International Journal of Nutrition and Food Sciences
2022; 11(5): 134-142
http://www.sciencepublishinggroup.com/j/ijnfs
doi: 10.11648/j.ijnfs.20221105.14
ISSN: 2327-2694 (Print); ISSN: 2327-2716 (Online)

Production and Evaluation of Mushroom Bread with Low Glycemic Index for Type Two Diabetes

Njeck Yvette Ashi[1], Mache Andre Gilles[2], Noumo Ngangmou Thierry[1], Njounkou Andre Ledoux[3], Ejoh Aba Richard[1, *]

[1]Department of Nutrition, Food and Bio-resource Technology, College of Technology, the University of Bamenda, Bambili, Cameroon

[2]Department of Biochemistry, University of Bamenda, Bambili, Cameroon

[3]Department of Biology, Faculty of Sciences, University of Bamenda, Bambili, Cameroon

Email address:

ejohrab62@gmail.com (Ejoh Aba Richard)

[*]Corresponding author

To cite this article:

Njeck Yvette Ashi, Mache Andre Gilles, Noumo Ngangmou Thierry, Njounkou Andre Ledoux, Ejoh Aba Richard. Production and Evaluation of Mushroom Bread with Low Glycemic Index for Type Two Diabetes. *International Journal of Nutrition and Food Sciences*. Vol. 11, No. 5, 2022, pp. 134-142. doi: 10.11648/j.ijnfs.20221105.14

Received: August 10, 2022; **Accepted**: August 25, 2022; **Published**: September 19, 2022

Abstract: With the increased incidence of Type 2 diabetes worldwide, many therapeutic foods have been used for the reduction of blood glucose amongst which are different varieties of mushrooms. This study determined the effect of *Termitomyces le-testui* bread on blood glucose reduction. *T. le-testui* powder was used at up to 30% to replace wheat flour to produce bread. The nutritional, phytochemical, and sensory properties of the bread were determined. The blood glucose reduction property was evaluated on diabetic male Wistar rats and the glycemic index was determined on healthy nondiabetic humans. Incorporated wheat flour with 0 to 25%. *T. le-testui* increased protein, insoluble dietary fiber, soluble dietary fiber, polyphenols, flavonoids, and vitamin A levels. There was also a significant increase in Mg, Zn, and Fe as the concentration of *T. le-testui* increased. The decrease in carbohydrates was associated with the increase in *T. le-testui* powder. The 5% incorporation showed the best sensory properties. *T. le-testui* reduced the rate of sugar released in non-diabetic humans and in male Wistar rats after 15 days of administration significantly ($p=0.001$) reduced blood glucose and serum lipids, and increased the HDL levels. *T. le-testui* mushroom bread can be used by diabetic patients to lower blood sugar levels.

Keywords: *Termitomyces le-testui* Bread, Diabetes, Glycemic Index, Phytochemicals, Sensory Properties

1. Introduction

Diabetes mellitus describes a metabolic disorder of multiple causes, characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Epidemiological data are upgrading and alarming: 442 million people with diabetes were diagnosed worldwide in 2014, and 516 thousand in Cameroon by the International Diabetes Federation. According to projections, an increase of 54% in the world and 80% in Cameroon is expected by 2035. Seventy-five percent (75%) of these deaths occurred in people under 60 years of age with a high rate among the active population of young people aged 20 to 39 [1].

It is a pathology that stands out with the potential of developing long-term complications [2]. At a microvascular level, patients with diabetes may develop ischemic heart disease, cerebrovascular disease, and peripheral vascular disease, which often lead to morbidity and mortality. At a macrovascular level, diabetes can lead to vision impairment (retinopathy), kidney disease (nephropathy), and neuronal damage (neuropathy), which are more common causes of irreversible blindness, chronic kidney disease, and non-traumatic lower-limb amputations. A healthy diet and a Stress-less lifestyle can prevent the development of such pathological conditions [3]. This proves the severity of diabetes, as the reported complications affect different systems in the body and the consequence can severely compromise the patient's quality of life. Type II diabetes mellitus (T2DM) can be considered one of the chronic

diseases of greater impact on the public health system. In addition to causing a high degree of morbidity and mortality, the metabolic control of diabetes and the treatment of its complications have a high cost for health services [4, 5].

Bakery products such as bread, cakes, and biscuits are a large family of popular food products, consumed all over the world due to their varied tastes, relatively long shelf-life, and low cost. Unfortunately, the rich carbohydrate content makes it not good for diabetics as it easily causes an increase in blood sugar levels. The enrichment with vitamins, minerals, proteins, polyphenols, and fibers may be achieved through the incorporation of other food components. The dried mushroom powder is one of the components with great potential to reduce blood sugar levels when incorporated with bakery products [6] like bread. Despite these rich compliments people do not consume this mushroom as much as they should due to ignorance, availability, and inability to cultivate them. There is a need to innovate a method for maximum utilization, by processing the mushroom into a powder that can be stored and used even in off-season.

Several studies have proven that mushrooms can help in the management of type two diabetes [6]. Most often it is the cultivated mushroom that is used in Africa., meanwhile the local wild edible mushroom *Termitomyces le-testui* is very nutritive and has antidiabetic potential [7]. *Termitomyces le-testui* mushroom is rich in proteins (27–36%) and ascorbic acid (10–18 mg/g) and also superior in taste [8]. Some of the ethnomedicinal uses of *Termitomyces* mushrooms can be related to certain bioactive compounds that these mushrooms contain including phenolic compounds, polysaccharides, cerebrosides, serine protease, ergostanes, saponins, and fatty acid amides [9]. *Termitomyces le-testui* has also been found to be potent antioxidants in the concentration range of 10-70μg/Ml [10]. These bioactive components also have antidiabetic potentials [11, 12]. This study was to produce and evaluate *T. le-testui* powder, use it to substitute wheat flour in bread, and determine its glycemic potential using humans and Wistar rats.

2. Materials and Methods

2.1. Sample Collection and Preparation

Fresh *Termitomyces le-testui* (mushroom) was bought from the local markets of Ndop and identified at the Botany department of the Faculty of Science, University of Bamenda. Commercial wheat flour (Amigo brand), yeast (NEVADA), and salt were bought from the Nkwen market Bamenda.

2.1.1. Preparation of Mushroom Flour

The mushroom powder was prepared according to the method reported by [13]. The dried mushrooms were ground into powder using a grinder (ROYALTY + LINE (Model no.: SME-600.6). The powder of all the mushrooms collected from different villages was mixed and sieved (using a sifter) to fine particle size and packaged in airtight containers, stored in the freezer -4℃ (to prevent the growth of molds) for further use in the preparation of bread.

