the following descriptive denominations: Very good is defined as 90–100, good as 80–90, satisfactory as 70–79, and questionable as less than 70.

### **Biological experiment**

#### **Animals and induction diabetes**

Male albino rats were housed in clean laboratory conditions in well-ventilated cages in the animal house of the Faculty of Agriculture, Zagazig University. In the

adaptation period for two weeks, the rats were fed on the basal diet, which was prepared in accordance with Campbell (1963) and Reeves *et al.* (1993). After the adaptation period, thirty-two male rats were injected by streptozotocin at a rate of 40 mg/Kg B.W. (Balamash *et al.*, 2018). After the streptozotocin injection, the injected rats consumed an ad libitum fructose solution of 10 % (wt/vol) for 3 days to overcome the drug according to Balasubramanian *et al.* (2004). The blood glucose rates were determined by Glucometer- elite commercial test (Fine test), by collecting blood samples from the tip of the tail of all rats. Animals were classified as diabetic if their blood glucose level was greater than 200 mg/dl (Fagbohun *et al.*, 2020), while non diabetic rats were re-injected.

#### **Experimental design and animal group**

The rats were divided into the following five groups at random, with eight rats in each group: Group (1) was given a basal diet as a negative control. Group (2) was a positive control group that received a basal diet after receiving a streptozotocin injection. Following a streptozotocin injection, groups 3, 4, and 5 were fed pan bread containing 5, 10, and 15% of the waste from okra, respectively, with 30% of their diet (Sayed-Ahmed, 2014). During the trial period of 30 days, the amounts of food intake and waste were recorded daily to determine the food intake. Whereas, the body weight was recorded every week. The body weight gain (B.W.G.%) was calculated according to Chapman *et al.* (1959), Food efficiency ratio (F.E.R) was calculated according to Lee and Nieman (1996).

#### **Biochemical examination of blood samples**

At the end of the experiment, the rats were fasted the whole night before being slaughtered. Blood samples were combined from the aorta via Wassermann and EIDTA tubes then centrifuged at 3000 rpm for 20 min to separate serum and plasma. The separated serum and plasma were precisely placed into dry, clean Eppendorf tubes, then kept freezing at -20°C until examination. The blood glucose levels were measured by the Glucometer Elite commercial test (Fine test) immediately after sacrificed each rat.

Glucose, insulin, HbA1c%, triglycerides, total cholesterol, HDL-C, LDL-C, VLDL-C, uric acid, creatinine, total protein, AST and ALT were measured according to the techniques of Pruden *et al.* (1995), Burgi *et al.* (1988), Sudhakar and Pattabiraman (1981), Stein (1987), Young (2001), Lopes *et al.* (1977), Friedewald *et al.* (1972), Patton and Crouch (1977), Murray (1984), Koller (1984) and Retiman and Frankel (1957), respectively.

### Histopathological examination

The histopathological preparation techniques were applied as elucidated by Bancroft and Stevens (2013). Tissues from the pancreas, liver, and kidney were cut to a thickness of 3–4 mm, preserved in 10% neutral buffered formalin (10% NBF), dried in varying ethanol concentrations, cleaned in xylene, and embedded in paraffin. In order to study general tissue structure, the paraffin blocks were sectioned using a microtome at a thickness of 4–6 $\mu$ m, and then stained with hematoxylin and eosin. At Cairo University's Faculty of Veterinary Medicine, H&E-stained sections were inspected, and sections of the central vein and portal areas were photographed for each group using a Leica microscope (CH9435 Hee56rbrugg) (Leica Microsystems, Switzerland).

### Statistical analysis

A statistical program called "SPSS" version 20 for Microsoft Windows was used for all statistical analyses according to Dominick and Derrick (2001). The mean  $\pm$  SD was used to express numerical data. The least significant difference (LSD) method was used to analyze the marker levels. The means for the same examination with different superscript letters differ significantly at  $P \leq 0.05$ , but the means with the same letters are not significant at  $P > 0.05$ .

## RESULTS AND DISCUSSION

### Chemical composition of raw materials

Chemical composition of okra pod waste powder and wheat flour are shown in Table (1). The results revealed that okra pod waste powder showed a higher content of protein (12.40%), fat (3.15%), ash (11.79%) and crude fiber (14.76%) than wheat flour, conversely, wheat flour recorded a higher content of moisture (13.64%) and carbohydrates (71.23%). The protein content of wheat flour in this study was higher than that determined by Shehata (2020) (10.05%), while it was equal to that determined by El-Gammal *et al.* (2016) (11.50%). The protein content (12.40%) in okra pod waste powder was lower than that in okra fruit flour (19.06%), while the fat, ash and fiber contents were higher than those in okra fruit flour (2.91, 9.06 and 12.91, respectively) (El-Sayed *et al.*, 2019). Also, the protein, fat and fiber contents in okra pod waste powder was lower than that in okra seeds powder (32.68, 8.46 and 32.39%, respectively) (Aydin *et al.*, 2023).

**Table 1. Chemical composition of okra pod waste powder and wheat flour (g/ 100g dry weight basis)**

Item	Okra pod waste	Wheat flour
Moisture	10.85 $\pm$ 0.19	13.64 $\pm$ 0.25
Crude protein	12.40 $\pm$ 0.47	11.50 $\pm$ 0.44
Crude fat	3.15 $\pm$ 0.09	2.44 $\pm$ 0.07
Ash	11.79 $\pm$ 0.11	0.71 $\pm$ 0.01
Crude fiber	14.76 $\pm$ 1.10	0.48 $\pm$ 0.04
Carbohydrates	47.05 $\pm$ 0.32	71.23 $\pm$ 0.24

Means of three replicates  $\pm$  standard deviation

### Total phenolic and flavonoid compounds and DPPH-free radical scavenging activity of okra pod waste powder

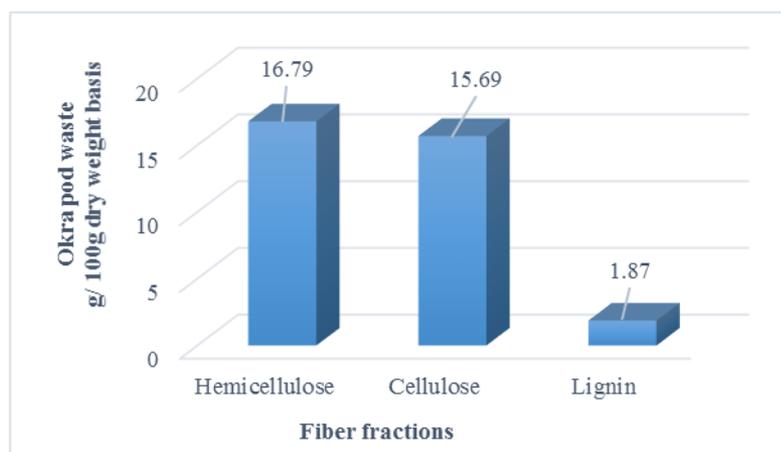
The results in Table (2) indicated that total phenolic and flavonoid compounds in okra pod waste powder were 227.08  $\mu$ g GAE  $g^{-1}$  and 123.37  $\mu$ g QE  $g^{-1}$ , respectively. In addition, the DPPH free radical scavenging activity ratio of okra pod waste powder was 88.74%. However, Ahiakpa *et al.* (2013) revealed that the content of total phenolics and flavonoids compounds in okra fruits were 12.99 mg GAE/ml extract and 2288.20 mg QE/ml extract. While okra seeds contain 16.5 mg GAE/100 g of total phenolic compounds (Aydin *et al.*, 2023). Also, Abouel-Yazeed (2019) established that the total phenolic and antioxidant activity in okra seeds powder were 34.29 mg GAE/100g and 50.43%, respectively.