2.1.2. Production of Mushroom Bread

T. le-testui flour and wheat flour were used in the production of bread. The flour was used in the ratio for *T. le-testui* to Wheat of 0:100, 5:95%, 10:90%, 15:85%, 20:80%, 25:75%, and 30:70%. The dough preparing method (AACC, 2000) was used with slight modification for the production of bread. The recipe consisted of wheat flour (500 g), salt (7.5 g), yeast (7.5 g) (NEVADA, Mexico by SAFMEX S.A. de C.V.), and water according to the amount needed to achieve a dough consistency of 500 FU. The dough was formed using a mixer (DITO SAMA B21) to mix wheat flour, mushroom powder, dry ingredients, yeast, and water at speed of 1 for 5 min, and then increase the mixer speed to three for another 10 min when the elastic dough is formed. After that, rested the dough for 30 min and then hand-kneaded and rested for another 15 min. Dough pieces were divided (100 g), hand-rounded, and then put into baking pans, which were greased with margarine ahead and placed in a proofer at 40°C for 60 min. The dough was then baked in an electric oven at 200°C for 20 minutes. After baking, the bread loaves were cooled at room temperature for 2 hours and then stored at 20°C for subsequent analysis [14].

2.2. Proximate Analysis

The bread samples were analyzed individually for protein [15], lipids [16], total carbohydrates [17], total dietary fiber, Soluble and insoluble fiber [18], minerals (K, Fe, Na, and Mg) [19], Vitamin A converted from beta carotene [19], total phenolic compounds [20], and flavonoids [21].

2.3. Sensory Evaluation

The sensory evaluation was carried out on the bread samples within 6-12 hours of baking using A 9-point hedonic scale [6]. The samples served were sliced (1.5 cm thick) and tested for their sensory properties on 40 (20 men and 20 women) randomly selected nondiabetic panelists with ages ranging from 18 to 45. Bread samples coded with four-digit random numbers were served on a tray in random orders. The sensory assessment was carried out under light and water was provided to clean the mouth between samples [6]. The overall acceptability of bread was calculated from the average values of all the above sensory parameters.

2.4. Glycemic Index Determination

Fourteen healthy nondiabetics volunteers aged 18- 45 (7 men and 7 women) were selected for the determination of the glycemic index. The exclusion criteria were fasting blood glucose (FBG) ≥ 5.5 mmol/L; those with chronic diseases; those following a special diet; those taking medication that affected glucose metabolism and those taking. Athletes, as well as pregnant or breastfeeding women, were also supplements excluded from the study. Other exclusion criteria included smoking, and those unwilling to adhere to study protocol. These criteria were assessed through oral

questioning of participants. Prior to obtaining the informed consent, all volunteers were educated on the nature and risks of the experimental procedures. Approval of Ethical clearance was done by the Ethical board of the University of Bamenda.

The procedure was a modification of that of [22]. Subjects were left overnight to fast for ten hours, and the fasting blood sample was done at 7:00 am. The subjects ate the test bread samples at a comfortable pace (max 15 min) and blood samples were collected again at 15, 30, 45, 60, 90, and 120 min after. The test meals were served with water (200ml). The water taken by everyone remained constant for each of the bread samples. with little modifications with no addition of sugar. The blood was obtained by finger-prick (plasma glucose) and a drop of blood was placed on the active side of a glucometer (PRODIGY Auto Code) to measure the blood glucose. The glucose measurement remained standard for the duration of the study for each sample [22].

2.5. Animal Experiment

Male Wistar albino rats of weight 150-200g were bought from the university of Yaounde 1 and were categorized into different control and test groups in labeled bowls, then placed on shelves. All experimental rats had a normal day: light (12:12) cycle and were kept at a temperature of 24 ± 2°C. The rats had access to feed and water for a baseline period of 3 weeks.

Diabetes was experimentally induced nutritionally through a high sugar diet for four months according to the method described by Kamgang *et al*., [23]. Seven groups containing five animals each were taken for the in vivo study and marked as control and test groups. For all the animals, blood glucose level and body weight were determined daily for 15 days using a glucometer (EZ-CHEK, ModelG-425-3) and a weighing balance (SEVEN STAR GERMANY) respectively. *T. le-testui* powdered bread with different proportions, (0-30% incorporation) of the powder was used to feed rats to test the antidiabetic potential of mushroom bread in the reduction of blood glucose levels for type 2 diabetes. Blood was gotten by a prick using a needle at the tail of the rats and placed on the active side of a glucometer (EZ-CHEK, ModelG-425-3).

After 28 days of treatment with mushroom bread, biochemical parameters like triglyceride, cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined [24]. On the 28th day, the animals were subjected to overnight fasting for 11- 12 hours, anesthetized intraperitoneally with Diazepam and ketamine in the ratio of 2: 1 according to body weight (60mg/kg). Thereafter the blood samples were collected using heparinized tubes and kept in test tube racks undisturbed for thirty minutes to obtain serum. The supernatant was used to test the lipid profile. The total cholesterol (TC) of the samples was estimated calorimetrically according to the method of Shahzad *et al*., [25].

The quantification of serum triglyceride was done by the formation of dihydroxyacetone phosphate and hydrogen peroxide from the oxidation of glycerol 3 phosphate [26].

High-density lipoprotein cholesterol content was measured from the supernatant that was separated by centrifugation [27]. Two hundred microliters of the samples were added to 500 microliters of precipitated reagent and centrifuged for 10 minutes at a speed of 4000 rpm.

Friedewald formular was used to calculate low-density lipoprotein [27].

2.6. Data Analysis

Experiments were conducted in triplicate. Analysis of results was done using SPSS version 2.1. Statistical differences were determined by analysis of variance (ANOVA) and relationship analysis using the Pearson correlation test (p <.05).

3. Results and Discussion

The nutritional and phytochemical compositions of bread supplemented with *T. le-testui* mushroom powder are presented in Table 1. Soluble and insoluble fibers, protein, polyphenols, and flavonoids increased as mushroom concentrations of the bread increased from 0 to 30%, while the carbohydrates content of the bread decreases as the concentration of mushroom content in bread decreased. There is a significant negative correlation between the quantity of mushroom powder used and the amount of carbohydrate in the bread (with r= -0.983 and p=0.000). This result is in agreement with that of Okafor *et al*., [28] and Agu *et al*., [29] who incorporated Nigerian oyster mushroom powder to wheat flour at levels 0-25%and had a significant reduction in carbohydrates.

Table 1. *Nutritional and phytochemical composition of mushroom bread in g/100g Dry weight.*

Bread samples	Carbohydrates	Protein	Lipids	Soluble Fiber	Insoluble Fiber	Polyphenols	Flavonoid
C0	71.33 ±0.09^{b}	3.77±0.05^{a}	0.007±0.005^{a}	3.91± 0.01^{a}	11.89±0.01^{a}	0.111±0.008^{a}	0.202±0.004^{a}
C5	63.33 ±0.57^{a}	7.92±0.01^{a}	0.013±0.005^{ab}	5.42± 0.01^{a}	36.37±1.011^{a}	0.998±0.005^{a}	0.276±0.003^{a}
C10	59.80±0.16^{a}	8.04±0.01^{a}	0.023±0.005^{bc}	6.47± 0.01^{a}	38.16±0.04^{a}	1.196±0.038^{a}	0.424±0.003^{a}
C15	57.40±0.16^{a}	10.19±0.01^{a}	0.033±0.005^{cd}	6.91± 0.01^{a}	55.31±0.08^{a}	1.671±0.006^{a}	0.664±0.005^{a}
C20	41.80±0.05^{a}	10.23±0.02^{a}	0.040±0.006^{d}	7.55± 0.03^{a}	58.10± 0.04^{a}	1.920±0.001^{a}	0.846±0.022^{a}
C25	38.10±0.34^{a}	11.30±0.01^{a}	0.047±0.005^{cd}	11.33±0.01^{a}	60.78±0.02^{a}	2.352±0.025^{a}	0.972±0.023^{a}
C30	33.03±0.08^{a}	13.40±0.00^{a}	0.063±0.012^{d}	26.89±0.01^{a}	83.52±0.09^{a}	2.484±0.022^{a}	1.388±0.002^{a}

Values are Mean ± standard deviation (n=5). (a); values with same superscript letters are not significantly different (P > 0.05) C0 = 0% T. le testui, C5= 5% T. le testui, C10 =10% T. le testui, C15= 15% T. le testui, C20= 20% T. le testui, C25= T. le testui, C30= 30%T. le testui.

There is a significant positive correlation between the amount of mushroom flour used and the amount of protein with r=0.951 and P=0.001. This means as the amount of mushroom flour increased, proteins in the mushroom bread also increased. The bread with 30% mushroom inclusion was observed to have the highest protein content (13.397), this was followed by 25% mushroom bread (11.295%), while the bread without mushroom inclusion had the lowest protein content (3.773%). This is similar to the findings of Okafo *et al.,* [28] who supplemented wheat flour with mushroom powder of (*Pleurotus plumonarius)* from 0-25% and found that the crude protein content increased significantly from 7.96-14.62%.

There is a positive correlation between the amount of mushroom powder used and the amount of lipids with r = 0.993 and P= 0.001. The lipid content of the bread samples was low and did not differ significantly as the mushroom concentrations increased. This result is lower than that of Okafor *et al.,* [28]. It is also in line with the results of Irakiza et al., [30] on fortification with mushroom flour (*Pleurotus ostreatus*).

There is a positive significant correlation between the quantity of mushroom flour in the bread, also soluble fiber (with r=0.801 and P= 0.030), and the amount of insoluble fiber with r=0.962 and P=0. 001. These results are similar to the findings of Ng *et al*., [31] who determined the effects of oyster mushroom (OM) powder addition on the nutritional values, in-vivo glycemic index, and sensorial properties of biscuits, and had appreciable amounts of insoluble dietary fiber (48.79%), soluble dietary fiber (8.21%) were detected in the osster mushroom powder. These results are also in line with those of Lu *et al.,* [32]. The restriction of starch for hydrolysis markedly reduced the glycemic index of bread.

There is a positive correlation relationship between the quantity of mushroom flour used and the amount of polyphenols content (with r = 0.977 and P= 0.000), and flavonoids in the mushroom bread samples (with r = 0.981 and P=0.000) (Table 1). These results are similar to those of Lu *et al.,* [32] who had a strong positive correlation ($p \leq 0.05$) for the total phenolic content of the bread produced. Studies have suggested that the presence of phytochemicals and antioxidants in many species is closely related to their anti-hyperglycemic potentials [33, 34]. In addition, polyphenolic compounds in the added mushroom powder could act as non-proteinaceous amylase inhibitors [35]. Choo *et al*. reported that polyphenols reduced starch hydrolysis by binding with amylases [36]. Several mechanisms have been proposed for the hypoglycemic effects of phytochemicals, such as inhibition of carbohydrate metabolizing enzymes, manipulation of glucose transporters, β-cell regeneration, and enhancing insulin-releasing activity and sensitivity [37]. Thus the phytochemicals and antioxidant properties of the *Termitomyces* may contribute to their anti-diabetic potential. If that is the case, then the discrepancies in the level of inhibition between various compositions can be attributed to the differences in the concentration of these bioactive components present in the different species of *Termitomyces* mushrooms. The presence of phytochemicals polyphenols increases insulin sensitivity [38] and flavonoids Inhibitory activity against α- glycosidase [39].

For the mineral and vitamin A composition of *T. le-testui* Bread, the result showed that potassium and sodium were the main mineral elements in the bread samples, and the mineral (potassium, magnesium, iron, and zinc) composition increases with the level of *Termitomyces le testui* mushroom addition (Table 2). Iron, Zn, and vitamin A are also present but in smaller quantities, as compared to Phosphorus, Sodium, and Magnesium.

Table 2. *Minerals and vitamin A composition (mg/100g) of Termitomyces le-testui mushroom bread.*

Mushroom Levels	Na (mg/100g)	Mg (mg/100g	Fe (mg/100g	Zn (mg/100g	K (mg/100g)	Vitamin A (µg/g)
C0	66.97±1.46^{a}	38.95±0.07^{a}	6.33±0.025^{a}	1.05±0.06^{a}	336.115±30^{a}	5.67±0.06^{a}
C5	68.41±0.08^{a}	88.29± 0.81^{a}	7.705±0.25^{a}	1.36±0.05^{b}	634.33±5.6^{a}	14.53±0.20^{b}
C10	112.10±1.02^{a}	68.07± 0.03^{a}	8.98±0.03^{a}	2.37±0.015^{a}	569.13±100^{a}	14.48±0.05^{b}
C15	147.40±0.61^{a}	117.33±0.69^{a}	8.77±0.14^{a}	1.45±0.01^{bc}	680.52±3.9^{a}	5.72±0.11^{a}
C20	147.02±0.23^{a}	164.66±0.58^{a}	9.810.205a	1.67±0.05^{a}	753.41±1.1^{a}	15.39±0.13^{c}
C25	21.02±0.010^{a}	166.13±0.89^{a}	14.62±0.05^{a}	1.49±0.05^{a}	987.18±1.5^{a}	15.40±0.14^{c}
C30	187.50±0.52^{a}	146.40±0.60^{a}	20.11±0.001^{a}	2.11±0.02^{a}	1012.25±19^{a}	16.395±0.505^{d}

Values are Mean ± standard deviation (n=5). (a); values with same superscript letters are not significantly different (P > 0. 05) C0 = 0% T. le testui, C5= 5% T. le testui, C10 =10% T. le testui, C15= 15% T. le testui, C20= 20% T. le testui, C25= T. le testui, C30= 30%T. le testui.