**Table 2. Total phenolic & flavonoid compounds and DPPH free radical scavenging activity of okra pod waste powder**

Raw material	Total phenolic ( $\mu$ g GAE $g^{-1}$ )	Total flavonoid ( $\mu$ g QE $g^{-1}$ )	DPPH %
Okra pod waste	227.08 $\pm$ 1.28	123.37 $\pm$ 0.41	88.74 $\pm$ 0.89

### Fiber fractions of okra pod waste powder

The insoluble fibers present in okra pod waste powder were hemicelluloses, cellulose and lignin with levels of 16.79, 15.69 and 1.87%, respectively (Fig. 1). Nikpayam *et al.* (2021) stated that the fiber conformation in okra pod is  $\alpha$ -cellulose, hemicellulose and lignin with levels of 67.5%, 15.4% and 7.1%, respectively.



**Fig. 1. Fiber fractions analysis results of okra pod waste powder**

### Rheological properties

Table (3) displays the effect of replacing 5, 10 and 15% of wheat flour by okra pod waste powder on the farinograph and extensograph parameters of pan bread. The water absorption exhibited a gradual increase with increasing the replacement rate of wheat flour with okra pod waste. This rise in water absorption can be due to the high fiber content which is substantiated by the chemical composition of okra pod waste, where the fiber contains several hydroxyl groups that react with hydrogen bonds of water, thereby supporting absorption (Porízka *et al.*, 2023). Also, okra pod waste contains mucilage that is a mixture of polysaccharides which absorb water (Alamri, 2014). Therefore, pan bread blends containing wheat flour and 15% okra pod waste powder had the highest water absorption (59%) than pan bread blends containing wheat flour only (52%). Arrival time is one of the important farinograph parameters, it is the time needed of the curve to reach the point of the highest torque after the commencement of mixing (the 500 BU mark), where higher arrival time point out slower gluten development during blending. Thus, this will make dough mixing time is more longer and delay bread making (Lotfy and Bayomy, 2017). The results in Table (3) indicated that the arrival time for the pan bread blends containing 5 and 10% okra pod waste powder (0.50 and 0.75 min, respectively) was lower than the control (1.00 min), pointing out quicker dough development and water absorption. While the pan bread blend containing 15% okra pod waste powder arrived at 1.00 min equally with the control.

Dough development began with the addition of water and the beginning of the mixing process. The dough development time indicates the period of time needed after blending the flour and other components until it reaches maximum consistency (Lotfy and Bayomy, 2017). The dough development time decreased from 12.00 min for the control to 10.00 for the dough containing 5 and 10% okra pod waste and 8.50 min for the dough contained 15% okra pod waste. The quantity and quality of gluten, flour particles and degree of milling influence on dough development time (Mospah *et al.*, 2023). Also, results showed that the highest stability time was for dough contained 5% okra pod waste (14.00 min) followed by dough contained 10 and 15% okra pod waste (13.00 min) then the control dough (12.00 min). The rise of stability time indicates to high dough quality. According to Zhang *et al.* (2019), the dough stability time point out the length of time the dough keeps its optimal consistency and is a reliable indicator of dough strength. The degree of softening, elasticity and energy decreased with increasing the replacement rate with okra pod waste. Concerning extensibility, it was decreased in dough contained 10 and 15% okra pod waste compared to the control and dough contained 5% okra pod waste. This decrease may be due to increase of fiber particles presence in dough or because of the mucilage presence in okra waste which influence on gluten network, where okra gum act as a binding agent with both of flour and water and formed a glutinous paste (Alamri, 2014 and Atta *et al.*, 2023).

**Table 3. Farinograph and extinsograph parameters of pan bread blends containing wheat flour and okra pod waste powder**

	Blends	Pan bread			
		100% WF	95% WF + 5% OPW	90% WF + 10% OPW	85% WF+ 15% OPW
Farinograph	Water absorption%	52.00	54.00	55.00	59.00
	Arrival time (min)	1.00	0.50	0.75	1.00
	Dough development (min)	12	10	10	8.5
	Stability time (min)	12	14	13	13
	Degree of softening (B.U)	90	70	50	50
Extinsograph	Elasticity (B.U)	560	450	420	310
	Extensibility (mm)	120	120	85	65
	P.N	4.66	3.75	4.94	4.76
	Energy (Cm <sup>2</sup> )	80	50	40	30

WF: What flour; OPW: Okra pod waste

### Physical properties of pan bread

The effect of okra pod waste powder addition on physical properties of pan bread is presented in Table (4). It could be observed that, a significant increase in the loaves weight of pan bread with increasing the replacement ratios of okra pod waste powder compared with the control bread. On the contrary, a significant decrease in loaves volume and specific volume values was found with increasing the okra pod waste powder ratios. Pan bread loaf containing 15% okra pod waste had the highest weight and lowest specific volume comparing to other bread loaves. These results are in conformity with those obtained by Sallam *et al.* (2019) and Alsuhaibani & Alshawi (2022), who discovered that when the dietary fiber increased, the bread's volume and specific volume decreased. Furthermore, Alamri (2014) and Xu *et al.*, (2020) indicated that the cause of the bread volume reduction by addition of okra flour attributed to the ability of okra mucilage to absorb the water, which negatively affects gluten during dough blending. Where, the high absorption of water and the fiber content in okra caused a decrease in the water required to connect the starch-gluten network among the bread manufacture. Hence, the gluten network did not evolve led to reduce the bread volume.

### Chemical composition of pan bread

The results in Table (5) showed that moisture, protein, fat, ash and fiber contents significantly increased with increasing the okra pod waste powder ratio in pan bread, while carbohydrates and energy contents significantly decreased compared with the

bread control. Pan bread samples containing 15 % okra pod waste powder had the highest protein, fat, ash and fiber contents compared with the control because protein, fat, ash and crude fiber levels in okra pod waste were higher than that in wheat flour. These results agree with Akoja and Coker (2018) they point out the addition of okra flour to biscuit led to a significant increase in protein, ash and fiber content, while energy and carbohydrate value decreased. Also, Owhero *et al.* (2023) made fried snack by mixing wheat flour with the okra seed flour at levels 10, 20,30, 40 and 50% and showed that the protein, ash and fiber content increased with raising okra seed flour levels. while there was reduction in the moisture, fat and carbohydrate content.

### DPPH free radical scavenging activity of prepared pan bread

The results of the DPPH free radical scavenging assay of pan bread are shown in Fig. (2). Pan bread containing 15% okra pod waste powder had the highest DPPH scavenging activity percentage (78.82%), while control pan bread had the lowest (56.10 %). It was noticed that adding okra pod waste powder led to a rise in DPPH scavenging activity. This increase in free radical scavenging activity may be attributed to the higher phenolic content (Meral and Köse, 2019) in okra pod waste than wheat flour. This result is agreement with Xu *et al.* (2020) who advertised that okra seed flour intensely increased the phenolic content, thus antioxidant activity of wheat bread.

**Table 4. Physical properties of pan bread containing okra pod waste**

Item	Pan bread				LSD
	Control	5% OPW	10% OPW	15% OPW	
Weight (g)	810.67± 6.51 <sup>d</sup>	846.67± 4.16 <sup>c</sup>	859.33± 6.66 <sup>b</sup>	873.33± 5.13 <sup>a</sup>	10.75
Volume (cm <sup>3</sup> )	1848.00±18.00 <sup>a</sup>	1694.00±25.53 <sup>b</sup>	1655.00±21.79 <sup>b</sup>	1583.00±17.09 <sup>c</sup>	39.30
Specific volume (cm <sup>3</sup> / g)	2.28± 0.02 <sup>a</sup>	2.00± 0.04 <sup>b</sup>	1.93± 0.04 <sup>c</sup>	1.81± 0.03 <sup>d</sup>	0.06

OPW: Okra pod waste

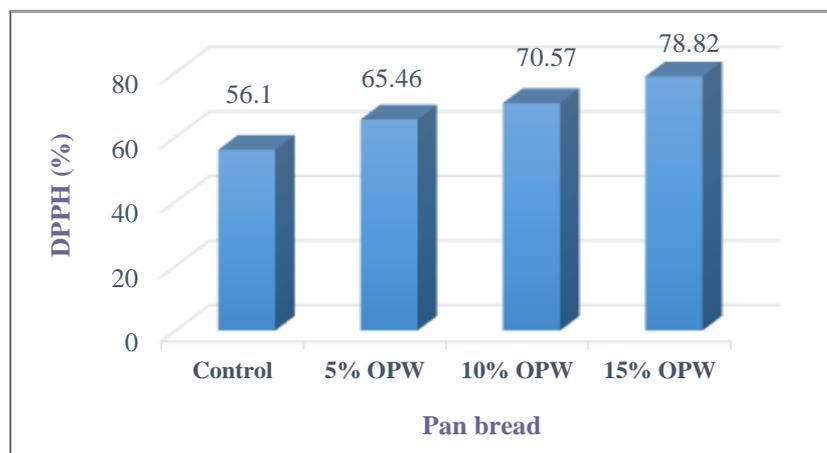
Mean values with different superscripts in the same row are significantly different ( $P \leq 0.05$ )

**Table 5. Chemical composition of pan bread containing okra pod waste powder**

Item	Pan bread				LSD
	Control	5% OPW	10% OPW	15% OPW	
Moisture (%)	27.52± 0.49 <sup>c</sup>	30.20± 0.54 <sup>b</sup>	31.41± 0.58 <sup>a</sup>	31.76± 0.55 <sup>a</sup>	1.02
Crude protein (%)	9.00± 0.30 <sup>c</sup>	9.32± 0.28 <sup>bc</sup>	9.70± 0.27 <sup>ab</sup>	10.04± 0.35 <sup>a</sup>	0.56
Crude fat (%)	4.77± 0.21 <sup>b</sup>	4.92± 0.25 <sup>b</sup>	5.09± 0.29 <sup>ab</sup>	5.42± 0.25 <sup>a</sup>	0.47
Ash (%)	0.52± 0.01 <sup>d</sup>	1.36± 0.01 <sup>c</sup>	1.63± 0.02 <sup>b</sup>	2.00± 0.02 <sup>a</sup>	0.03
Crude fiber (%)	0.67± 0.07 <sup>c</sup>	1.83± 0.13 <sup>b</sup>	2.05± 0.15 <sup>b</sup>	2.82± 0.21 <sup>a</sup>	0.28
Carbohydrates (%)	57.53± 0.69 <sup>a</sup>	52.37± 0.36 <sup>b</sup>	50.12± 0.57 <sup>c</sup>	47.97± 0.25 <sup>d</sup>	0.94
Energy kcal/100g	309.01± 1.03 <sup>a</sup>	291.06± 3.56 <sup>b</sup>	285.09± 2.92 <sup>c</sup>	280.76± 2.58 <sup>c</sup>	5.06

OPW: Okra pod waste

Mean values with different superscripts in the same row are significantly different ( $P \leq 0.05$ )



**Fig. 2. DPPH free radical scavenging assay of pan bread containing okra pod waste powder**

**Color evaluation of prepared pan bread**

The effect of okra pod waste powder addition on pan bread color is presented in Table (6) and Fig. (3). Concerning crust color, it was noticed that by increasing the addition ratio of okra pod waste powder, the value of  $L^*$  significantly decreased from 46.74 of the control pan bread to 38.68 of pan bread containing 15% okra pod waste powder. Moreover, by increasing the addition ratio of okra pod waste powder, there were a significant decline in the values of  $a^*$  and  $b^*$ . This is may be referring to Millard and caramelization reactions. These reactions attributed or refer to the degree of polymerization and the ratio of low molecular weight of

sugars in the formulation (Zhang *et al.*, 2019 and Shobeiri *et al.*, 2023).

In crumb color, there were a significant decreased in  $L^*$  values of pan bread containing okra pod waste compared to the control pan bread. The reduction of crumb lightness may be referred to increase the ratio of okra pod waste to dough which had greater amount of moisture caused lower lightness. Moreover, the increase in the ratios of okra pod waste addition caused a significant increase in crumb  $a^*$  values and reduction in crumb  $b^*$  values. The obtained results are harmonized with Shobeiri *et al.* (2023) who stated that the adding okra flour to cake led to increase in  $a^*$  values and decrease in  $b^*$  values.

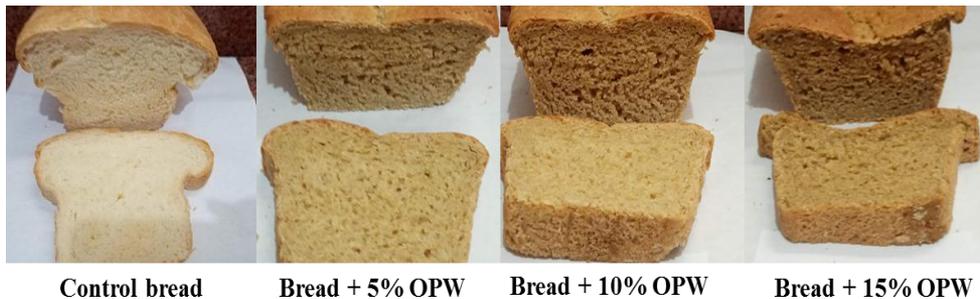
**Table 6. Color characteristics of pan bread containing okra pod waste**

Item		Pan bread				LSD
		Control	5% OPW	10% OPW	15% OPW	
Crust	$L^*$	46.74± 1.86 <sup>a</sup>	44.36± 1.58 <sup>a</sup>	40.20± 0.75 <sup>b</sup>	38.68± 2.91 <sup>b</sup>	3.65
	$a^*$	14.29± 1.80 <sup>a</sup>	12.75± 0.67 <sup>ab</sup>	10.89± 0.09 <sup>b</sup>	8.18± 0.87 <sup>c</sup>	1.99
	$b^*$	22.12± 2.11 <sup>a</sup>	21.23± 0.68 <sup>a</sup>	18.16± 0.32 <sup>b</sup>	17.11± 1.59 <sup>b</sup>	2.58
Crumb	$L^*$	67.73± 155 <sup>a</sup>	54.89± 1.80 <sup>b</sup>	49.35± 1.05 <sup>c</sup>	43.04± 0.88 <sup>d</sup>	2.58
	$a^*$	-1.37± 0.10 <sup>d</sup>	0.37± 0.13 <sup>c</sup>	1.35± 0.21 <sup>b</sup>	1.64± 0.11 <sup>a</sup>	0.27
	$b^*$	22.54± 0.71 <sup>a</sup>	20.90± 0.60 <sup>b</sup>	19.23± 0.39 <sup>c</sup>	17.56± 0.22 <sup>d</sup>	0.97

$L^*$ : lightness,  $a^*$ : redness,  $b^*$ : yellowness.