Table 2 shows the mineral composition and vitamin A content of the composite bread. A significant positive correlation existed between the amount of mushroom powder used in the bread made and the quantity of potassium, magnesium, Iron, (with significant P=0.000 and r=0.964), and Zinc (r= 0.857 and P=0.014) in the mushroom bread, while there was a positive but insignificant correlation between the amount of mushroom used and the quantity of Sodium used (with r = 0.464 and P=0.294). These results are similar to that of Bello *et al*., [40] who made bread and biscuit from wheat and oyster mushroom powder, but the potassium content of this study is higher than that of Bello *et al*., [40]. This may be due to the difference in the species, *Termitomyces le testui* has a higher concentration of potassium than oyster mushrooms.

There was a positive and insignificant correlation between the amount of mushrooms used and the amount of Vitamin A found (r= 0.234 and P= 0.613). Consumption of magnesium-rich foods reduces the resistance of insulin thereby reducing the risks of metabolic syndrome and type two diabetes

mellitus [41].

Zinc is an essential micronutrient for metabolism that regulates more than 100 enzymes for protein folding, and gene expression, as well as in the production and neutralization of reactive oxygen species (ROS). Zinc is essential for the proper processing, storage, secretion, and activities of pancreatic cells of mammals [42]. Also, Magnesium as a cofactor is needed for the metabolism of carbohydrates, and the movement of glucose into the cell. Therefore insufficient intake is a risk factor for those with diabetes [43]. Magnesium deficiency leads to oxidative damage that prevents cellular defenses, consequently leading to decreased strength and oxidative stress in those with diabetes [41].

The sensory parameters of the mushroom bread are presented in table 3. The Increased in mushroom concentrations affected the taste, texture, and mouthfeel thus reducing the overall acceptability of the bread. Meanwhile, the color and aroma were increased with increased mushroom concentrations in the bread. A significant ($p<0.05$) difference between 100% WF bread (control) and those incorporated with 15, 20, 25, and 30% MP (table 3). In all the quality attributes analyzed bread with 5% MP did not differ significantly from 100% WF bread in color, taste, texture, aroma, and overall acceptability. Similarly, there was no significant ($p>0.05$) difference between the 10% MP supplemented bread and the control in aroma, and texture. Bread with 25% and 30% MP addition had significantly poor loaf size, dark color, and pronounced mushroom taste and flavor. Although the 15% MP fortified bread had a low rating in most of the quality attributes, they were, however, acceptable. Generally, the mushroom powder gave the bread a unique taste and texture that make them look and feel like cake bread, particularly the 20, and 25% mushroom powder formulation.

Bread with Proper color change might attract consumers' attention and affect their preference and acceptability. The bread with *Termitpmyces le-testui* mushroom had a distinctive aroma increase, as the concentration of mushroom powder increased (from 3.67±0.33-2.91±0.06). This result is similar to that of Okafor et al. [28] who studied the quality characteristics of bread made from wheat and Nigerian oyster mushroom (*pleurotus plumonarius*) powder and supplemented wheat flour with mushroom powder from 0-25% and observed a significant difference ($p<0.05$) between 0% mushroom bread and those fortified with 15-25% mushroom powder in all the quality attributes analyzed, implying bread with 5 and 10 percent had better results in all the attributes. These findings are also similar to that of Salehi [6] who studied the characterization of different mushroom powder and their application in bakery products and found out that, the bread containing 5% powdered osyster mushroom showed a considerably better result for texture and total acceptability.

Table 3. *Sensory evaluation of mushroom bread.*

Formulations	Colour	Taste	Mouth feel	Aroma	Texture	Overall Acceptability
C0	7.31±0.21^{a}	7.97±0.59^{a}	7.75±0.16^{a}	6.67±0.33^{a}	7.44±0.54^{a}	7.74± 0.38^{a}
C5	7.55±0.22^{ab}	6.97±0.07^{a}	6.61±0.18^{a}	6.38±0.49^{a}	7.86±0.53^{a}	6.36± 0.59^{a}
C10	7.68±0.53^{a}	5.45±0.27^{a}	6.92±0.24^{a}	6.35±0.13^{a}	7.98±0.42^{a}	6.61± 0.96^{a}
C15	7.74±0.19^{ab}	5.82±0.36^{a}	5.28±0.10^{a}	6.25±0.46^{a}	7.76±0.42^{a}	6.23± 0.79^{a}
C20	6.65±0.23^{b}	5.90±0.36^{ab}	5.69±0.47^{a}	7.58±0.43^{a}	6.77±0.11^{a}	5.25± 0.56^{a}
C25	6.83±0.53^{b}	5.91±0.38^{a}	5.79±0.60^{a}	6.15±0.25^{a}	6.50±0.73^{a}	5.03± 0.84^{a}
C30	5.09±0.53^{b}	4.06±0.33^{a}	5.85±0.25^{a}	7.91±0.06^{a}	6.82±0.10^{a}	5.02± 0.80^{a}

Values are Mean ± standard deviation (n=5). (a); values with same superscript letters are not significantly different ($P > 0.05$ C0 = 0% T. le-testui, C5= 5% T. le-testui, C10 =10% T. le-testui, C15= 15% T. le-testui, C20= 20% T. le-testui, C25= T. le-testui, C30= 30%T. le-testui.

The results from the glycemic index of the mushroom bread evaluated in non-diabetic humans are illustrated in figure 1. From 0-30 minutes, there is an increase in the blood glucose levels for all the animals fed different bread samples. From 30-120 minuites, the blood.