OPW: Okra pod waste

Mean values with different superscripts in the same row are significantly different ( $P \leq 0.05$ )



**Fig. 3. Control bread and bread containing okra pod waste (OPW)**

### Sensory evaluation of pan bread

From the essential encouraging factors for consumers to acceptability of the made pan bread is the sensory properties. Crust color, crumb color, crumb distribution, taste, flavor, general appearance and overall acceptability of pan bread made by replacing wheat flour with 5, 10, and 15% okra pod waste powder are given in Table (7). The results revealed that the control pan bread recorded the highest of all sensory properties scores compared to pan bread containing okra pod waste. No significant differences were found in all sensory evaluation scores between pan bread containing 5% okra pod waste and the control. Pan bread containing 15% okra pod waste recorded the lowest scores of sensory evaluations but it was satisfactory. The color scores were reduced with incorporation of okra pod waste in pan bread cause of the green color of okra pod waste which produced dark color. Moreover, the darkness increased with raising the okra pod waste ratio in bread, hence it was reflected on  $L^*$  values (Table 6). This may be owing to the non-enzymatic browning reactions, which are influenced by the water distribution and the reaction between reducing sugars and amino acids (Martínez-Girón *et al.*, 2017 and Omran *et al.*, 2020). In addition, it could be noted that no significant differences were found in flavor for all pan bread samples, this may be due to the low content of volatile compounds in okra pod waste which led to flavor acceptable. Crumb distribution scores decrease of pan bread containing okra pod waste may be due to the

addition of dietary fiber (Omran *et al.*, 2020). These results are in line with those obtained by Akoja and Coker (2018) they reported that presence of the okra flour to biscuit at ratios 5, 10, 15, 20 and 25% led to a significant difference in the sensory quality, but all biscuit samples were acceptable with high rating grade of likeness.

### Body weight gain and food intake of diabetic rats

Results illustrated in Table (8) show the effect of feeding on pan bread containing okra pod waste on body weight gain, food intake and FER of diabetic rats. The mean values of weight gain, body weight gain%, food intake and FER were significantly reduced in the positive control group (G2) compared to the negative control group (G1) and these results are matched with those obtained by Abul-Fadl *et al.* (2016) and Shehata (2020) who observed that body weight gain, food intake and FER were reduced in diabetic rats compared to the negative control group. Diabetic rats fed on pan bread containing 10 and 15% okra pod waste caused a significant increase in these parameters compared to the positive control group. Also, a significant difference was observed between the G5 which fed on pan bread containing 15% okra pod waste for body weight gain and FER compared to the negative control group. While, there were no significant differences for BWG% and food intake in G5 compared to G1.

The obtained results are consistent with those of Uadia *et al.* (2020) who found a rise in the body weight of diabetic rats fed on a basal diet containing okra

**Table 7. Sensory evaluation of pan bread containing okra pod waste powder**

Item	Pan bread				LSD
	Control	5% OPW	10% OPW	15% OPW	
Crust color (15)	14.75± 0.71 <sup>a</sup>	14.13± 0.99 <sup>ab</sup>	13.25± 1.38 <sup>bc</sup>	12.38± 1.41 <sup>c</sup>	1.16
Crumb color (15)	14.63± 0.74 <sup>a</sup>	13.75± 0.89 <sup>ab</sup>	13.00± 0.93 <sup>bc</sup>	12.25± 1.04 <sup>c</sup>	0.93
Crumb distribution (15)	14.38± 0.74 <sup>a</sup>	13.63± 1.30 <sup>a</sup>	12.38± 0.74 <sup>b</sup>	10.75± 0.89 <sup>c</sup>	0.97
Taste (20)	18.63± 1.41 <sup>a</sup>	18.50± 0.93 <sup>a</sup>	18.63± 1.85 <sup>a</sup>	14.75± 1.39 <sup>b</sup>	1.46
Flavor (15)	14.00± 1.69 <sup>a</sup>	14.13± 1.13 <sup>a</sup>	13.50± 2.51 <sup>a</sup>	12.63± 2.45 <sup>a</sup>	2.07
General appearance (20)	19.25± 0.89 <sup>a</sup>	19.13± 0.83 <sup>a</sup>	17.63± 1.06 <sup>b</sup>	16.25± 1.49 <sup>c</sup>	1.12
Overall acceptability (100)	95.63± 5.34 <sup>a</sup>	93.25± 4.40 <sup>a</sup>	88.38± 3.07 <sup>b</sup>	79.00± 5.04 <sup>c</sup>	4.66
Grade	Very good	Very good	Good	Satisfactory	

OPW: Okra pod waste

Mean values with different superscripts in the same row are significantly different ( $P \leq 0.05$ )

**Table 8. Effect of feeding with pan bread containing okra pod waste on body weight gain, food intake and FER of diabetic rats**

Groups	Body weight gain (g/ 30 days)	Body weight gain %	Food intake (g/ day)	Food intake (g / 30 days)	Food efficiency ratio (FER)
G1	77.67±4.51 <sup>a</sup>	29.47±2.61 <sup>a</sup>	27.56±1.38 <sup>a</sup>	826.80±41.32 <sup>a</sup>	0.094±0.005 <sup>a</sup>
G2	37.00±1.73 <sup>d</sup>	17.73±1.21 <sup>c</sup>	22.56±0.64 <sup>cd</sup>	676.80±19.24 <sup>cd</sup>	0.055±0.002 <sup>d</sup>
G3	40.33±3.21 <sup>d</sup>	16.42±1.66 <sup>c</sup>	20.41±2.21 <sup>d</sup>	612.30±65.83 <sup>d</sup>	0.066±0.002 <sup>c</sup>
G4	46.33±1.53 <sup>c</sup>	24.00±3.30 <sup>b</sup>	23.74±0.33 <sup>bc</sup>	712.20±9.99 <sup>bc</sup>	0.065±0.001 <sup>c</sup>
G5	56.67±1.15 <sup>b</sup>	26.56±1.16 <sup>ab</sup>	25.81±0.76 <sup>ab</sup>	774.30±22.74 <sup>ab</sup>	0.073±0.003 <sup>b</sup>
LSD	4.97	3.92	2.23	68.26	0.005

G1: Negative control group.

G2: Positive control group.

G3, G4 and G5: Fed on pan bread containing 5, 10 and 15 % okra pod waste, respectively.

Mean values with different superscripts in the same column are significantly different ( $P \leq 0.05$ ).

powder compared to diabetic rats fed on a basal diet without okra powder. Also, it was mentioned that the weight increase in diabetic rats fed an okra-based diet was likely caused by the high nutrient content of the okra powder, which included minerals, vitamins, tryptophan, lysine, and linoleic acid, all of which are known to improve health. The weight loss in the positive control group of rats may have been caused by unchecked lipolysis, particularly in peripheral tissues like the skeletal muscle.

**Glucose, HbA1c and insulin of diabetic rats**

Data presented in Table (9) shows the effect of feeding diabetic rats pan bread blended with different ratios of okra pod waste on glucose, HbA1c% and insulin levels. It could be observed that the rat injection with streptozotocin led to diabetes induction and a significant increase in both glucose levels and HbA1c% to be 262.17 mg /dl and 9.93%, respectively in the positive control group compared to the negative control group (83.73 mg /dl and 5.73%, respectively). At the same time, the insulin level recorded a significant decrease to 6.18 U/ml for the positive control group compared to 17.46 U/ml for the negative control group. This is due to the toxicity of streptozotocin which causes an inflammatory process lead to selective destruction of insulin producing islet β-cells in the pancreas. As a result of this process, insulin levels

decreased while glucose and HbA1c% levels increased in diabetic rats (Furman, 2015 and Akinlade *et al.*, 2021).

Feeding diabetic rats on pan bread with different levels (5, 10 and 15%) of okra pod waste resulted in a significant decline in the blood glucose levels and HbA1c% compared to the positive control group. There were no significant differences between HbA1c% for diabetic rats fed on 15% okra pod waste and negative control group. A significant increase was found in insulin levels of diabetic rats fed on pan bread containing different ratios of okra pod waste than that of the positive control group.

The reduction in glucose levels or HbA1c% and rising insulin levels of diabetic rats fed pan bread with okra pod waste may be attributed to the presence of myricetin, which is the main glucose-lowering agent in okra, adding to some other agents like β- sitosterol, kaempferol and oleanolic acid (Uadia *et al.*, 2020). Okra extract is rich with antioxidant components which destroy oxidative stress and insulin resistance, thus improving the glucose levels of diabetic rats (Tian *et al.*, 2015). As well as, the okra is rich with mucilage and fiber that produce sticky gels which bind glucose, thus retarding their absorption from the intestinal mucosa into the blood (Prabhune & Sharma, 2017).

**Table 9. Effect of feeding with pan bread containing okra pod waste on glucose, HbA1c and insulin of diabetic rats**

Groups	Glucose (mg /dl)	HbA1c %	Insulin (U /ml)
G1	83.73±7.06 <sup>c</sup>	5.73±0.81 <sup>d</sup>	17.46±1.95 <sup>a</sup>
G2	262.17±5.39 <sup>a</sup>	9.93±0.35 <sup>a</sup>	6.18±1.00 <sup>c</sup>
G3	192.03±7.17 <sup>b</sup>	7.83±0.49 <sup>b</sup>	8.81±0.78 <sup>d</sup>
G4	140.50±3.50 <sup>c</sup>	6.97±0.15 <sup>bc</sup>	11.61±0.55 <sup>c</sup>
G5	102.67±3.88 <sup>d</sup>	6.17±0.49 <sup>cd</sup>	14.89±0.72 <sup>b</sup>
LSD	10.21	0.92	2.14

G1: Negative control group.

G2: Positive control group.

G3, G4 and G5: Fed on pan bread containing 5, 10 and 15 % okra pod waste, respectively.

Mean values with different superscripts in the same column are significantly different ( $P \leq 0.05$ )

Furthermore, Saatchi *et al.* (2022) stated that the antidiabetic activity of okra is due to the rhamnogalacturonan component, which is the main polysaccharide in okra but with blurred mechanism. Furthermore, Wu *et al.* (2020) found that the aqueous okra pod extract was responsible for encouraging insulin excretion and decreasing blood glucose levels. As well, Mokgalaboni *et al.* (2023) reported that okra had a potential to regulate hyperglycemia, so it can be used as a supplemental dietary to improvement of the glycemic in patients with pre-diabetes or Type 2 diabetes.

#### Lipid profile of diabetic rats

The effect of feeding pan bread containing okra pod waste on lipid profile of diabetic rats is presented in Table (10). It could be noted a markedly significant increase in the mean values of TG, TC, LDL and VLDL for the positive group (G2) compared to the negative control group. Concerning, the mean values of HDL for the positive group (G2) (23.40 mg/dl) recorded a significant decline compared to the negative control group (53.91 mg/dl). Feeding diabetic rats on pan bread containing okra pod waste caused a significant improvement in the mean values of lipid profile parameters compared to the positive control group, and at the same time, it was near to the normal groups values. There was no significant difference in HDL levels between negative control group and diabetic rats fed on bread containing 10 and 15%. The improvement in lipid profile parameters of diabetic rats fed on pan bread containing okra pod waste perhaps are due to high content of fiber, mucilage and polyphenols in okra pod waste. Where, Prabhune & Sharma (2017) established that okra is rich with mucilage and fiber that form sticky gels that bind lipids, thus delaying their absorption it from the intestinal mucosa and accelerating faecal excretion. According to El-Sayed *et al.* (2019), okra is high in flavonoids, which may lower the risk of obesity, and its flour contains a high fiber content that promotes the breakdown of cholesterol to fecal bile acids. The

obtained results are in line with El-Sayed *et al.* (2019) who noted that the TG, TC, LDL and VLDL values of rats group consumed balady bread containing okra flour were reduced when compared it with rats group consumed balady bread containing 100% wheat flour. Furthermore, Esan *et al.* (2021) assured that aqueous okra fruit extracts caused a significant decrease in plasma cholesterol and triglycerides levels in diabetic rats. Also, Sabitha *et al.* (2011) confirmed that okra peels and seeds act to organizing blood glucose and reduce the lipid profile in diabetic rats.

#### Kidney and liver function of diabetic rats

As shown in Table (11), the mean levels of urea and creatinine in the positive group were significantly increased as compared to the negative control group and this was due to protein catabolism, degenerative changes and dysfunction of the kidneys, thus establishing the hypothesis that diabetes impairs kidney function (Abul-Fadl *et al.* 2016). These results are harmonized with Shehata and El-sayed (2020). Feeding diabetic rats on pan bread containing 5, 10 and 15% of okra pod waste resulted in a significant decrease for serum urea and creatinine levels compared to the positive control group. Additionally, insignificant differences were found between diabetic rats fed bread containing 15% okra pod waste and negative control group (G1), these results are confirmed by El-Sayed *et al.* (2019) who observed that the urea and creatinine values of rats group consumed balady bread containing okra flour reduced when compared it with rats group consumed balady bread containing 100% wheat flour.

Concerning liver function in the same Table, it was observed that the diabetic rats (positive control group) showed a significant increase in liver parameters including ALT and AST enzymes to be 41.92 and 208.43 U/l, respectively compared to the negative control group (24.19 and 91.96 U/l, respectively). Similar results were reported by Yazdi *et al.* (2019). This enzyme levels significantly reduced in diabetic rat

groups fed on pan bread containing different ratio of okra pod waste than that in the positive control group.