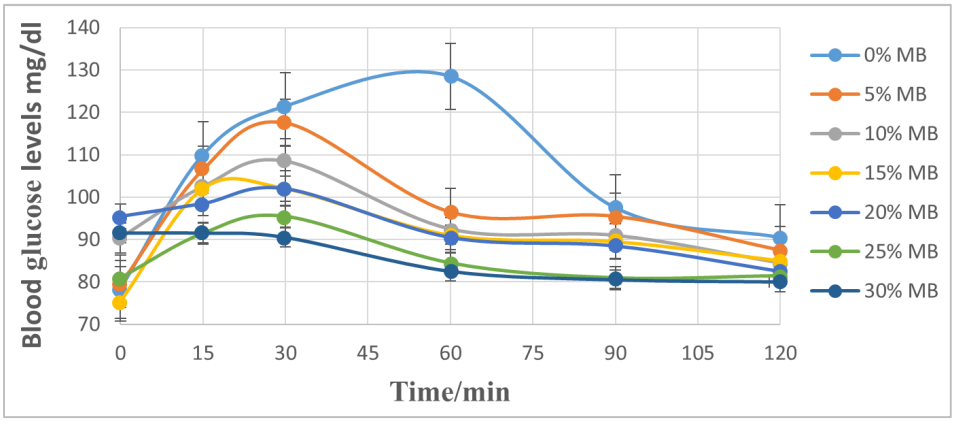


Figure 1. *Effects of mushroom bread compositions on the reduction of glucose release.*

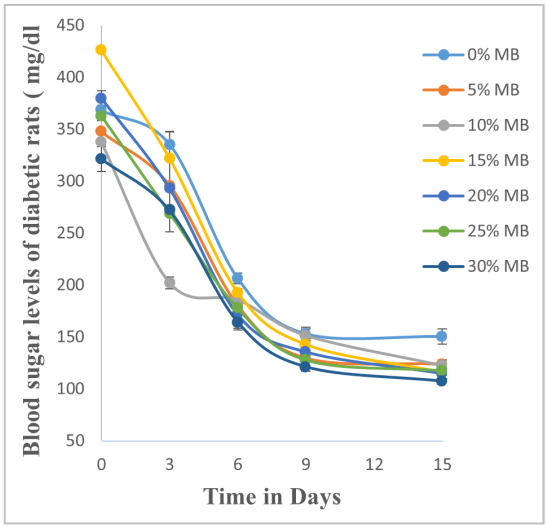
Increasing mushroom powder substitution levels decreased sugar release thereby decreasing the area under the curve. The substitution of wheat flour with mushroom powder caused a decrease in the total carbohydrate digestibility time as illustrated in figure 1. Lower levels of sugars were released from all the mushroom-enriched bread compared to the control (100% wheat bread). The levels of sugar release in the control bread were significantly higher, at 60 and 120 min in the in vivo digestion, compared to the mushroom enriched bread. The highest decrease in sugar release was observed in the 30% substitution level. The rapidly digested starch during the first 15 min in the mushroom enriched samples 5-30% was reduced compared to the control (0%MP). The amounts of the slowly digested carbohydrates fractions, over 15–120 min, were lower in the mushroom bread samples when compared to the control. The values of the 25% and 30% mushroom bread samples were significantly lower than the control at 120 min. There was a positive correlation between the quantity of mushroom flour used and fasting blood sugar level after 15 minutes, with very little significant difference (r=-0.991, P=0.328). Also, a significant negative correlation existed between the quantity of mushroom flour used and fasting blood sugar levels after 30, 60, 90, and 120 minutes (r= -0.991, P=0.000) and 90 minutes (r = -1.000, P=0.000).

These results are similar to those of Lu *et al*., [32] who worked on enhancing the nutritional properties of bread by incorporating mushroom bioactive compounds. The results showed that a significant positive correlation existed between the total carbohydrate content and area under the curve value ($r = 0.699$, at $p \leq 0.001$), meanwhile in this study a negative correlation existed between the total starch content and area under the curve value ($r = -0.773$ and -0.586, respectively, at $p \leq 0.001$).

These phenomena might be partly attributed to the significantly lower total carbohydrate content and more insoluble dietary fibre and soluble dietary fiber levels in the mushroom bread than in the control (0% mushroom). Interestingly, compared with soluble dietary fiber, insoluble dietary fibre played a more important role in modulating the starch digestibility of the final bread samples. This may be due to the fiber that protects the starch granules from human physical digestive actions inside the stomach by increasing the viscosity of the digesta and forming a physical "barrier" to the digestion of starch granules [44]. In addition, dietary fiber could form a close and compact matrix with other ingredients in bread, such as protein and starch, and such complex formations play a pivotal role in inhibiting enzyme accessibility to starch granules [35, 45]. It has been observed that even small amounts of protein in food products were enough to alter the starch digestibility and other functional properties [35] in that the protein matrix can entrap the starch granules and so reduce accessibility to enzyme attack. Continuous protein strands have an encapsulation effect on large starch granules, resulting in well-formed protein-starch complexes; thus, limiting starch degradation and sugar liberation [45]. Choo *et al*.,[36] reported that polyphenols reduced starch hydrolysis by binding with amylases. Several mechanisms have been proposed for the hypoglycemic effects of phytochemicals, such as inhibition of carbohydrate metabolizing enzymes, manipulation of glucose transporters, β-cell regeneration, and enhancing insulin-releasing activity and sensitivity [37]. Thus, the phytochemicals and antioxidant properties of the *Termitomyce le testui* may contribute to their anti-diabetic potential. If that is the case, then the distinctions in the level of inhibition amongst the different compositions might be because of the differences in the concentration of these bioactive components present in the different species of *Termitomyces* mushrooms.

Summarily, this study shows that different concentrations of *Termitomyces le-testui* mushroom appreciably inhibited the rapid release of sugar in blood from carbohydrates in the bread. Therefore, the *Termitomyces le-testui* mushroom can play a significant role in the fight against diabetes.

Figure 2 shows blood sugar reduction in diabetic rats. There was a reduction in blood sugar levels to normal levels, for the rats who ate bread with mushrooms from day one to day fifteen. The blood sugar levels of the rats who ate bread without mushrooms were reduced but the rats were still diabetic at the end. The effect of glucose reduction of *T. le-testui* bread was studied on the diabetic rat (figure 2). In experimental animals, diabetes was recognized as a rise in Blood Glucose Levels (BGL) of more than 200 mg/dl after the administration of a high sugar diet. Later, BGL of Group II, III, IV, V, VI, and VII after receiving treatments for 15 days, was reduced to normal levels. For group I, the blood sugar level was (150.4±2.1 mg/dl) on day 15, this was higher compared to those groups who ate bread with different proportions of mushrooms. There was a positive correlation (figure 2) between the quantity of high sugar diet after induction and blood sugar levels (r=-0.991, P=0.328). The was an insignificant negative correlation between the quantity of mushroom flour used and the blood sugar levels on the 3rd and 6th day (r=-0.429, P=0.176, r= -0.619, P=0.051 respectively).