**Table 10. Effect of feeding with pan bread containing okra pod waste on lipid profile of diabetic rats**

Groups	Triglyceride (mg /dl)	Total cholesterol (mg /dl)	HDL (mg /dl)	LDL (mg /dl)	VLDL (mg /dl)
G1	88.53±1.70 <sup>e</sup>	93.61±2.67 <sup>e</sup>	53.91±2.04 <sup>a</sup>	22.00±1.00 <sup>e</sup>	17.71±0.34 <sup>e</sup>
G2	194.77±4.90 <sup>a</sup>	185.17±2.05 <sup>a</sup>	23.40±0.55 <sup>c</sup>	122.82±2.40 <sup>a</sup>	38.95±0.98 <sup>a</sup>
G3	145.77±3.48 <sup>b</sup>	144.08±1.77 <sup>b</sup>	33.42±1.91 <sup>b</sup>	81.51±2.77 <sup>b</sup>	29.15±0.70 <sup>b</sup>
G4	128.07±1.86 <sup>c</sup>	125.47±2.41 <sup>c</sup>	51.74±0.68 <sup>a</sup>	48.07±2.02 <sup>c</sup>	25.86±0.40 <sup>c</sup>
G5	96.33±3.04 <sup>d</sup>	99.53±2.81 <sup>d</sup>	53.94±4.49 <sup>a</sup>	26.33±2.79 <sup>d</sup>	19.27±0.61 <sup>d</sup>
LSD	5.85	4.32	4.36	4.17	1.22

G1: Negative control group. G2: Positive control group.  
 G3, G4 and G5: Fed on pan bread containing 5, 10 and 15 % okra pod waste, respectively.  
 Mean values with different superscripts in the same column are significantly different ( $P \leq 0.05$ )

**Table 11. Effect of feeding with pan bread containing okra pod waste on kidney and liver function of diabetic rats**

Group	Kidney function		Liver function	
	Urea (mg /dl)	Creatinine (mg /dl)	ALT (U/l)	AST (U/l)
G1	25.79±0.68 <sup>d</sup>	0.59±0.02 <sup>d</sup>	24.19±1.74 <sup>e</sup>	91.96±7.88 <sup>e</sup>
G2	45.44±1.56 <sup>a</sup>	1.05±0.08 <sup>a</sup>	41.92±2.81 <sup>a</sup>	208.43±2.80 <sup>a</sup>
G3	35.62±1.32 <sup>b</sup>	0.82±0.02 <sup>b</sup>	36.88±1.29 <sup>b</sup>	186.53±2.93 <sup>b</sup>
G4	30.94±0.86 <sup>c</sup>	0.71±0.02 <sup>c</sup>	32.59±0.64 <sup>c</sup>	153.67±3.45 <sup>c</sup>
G5	27.70±0.87 <sup>d</sup>	0.63±0.02 <sup>d</sup>	28.96±1.77 <sup>d</sup>	122.03±3.25 <sup>d</sup>
LSD	2.01	0.07	3.27	8.17

G1: Negative control group. G2: Positive control group.  
 G3, G4 and G5: Fed on pan bread containing 5, 10 and 15 % okra pod waste, respectively.  
 Mean values with different superscripts in the same column are significantly different ( $P \leq 0.05$ )

Although the feeding diabetic rats on pan bread containing okra pod waste led to improve the liver parameters but still significantly high than that in the negative control group. From these results, it could be concluded that pan bread containing okra pod waste had the ability to protect liver from failure and restore the normal functional status. The obtained results are matched with El-Sayed *et al.* (2019) who noted that, there was an improvement in liver enzymes for rats fed on balady bread containing 10% okra flour compared to the positive control group and rats fed on balady bread containing 100% wheat flour.

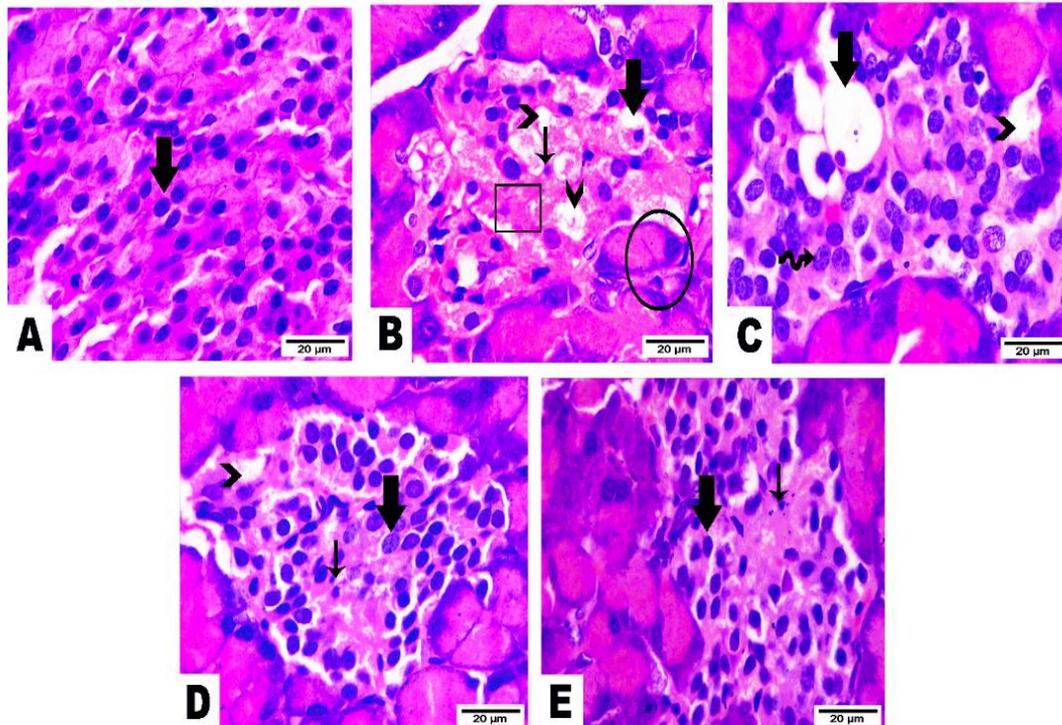
**Histopathological results**

The histological examination of the pancreas, liver and kidney for normal and diabetic group rats are exposed in Fig. (4,5 and 6).

**Pancreas**

Fig. (4. A) appears sections from pancreas of negative control group which reveals the standard histological structure of the pancreatic islets with its normal size homing alpha cells, delta cells, F cells, and beta cells centrally (arrow). Whereas Fig. (4. B) shows the positive control group, which highlights severe degenerative changes in this group including atrophy in size, areas of necrosis (cube), vacuolations between cells (arrowhead), degenerative effect in beta cells (thin arrow) and

cytoplasmic vacuolations encircled alpha cells (thick arrow). Notice normal structure of pancreatic acinar cells surrounding islets of Langerhans (circle). This result was in accordance with previous studies which confirmed that hyperglycemia led to a progressive decline in  $\beta$ -cells function (Anjani *et al.*, 2018). This is due to the toxicity of streptozotocin which causes an inflammatory process causes selectively destruction insulin-producing islet  $\beta$ -cells in the pancreas. Also, streptozotocin causes increases oxidative stress, inflammation and endothelial dysfunction (Eleazu *et al.*, 2013). Fig. (4. C) of diabetic rats group feeding bread consisting of 5% okra pod waste marks some islets cells with pyknosis and cytoplasmic vacuolations (arrow) while others exist with its normal shape (wave arrow). Vacuolations between islet cells (arrowhead) are also declined. Also, Fig. (4. D) of diabetic rats group feeding pan bread consisting of 10% okra pod waste displays most of islet's cells in a normal assembly (thick arrow) except few cells with pyknosis (thin arrow). Vacuolations between islet cells (arrowhead) are greatly reduced. Moreover, Fig. (4. E) of diabetic rats group feeding bread consisting of 15% okra pod waste signifies a noticeable improvement in islets cell's structure (thick arrow) and a few pyknotic cells (thin arrow) are also observed. Hence, the diabetic group rats fed on pan bread containing okra pod waste present remarkably reduced histopathological alterations than that noted in positive control group.