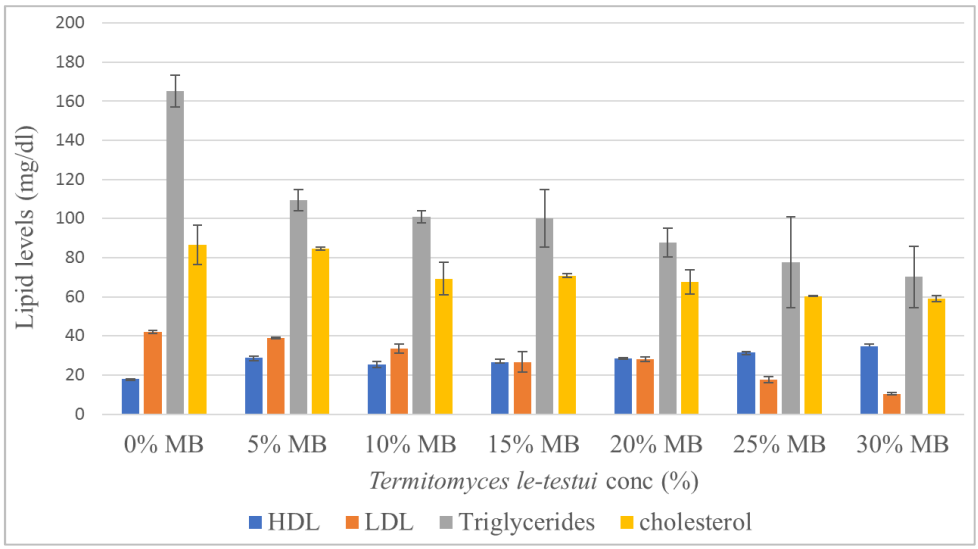
MB=Mushroom bread

Figure 2. *Effect of Termitomyces le-testui mushroom bread on blood sugar (mg/dl).*

There was a negative and significant correlation between the quantity of mushroom flour used and the blood sugar levels on the 9th and 15th days of treatment (r=-0.714, P=0.024 and r=-0.810, P=0.011 respectively). These results are in line with those of Balaji et al., [46] who used hot water extract (HWE) of *Pleurotus pulmonarius* mushroom on streptozotozin-induced diabetic rats, and found that 200 mg of hot water extract on averagely reduced the level of blood sugar from day 4 onwards with maintained effect till the end of treatment. The 400 mg/kg of hot water extract had a significant value ($P < 0.001$), with 97.19 ± 5.52 mg/dl when compared with that of the reference control (106.60 ± 8.43 mg/dl). This could be due to the presence of high fiber in the mushroom, the presence of phytochemicals, polyphenols which increase insulin sensitivity [38], and flavonoids' inhibitory activity against α- glycosidase [39].

Lipid profiles of the serum of diabetic rats after consumption of different compositions of mushroom bread are shown in figure 3. There was an increase in HDL, and a decrease in LDL, Trygliceride, and Cholesterol in the blood with increased mushroom concentrations. Dyslipidemia, heart diseases, and some other connected difficulties in diabetics are caused by the metabolism of unusual fatty acids, which are characterized by a high difference in serum lipid profiles [47]. High-density lipoprotein (HDLC) reduces the risk of atherosclerosis, and heart diseases, and plays a big role in cholesterol transfer to the liver for disposal [48].

Figure 3 shows that as the composition of the mushroom bread increases from 0% to 30% the low-density lipoproteins decreases while the high-density lipoproteins increase. Again, the Triglyceride and cholesterol levels are decreasing as the composition of mushroom flour in the bread samples are increasing. There was a significant positive correlation between the percentage composition of mushroom bread and the high-density lipoprotein of the rat (r =0.857, P=0.014). There was also a significant negative correlation between the percentage composition of mushroom bread and low-density lipoprotein (r = -0.976, P=0.001).



HDL=High Density Lipoprotein, LDL=Low Density Lipoprotein, MB= Mushroom Bread

Figure 3. *Values show the changes that occur in the lipids level of diabetic rats when different compositions of the mushroom bread were consumed.*

On the other hand, there was a negative correlation between the percentage composition of mushroom bread and the Triglyceride level (r = -0.893, P=0.007). similarly, there was a significant negative correlation between the percentage composition of mushroom bread and cholesterol (r = -0.949, P=0.001). These results are similar to those of Balaji et al., [46] who during the study period, found that hot water extract and alcoholic extract of *Pleurotus pulmonarius* led to a decrease in Total cholesterol, Triglyceride, Low-density lipoprotein cholesterol, and Very low-density lipoprotein levels in diabetic rats.

4. Conclusion

T. le-testui bread has good nutritional properties, with protein (3.77-13.97%), insoluble fibers (11.89-83.52%), soluble fibers (3.91-26.89), mineral (potassium, sodium, iron, zinc, magnesium), vitamin A content and phytochemicals, Polyphenols (0.11-2.48%) and flavonoids (0.20-1.39%). These nutritional and phytochemical properties increased with increased mushroom concentrations with 30% mushroom bread having the highest values. Sensory evaluation indicated 5% as the most acceptable in all the sensory attributes tested (color, taste, mouthfeel, texture aroma). Meanwhile, 30% of MB had a good glycemic index with non-diabetic humans. Furthermore, mushroom inclusion levels led to a progressive decrease in blood sugar levels in diabetic rats with 30% MB reducing the most, also an overall decrease in LDL, Triglycerides, Cholesterol, and an increase in HDL with the increase in mushroom concentration with 30% having the best. The studied *T. le-testui* mushroom bread has good potential in reducing blood sugar in diabetic patients and can thus be used in the management of diabetes mellitus.

Conflict of Interest

The authors declare that they have no competing interests.

Ethic Al Approval

This study conforms to the Declaration of Helsinki, European Medicine Guidelines for human subjects. The study was approved by the Bamenda Regional Hospital Institutional Review Board (014/ APP/RDPH/RHB/IRB, of 10/03/2019). Authorization to conduct the research was granted by the College of Technology, University of Bamenda. Verbal informed consent was obtained from all the study participants prior to their inclusion in the study.

Acknowledgements

Our sincere appreciation to the Regional delegate of Public Health for the NWR (Cameroon), and Mr. Oumah Mahamad. We also appreciate the study participants for their cooperation.

References

[1] Yetendje L. C. *et al.*, "In vivo antidiabetic activity and mechanism of action of three Cameroonian medicinal plant extracts," *int. j. Res. -granthaalayah*, vol. 7, no. 8, 2019, doi: 10.29121/granthaalayah.v7.i8.2019.695.

[2] American Diabetes Association, "Diagnosis and classification of diabetes mellitus," *Diabetes Care*, vol. 33, no. SUPPL. 1. 2010. doi: 10.2337/dc10-S062.

[3] Badalyan S. M. and Rapior S., "Agaricomycetes medicinal mushrooms with potential neuroprotective activity growing in armenia," *Proc. YSU B Chem. Biol. Sci.*, vol. 54, no. 3 (253), 2020, doi: 10.46991/pysu:b/2020.54.3.196.

[4] Silva E. F. F., Ferreira C. M. M., and De Pinho L., "Risk factors and complications in type 2 diabetes outpatients," *Rev. Assoc. Med. Bras.*, vol. 63, no. 7, 2017, doi: 10.1590/1806-9282.63.07.621.

[5] Dubey S. K., Chaturvedi V. K., Mishra D., Bajpeyee A., Tiwari A., and Singh M. P., "Role of edible mushroom as a potent therapeutics for the diabetes and obesity," *3 Biotech*, vol. 9, no. 12. 2019. doi: 10.1007/s13205-019-1982-3.

[6] Salehi F., "Characterization of different mushrooms powder and its application in bakery products: A review," *International Journal of Food Properties*, vol. 22, no. 1. 2019. doi: 10.1080/10942912.2019.1650765.

[7] Kansci G., Mossebo D. C., A. B. Selatsa, and M. Fotso, "Nutrient content of some mushroom species of the genus Termitomyces consumed in Cameroon," *Nahrung - Food*, vol. 47, no. 3, 2003, doi: 10.1002/food.200390048.

[8] Hsieh H. M. and Ju Y. M., "Medicinal components in Termitomyces mushrooms," *Applied Microbiology and Biotechnology*, vol. 102, no. 12. 2018. doi: 10.1007/s00253-018-8991-8.

[9] Elkhateeb W. A. and Daba G. M., "Termitomyces Marvel Medicinal Mushroom Having a Unique Life Cycle," *Pharm. Res.*, vol. 14, no. 1, 2020, doi: 10.23880/oajpr-16000197.

[10] Gebreyohannes G., A. Nyerere, C. Bii, and Sbhatu D. B., "Investigation of Antioxidant and Antimicrobial Activities of Different Extracts of Auricularia and Termitomyces Species of Mushrooms," *Sci. World J.*, vol. 2019, 2019, doi: 10.1155/2019/7357048.

[11] Aba P. E. and Asuzu I. U., "Mechanisms of actions of some bioactive anti-diabetic principles from phytochemicals of medicinal plants: A review," *Indian J. Nat. Prod. Resour.*, vol. 9, no. 2, 2018.

[12] Gaikwad S. B., Krishna Mohan G., and Rani M. S., "Phytochemicals for Diabetes Management," *Pharm. Crop.*, vol. 5, no. 1, 2014, doi: 10.2174/2210290601405010011.

[13] Lu X., Brennan M. A., Serventi L., and Brennan C. S., "Incorporation of mushroom powder into bread dough—effects on dough rheology and bread properties," *Cereal Chem.*, vol. 95, no. 3, 2018, doi: 10.1002/cche.10043.

[14] Chinma C. E., Anuonye J. C., Simon O. C., Ohiare R. O., and Danbaba N., "Effect of germination on the physicochemical and antioxidant and characteristics of rice flour from three rice varieties from Nigeria," *Food Chem.*, vol. 185, 2015, doi: 10.1016/j.foodchem.2015.04.010.

[15] Mæhre H. K., Dalheim L., Edvinsen G. K., Elvevoll E. O., and Jensen I. J., "Protein determination—method matters," *Foods*, vol. 7, no. 1, 2018, doi: 10.3390/foods7010005.

[16] Axelsson M. and Gentili F., "A single-step method for rapid extraction of total lipids from green microalgae," *PLoS One*, vol. 9, no. 2, 2014, doi: 10.1371/journal.pone.0089643.

[17] Jain V., Karibasappa G., Dodamani A., and Mali G., "Estimating the carbohydrate content of various forms of tobacco by phenol-sulfuric acid method," *J. Educ. Health Promot.*, vol. 6, no. 1, 2017, doi: 10.4103/jehp.jehp_41_17.

[18] Brennan M. A., Monro J. A., and Brennan C. S., "Effect of inclusion of soluble and insoluble fibres into extruded breakfast cereal products made with reverse screw configuration," *Int. J. Food Sci. Technol.*, vol. 43, no. 12, 2008, doi: 10.1111/j.1365-2621.2008.01867.x.

[19] A.O.A.C, "AOAC-Association of official analytical chemists," *Official Methods of Analysis of AOAC International 18th ed, Gaithersburg, Maryland, USA*, vol. 45. 2005.

[20] Ballard T. S., Mallikarjunan P., Zhou K., and O'Keefe S., "Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins," *Food Chem.*, vol. 120, no. 4, 2010, doi: 10.1016/j.foodchem.2009.11.063.

[21] Kamtekar S., Keer V., and Patil V., "Estimation of phenolic content, flavonoid content, antioxidant and alpha-amylase inhibitory activity of marketed polyherbal formulation," *J. Appl. Pharm. Sci.*, vol. 4, no. 9, 2014, doi: 10.7324/JAPS.2014.40911.

[22] Wolever T. M. S. *et al.*, "Determination of the glycaemic index of foods: Interlaboratory study," *Eur. J. Clin. Nutr.*, vol. 57, no. 3, 2003, doi: 10.1038/sj.ejcn.1601551.

[23] Kamgang R., Mboumi R. Y., Mengue N'dillé G. P. R., and Yonkeu J. N., "Cameroon local diet-induced glucose intolerance and dyslipidemia in adult Wistar rat," *Diabetes Res. Clin. Pract.*, vol. 69, no. 3, 2005, doi: 10.1016/j.diabres.2005.02.005.

[24] Couderc R., M. Antar, Bonnefont-Rousselot D., Paul J.-L., and Therond P., "Blood lipid tests in 2017," *Ann. Biol. Clin. (Paris).*, vol. 75, no. 6, 2019, doi: 10.1684/abc.2017.1303.

[25] Shahzad F., S. Tawwab, and Ahsan U., "Lipid profiles of non-diabetic healthy and ischaemic heart disease patients," *J. Coll. Physicians Surg. Pakistan*, vol. 23, no. 4, 2013.