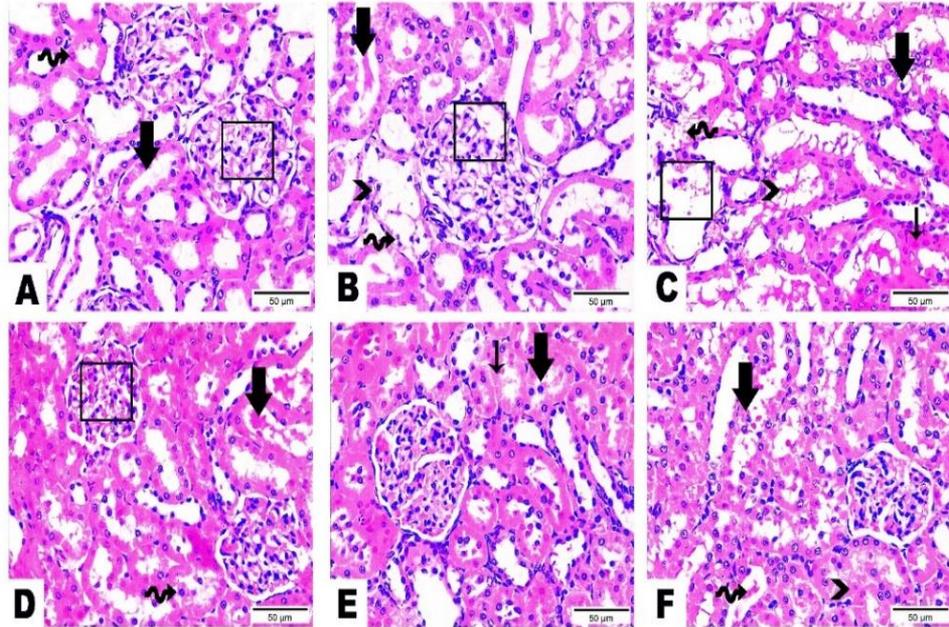
**Fig. 4. Photomicrographs of the histopathological differences in pancreatic tissue sections of studied rat groups. A (negative control group), B (positive control group), C (Diabetic rats group fed on pan bread containing 5% okra pod waste), D (Diabetic rats group fed on pan bread containing 10% okra pod waste) and E (Diabetic rats group fed on pan bread containing 15% okra pod waste). (Hematoxylin and Eosin Stain, Magnification Power = x1000, Scale bar= 20µm)**

This may be due to antioxidant activity of okra pod waste, that led to rise the  $\beta$ -cells number by increasing the rectify and renovation of  $\beta$ -cells and maintain cell membranes from oxidative damage, thus promoting insulin secretion. These results agree with Sheteewy *et al.* (2018) and Wu *et al.* (2020) they stated that okra extract intake by diabetic rats led to renovate destructed  $\beta$  cell pancreas induced by streptozotocin injection. Also, Uadia *et al.* (2020) reported that okra could regenerate damaged pancreatic cells with consequent increased secretion of insulin by pancreatic  $\beta$ -cells.

#### **Kidney**

Fig. (5. A) appears sections from kidney of negative control group which demonstrates the normal histological structure of renal corpuscle (cube), proximal (wave arrow), and distal (thick arrow) convoluted tubules. The sections from kidney of positive control group are seen in Fig. (5. B&C). Fig. (5. B) shows vacuolation of mesangial cells of renal corpuscles (cube), degeneration of renal tubules (arrowhead), desquamation of tubular epithelium (wave arrow) in addition to the presence of hyaline cast (thick arrow). While, Fig. (5. C) shows some sections of the renal cortex that reveal a loss of renal corpuscle (cube),

mild congestion (wave arrow), necrosis of renal tubules (thin arrow), vacuolar degeneration of renal cells with pyknotic nuclei (thick arrow), and desquamated lining epithelium (arrowhead). This is due to the toxicity of streptozotocin which causes a rise in glomerular protein kinase C activity that plays a main turn in the evolution of diabetic nephropathy (Zafar *et al.*, 2009). Fig. (5. D) of diabetic rats group feeding pan bread consisting of 5% okra pod waste demonstrates normal renal cortex and glomerular tufts (cube). The intratubular hyaline cast (thick arrow) and desquamated tubular lining (wave arrow) reduced to a greater extent (remarkably decreased). As well as Fig. (5. E) of diabetic rats group feeding pan bread consisting of 10% okra pod waste exhibits markedly reduced renal injury with limited renal degeneration, hyalinization (thin arrow), and tubular necrosis (thick arrow). Also, Fig. (5. F) of diabetic rats group feeding pan bread consisting of 15% okra pod waste represents a normal histological architecture of the renal cortex except for some areas highlighted with hyaline cast in one tubule (wave arrow), few degeneration (arrowhead), and desquamation of lining epithelium (thick arrow).



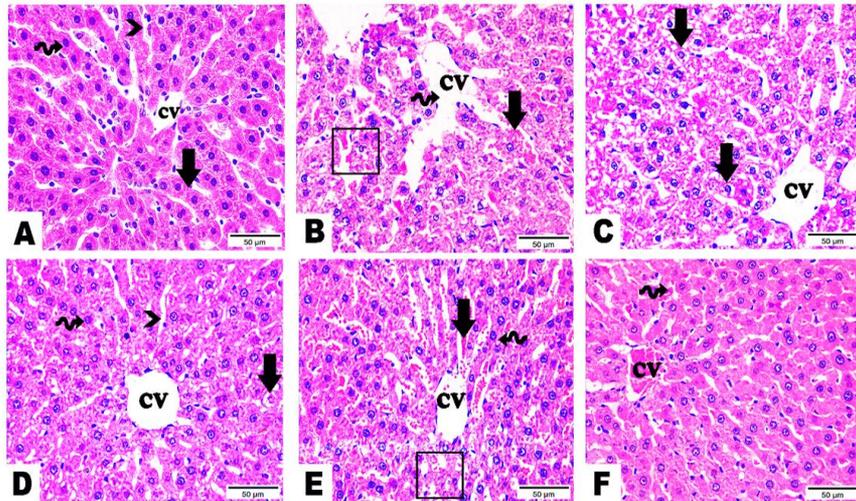
**Fig. 5. Photomicrographs of the histopathological differences in kidney tissue sections of studied rat groups. A (negative control group), B and C (positive control group), D (Diabetic rats group fed on pan bread containing 5% okra pod waste), E (Diabetic rats group fed on pan bread containing 10% okra pod waste) and F (Diabetic rats group fed on pan bread containing 15% okra pod waste). (Hematoxylin and Eosin Stain, Magnification Power = x400, Scale bar= 50µm)**

Huang *et al.* (2018) indicated that total flavone glycoside obtained from okra pod had a significant effect in improvement of the kidney tissue to diabetic rats.