[26] Tietz E., Norbert W., Finley, Pruden P. R, "Clinical Guide to Laboratory Tests, Second Edition," in *Clinical Guide to Laboratory Tests, Second Edition*, Second., vol. 10, no. 1, Philadelphia, PA: W. B. Saunders, 1990, pp. 304–306. doi: 10.1097/00004347-199101000-00013.

[27] Miller W. G. *et al.*, "Seven direct methods for measuring HDL and LDL cholesterol compared with ultracentrifugation reference measurement procedures," *Clin. Chem.*, vol. 56, no. 6, 2010, doi: 10.1373/clinchem.2009.142810.

[28] Okafor J. N. C., Okafor G. I., Ozumba A. U., and Elemo G. N., "Quality characteristics of bread made from wheat and Nigerian oyster mushroom (Pleurotus plumonarius) powder," *Pakistan J. Nutr.*, vol. 11, no. 1, 2012, doi: 10.3923/pjn.2012.5.10.

[29] Agu H., Ukonze J., and Paul K., "Quality Characteristics of bread made from Wheat and Fluted Pumpkin seed flour," *Niger. Food J.*, vol. 28, no. 1, 2010, doi: 10.4314/nifoj.v28i1.57430.

[30] Irakiza P. N. *et al.*, "Fortification with mushroom flour (Pleurotus ostreatus (Jacq.) P. Kumm) and substitution of wheat flour by cassava flour in bread-making: Nutritional and technical implications in eastern DR Congo," *Agric. Food Secur.*, vol. 10, no. 1, 2021, doi: 10.1186/s40066-021-00301-0.

[31] Ng S. H., Robert S. D., Wan Ahmad W. A. N., and Wan Ishak W. R., "Incorporation of dietary fibre-rich oyster mushroom (Pleurotus sajor-caju) powder improves postprandial glycaemic response by interfering with starch granule structure and starch digestibility of biscuit," *Food Chem.*, vol. 227, 2017, doi: 10.1016/j.foodchem.2017.01.108.

[32] Lu X., Brennan M. A., Guan W., Zhang J., Yuan L., and Brennan C. S., "Enhancing the nutritional properties of bread by incorporating mushroom bioactive compounds: The manipulation of the pre-dictive glycaemic response and the phenolic properties," *Foods*, vol. 10, no. 4, 2021, doi: 10.3390/foods10040731.

[33] El Omari N. *et al.*, "Evaluation of in vitro antioxidant and antidiabetic activities of Aristolochia longa extracts," *Evidence-based Complement. Altern. Med.*, vol. 2019, 2019, doi: 10.1155/2019/7384735.

[34] Gudise V., B. Chowdhury, and Manjappa A. S., "In vitro free radical scavenging and antidiabetic activity of aqueous and ethanolic leaf extracts: a comparative evaluation of Argyreia pierreana and Matelea denticulata," *Futur. J. Pharm. Sci.*, vol. 5, no. 1, 2019, doi: 10.1186/s43094-019-0014-9.

[35] Dhital S., Brennan C., and Gidley M. J., "Location and interactions of starches in planta: Effects on food and nutritional functionality," *Trends in Food Science and Technology*, vol. 93. 2019. doi: 10.1016/j.tifs.2019.09.011.

[36] Choo C. L. and Aziz N. A. A., "Effects of banana flour and β-glucan on the nutritional and sensory evaluation of noodles," *Food Chem.*, vol. 119, no. 1, 2010, doi: 10.1016/j.foodchem.2009.05.004.

[37] Tiwari A. K. and Rao J. M., "Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects," *Current Science*, vol. 83, no. 1. 2002.

[38] Huang D., Jiang Y., Chen W., Yao F., Huang G, and Sun L., "Evaluation of hypoglycemic effects of polyphenols and extracts from *Penthorum chinense*," *J. Ethnopharmacol.*, vol. 163, 2015, doi: 10.1016/j.jep.2015.01.014.

[39] Juárez-Reyes K. *et al.*, "Hypoglycemic, antihyperglycemic, and antioxidant effects of the edible plant Anoda cristata," *J. Ethnopharmacol.*, vol. 161, 2015, doi: 10.1016/j.jep.2014.11.052.

[40] Bello M., Oluwamukomi M., and Enujiugha V., "Nutrient Composition and Sensory Properties of Biscuit from Mushroom-Wheat Composite Flours," *Arch. Curr. Res. Int.*, vol. 9, no. 3, 2017, doi: 10.9734/acri/2017/35686.

[41] Hata A. *et al.*, "Magnesium intake decreases Type 2 diabetes risk through the improvement of insulin resistance and inflammation: The Hisayama Study," *Diabet. Med.*, vol. 30, no. 12, 2013, doi: 10.1111/dme.12250.

[42] Li Y. V., "Zinc and insulin in pancreatic beta-cells," *Endocrine*, vol. 45, no. 2. pp. 178–189, 2014. doi: 10.1007/s12020-013-0032-x.

[43] Lopez-Ridaura R. *et al.*, "Magnesium Intake and Risk of Type 2 Diabetes in Men and Women," *Diabetes Care*, vol. 27, no. 1, 2004, doi: 10.2337/diacare.27.1.134.

[44] Bharath A. P., Kathalsar A. K., Chandrashekhar S., and Prabhasankar, "Influence of tetraploid wheat (Triticum dicoccum) on P. low glycaemic index pizza base processing and its starch digestibility," *Int. J. Food Sci. Technol.*, vol. 56, no. 5, 2021, doi: 10.1111/ijfs.14845.

[45] Cleary L. and Brennan C., "The influence of a (1 → 3)(1 → 4)-β-D-glucan rich fraction from barley on the physico-chemical properties and in vitro reducing sugars release of durum wheat pasta," *Int. J. Food Sci. Technol.*, vol. 41, no. 8, 2006, doi: 10.1111/j.1365-2621.2005.01141.x.

[46] Balaji P. *et al.*, "Evaluation of antidiabetic activity of Pleurotus pulmonarius against streptozotocin-nicotinamide induced diabetic wistar albino rats," *Saudi J. Biol. Sci.*, vol. 27, no. 3, 2020, doi: 10.1016/j.sjbs.2020.01.027.

[47] Ktari N. *et al.*, "Antioxidative and ACE inhibitory activities of protein hydrolysates from zebra blenny (Salaria basilisca) in alloxan-induced diabetic rats," *Process Biochem.*, vol. 49, no. 5, 2014, doi: 10.1016/j.procbio.2014.01.032.

[48] März W. *et al.*, "HDL cholesterol: reappraisal of its clinical relevance," *Clinical Research in Cardiology*, vol. 106, no. 9. 2017. doi: 10.1007/s00392-017-1106-1.