**Liver**

Liver tissue sections of rats are seen in Fig. (6), negative control group (Fig. 6. A) demonstrates the normal histological structure of the central vein (C.V.) with regular hepatic cords (thick arrow) that are separated by blood sinusoids (arrowhead). The hepatic cords contain normal hepatocytes with central, spherical, and lightly stained nuclei (wave arrow). Positive control group shows dilated central vein (C.V.) (wave arrow) surrounded by irregular hepatic cords (cube) that are separated by dilated and congested (thick arrow) blood sinusoids (Fig. 6. B). Also, Fig. (6. C) reveals moderate hydropic degeneration of hepatocytes (thick arrows). This is attributed to production of free radicals during hyperglycemia leads to oxidative transformation of cellular macromolecules (proteins, lipids and carbohydrates), hence causes inflammatory reactions that destroy liver cells (Tangvarasittichai, 2015).

Concerning to liver tissues of diabetic rats group fed on pan bread containing 5% okra pod waste, it is observing nearly normal central vein (C.V.) and blood sinusoids (arrowhead). Some hepatocytes appear nearly normal (wave arrow), while others show mild vacuolar degeneration (thick arrow) (Fig. 6. D). While, diabetic rats group fed on pan bread containing 10% okra pod waste shows restoration of most histological architecture (wave arrow) except dilatation and congestion of blood sinusoids (thick arrow) as well as few degenerated hepatic cords (cube) (Fig. 6. E). As well as, diabetic rats group fed on pan bread containing 15% okra pod waste represents a marked improvement in hepatic parenchyma. Moreover, the hepatic sinusoids appear less congested (wave arrow). The congestion of the central vein (C.V.) is also observed (Fig. 6. F). Subsequently, feeding diabetic rats on pan bread containing okra pod waste exhibit remarkably decreased histopathological changes than observed in positive control group, this is turns out by biochemical analysis of blood samples. The obtained results are matched with Wu *et al.* (2020) who stated that aqueous okra extract ameliorated the liver morphology of diabetic rats.



**Fig. 6. Photomicrographs of the histopathological differences in liver tissue sections of studied rat groups. A (negative control group), B and C (positive control group), D (Diabetic rats group fed on pan bread containing 5% okra pod waste), E (Diabetic rats group fed on pan bread containing 10% okra pod waste) and F (Diabetic rats group fed on pan bread containing 15% okra pod waste). (Hematoxylin and Eosin Stain, Magnification Power = x400, Scale bar= 50µm)**

## Conclusion

This study concluded that substituting wheat flour with okra pod waste powder had significant effects on the physicochemical, rheological and sensory properties of pan bread. Hence, acceptable bread from the composite of wheat flour and okra pod waste powder as a cheap source of dietary fiber can be produced by the lowest cost. Feeding diabetic rats on pan bread containing 5,10 and 15% of okra pod waste led to reduce the glucose, HbA1c and increase insulin levels in blood samples. Also, it caused a significant ameliorate in the lipid profile, kidney and liver functions, also pancreas, kidney and liver tissue sections of diabetic rats. This study recommends to find a new source of dietary fiber and antioxidants from food factories wastes and its used into processing some of functional food products, which can be improve therapeutic management of diabetic diseases.

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## الملخص العربي

### تقييم التأثيرات التكنولوجية والخافضة لسكر الدم لخبز القالب المدعم بمخلف قرون البامية على ذكور

#### الفئران المصابة بمرض السكري

عزة صبيح عبد الغنى، داليا أحمد زكى

والنشاط المضاد للأكسدة، ولم يكن هناك أى فروق معنوية في جميع درجات التقييم الحسي بين عينات الخبز المحتوي على ٥% من مخلف قرون البامية والكنترول، وسجل خبز القالب المحتوى على ١٥% من المخلف أقل درجات التقييم الحسي ولكنه كان مقبولاً، وأظهرت نتائج الاختبارات البيولوجية إن تغذية الفئران المصابة بمرض السكري على خبز القالب المدعم بمخلف قرون البامية أدت إلى انخفاض معنوي في مستوي كلا من الجلوكوز و HbA1c وزيادة في مستوي الأنسولين في الدم. كما أحدثت تحسناً معنوياً في مستويات الدهون ووظائف الكلى والكبد وكذلك فى أنسجة البنكرياس والكلى والكبد للفئران المصابة بمرض السكري. وأخيراً نستخلص من نتائج هذه الدراسة أن التغذية على خبز القالب المدعم بمخلف قرون البامية أدت إلى تحسين مستوى الجلوكوز ويمكن أن تحمي من أمراض السكري ومضاعفاتها، وبالتالي يمكن التوصية بإمكانية استخدام مخلف قرون البامية كمصدر جديد ورخيص فى تدعيم الخبز بأنواعه المختلفة وغيره من منتجات المخابز لتحسين صحة مرضى السكري، بالإضافة الى الحاجة لدراسات مستقبلية لإيجاد مصادر جديدة للمركبات النشطة بيولوجياً من مخلفات التصنيع الغذائى واستخدامها في تصنيع العديد من المنتجات الغذائية الوظيفية.

**الكلمات المفتاحية:** مخلفات قرون البامية، خبز القالب، الخواص البيولوجية، التقييم الحسي، مرض السكري.

تعتبر مخلفات تصنيع الخضروات مصدرًا رخيصًا للمواد النشطة بيولوجيًا والتي يمكن استخدامها كإضافات في تصنيع بعض الأغذية الوظيفية. لذا أجريت هذه الدراسة بهدف استخدام مخلف قرون البامية بنسب استبدال ٥ و ١٠ و ١٥% من دقيق القمح في صناعة خبز القالب. وتمت دراسة كلا من الخصائص الفيزيائية والكيميائية والبيولوجية والحسية للخبز المصنع. كما تم تقييم تأثير تغذية الفئران المصابة بمرض السكري بالخبز المدعم على الاختبارات البيوكيميائية لدم الفئران والتغيرات التشريحية لأنسجة كلا من البنكرياس والكلى والكبد. وقد تبين من النتائج التي تم الحصول عليها أن محتوى مسحوق مخلف قرون البامية من البروتين والدهن والرماد والألياف الخام كان أعلى من دقيق القمح، وكان النشاط المضاد للأكسدة لتلك المخلف حوالى ٨٨,٧٤%، وأشارت نتائج الفارينوجراف والاكستنسوجراف أن إضافة مخلف قرون البامية الى دقيق القمح أدى إلى انخفاض في تطور العجين ودرجة الليونة والمرونة والتمدد، وزيادة في امتصاص الماء ووقت الثبات مقارنة بالعينة المحتوية على دقيق القمح فقط، وسجل خبز القالب المحتوى على ١٥% من مخلف قرون البامية أعلى وزن للرغيف (٨٧٣,٣٣ جم) وأقل حجم نوعى (١,٨١ سم<sup>٣</sup>/جم) مقارنة بالخبز الكنترول (٨١٠,٦٧ جم و ٢,٢٨ سم<sup>٣</sup>/جم على التوالي). وأدى زيادة نسبة مخلف قرون البامية في خبز القالب إلى ارتفاع كبير في محتوى الرطوبة والدهن والبروتين والألياف والرماد