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Technology Application and Biological Response of Prebiotic/Gluten-Free Snack Rolls Processed by Value-Added Composite Flour in Normal and Celiac-Diseased Rats

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Abstract

This study aimed to formulate novel snack rolls that are both prebiotic and gluten-free by incorporating value-added composite flours, including rice flour (RF), barley flour (BF), tiger nut flour (TF), and green banana flour (GBF), to enhance the individuals with celiac disease. Physical properties, baking quality, sensory acceptability, and chemical properties were evaluated for the prebiotic formula **(**P**)** and prebiotic/gluten-free formula (P/GF**).** For the *in vivo* study, two different protocols were used: first protocol the rats were allocated into three distinct groups, each consisting of six rats: the normal control, group (1) fed on prebiotic formula, and group (2) fed on prebiotic/gluten-free formula. The second protocol concluded three groups of six rat pups, each selected for celiac disease induction, a normal control, a diseased group, and a treatment group fed on a prebiotic/gluten-free formula. Results proved that green bananas and tiger nut flours could have functional uses in the food industry and be applied as good ingredients for healthy food products. The biological findings revealed that both formulas improved the rats' health and can be effective in treating celiac disease. In conclusion, prebiotic and prebiotic/gluten-free formulas are considered safe and healthy for celiac disease patients.

Keywords: Biological Evaluation; Celiac Disease; Gliadin; Gluten-free; Health Snacks

1. Introduction

Prebiotics can include fructooligosaccharides (FOS), resistant starch, β-glucans, dietary fiber, α-linolenic acid-modified dextrins, and various other ingredients that influence selective growth and probiotic bacteria activity **[1]**. Prebiotic compounds may be beneficial due to their nutritional value and the potential to improve some sensory attributes of food formulations, increasing product acceptance. Many food products have already been reported to be fortified with prebiotic elements in order to be better and healthier **[2]**. So, to take advantage to produce formula that combines all these features for celiac patients. The processing of gluten-free products faces great challenges in the industry to find suitable alternative gluten **[3]**, where Gluten plays a pivotal role in the water absorption capacity, cohesivity, viscosity, along with elasticity of dough **[4]**. One of the types of flour that is commonly utilized in gluten-free recipes is tiger nut flour. Tiger nut tubers possess a notable abundance of dietary fiber, omega-6 fatty acids, and a characteristic absence of gluten. Consequently, they hold promise as a valuable source material for the development of functional foods catering to individuals with specific nutritional requirements **[5-7]**. Actually, several studies attempted to take use of such characteristics by creating applications for free-from prebiotic foods **[7-9]**. Due to its superior supply of digestible proteins and excellent sensory properties, rice flour is used as an active ingredient in the creation of several gluten-free food items **[10]**. Several studies have been conducted with the objective of advancing the production of gluten-free food products utilizing rice flour as a primary ingredient **[11-13]**.

On the contrary, numerous cereal flours, including unripe banana (Musa spp.), are classified as prebiotic foods and are recognized for their significant contribution of macro elements, particularly potassium. These cereal flours possess health-promoting components such as oligosaccharides (specifically fructooligosaccharides), dietary fibers, resistant starch, and rapidly digestible starch **[14]**.

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Recently studies showed the possibility to use unripe banana flour as a prebiotic ingredient **[15, 16]**. In this context, food products based on β-glucans as a prebiotic effect are greatly dependent on barley flour **[17-19]**. Also, Barley grain soluble β-glucans have been shown to improve lipid metabolism, lower plasma cholesterol, lower the glycemic index, reduce lipid and bile acid absorption, and stimulate the immune system **[20]**. Flaxseed is a seed that contains bioactive components such as dietary fiber, lignans, and α-linolenic acid, which offer various health benefits. It is particularly rich in Omega 3 fatty acids, constituting approximately 48% of all lipids present in the seed. These Omega 3 fatty acids are considered essential and need to be incorporated into a regular diet **[21, 22]**. Generally, recent literature stated potential can use flaxseed as prebiotic **[23, 24]**. The development and planning of gluten-free food products are attracting more attention due to their increased consumption, which can be attributed to various causes including celiac disease, wheat gluten allergy and lifestyle as a gluten-free diet **[25]**.

Celiac disease (CD) is a persistent immune-mediated enteropathy of the small intestine that is triggered by the consumption of gluten in individuals who have a genetic susceptibility. At present, the sole efficacious approach for managing CD entails adherence to a rigorous and enduring gluten-free diet (GFD) **[26].** Gluten-free products, which should provide all the necessary nutrients, play a vital part in treatment. Gluten proteins are made up of gliadins and glutenins and are plentiful in barley- and wheat-based foods; gliadin is primarily composed of glutamine and possesses a notable proline content, rendering it resistant to digestion within the gastrointestinal tract of humans **[27, 28].** Celiac disease patients are focused on several of these gliadin peptides, which contain non-immunodominant peptide fragments resistant to gastric, pancreatic, and intestinal digestion and can initiate both a stress response and an innate immune response **[29].** Because celiac disease is largely human, numerous animal models to investigate it had been proposed, none of which are perfect **[30]**. In the case of suckling rats and mice, it has been observed that the repeated administration of gliadin orally leads to morphological and immunological changes that are similar to those observed in individuals with celiac disease **[31]**. Nevertheless, there is a mounting apprehension regarding the prolonged dietary patterns and food preferences of individuals with celiac disease. Numerous studies have demonstrated that adhering to a gluten-free diet (GFD) does not adequately fulfill the necessary nutritional requirements **[32]**. Following an extended period of adherence to a gluten-free diet (GFD), an analysis revealed the presence of inadequacies in essential components such as dietary fiber, complex carbohydrates, minerals, and vitamins **[33]**. These deficits are linked to gluten-free diets' high starch or refined flour content with deficient in fibers and vitamins B **[34]**. A lack of proteins, minerals (calcium, iron), vitamins (folic acid, vitamin B12, and lipid-soluble vitamins), and dietary fiber is a problem for persons who follow a gluten-free diet, related to the results of the different surveys **[35]**.

In order to provide a nutritional product that meets the needs of consumers who have grown more aware of prebiotic products, we propose a new product, which is a snack roll prepared from composite flour; tiger nut with green bananas, barley and flaxseed. The same formula was employed in the same proportions, but with a precise substitution of barley for rice flour to make the product gluten-free, making it safe for people suffering from celiac disease. Many previous studies dealt with the idea of using rice flour and green banana flour for the production of gluten-free products, others mentioned using barley flour and flaxseed as health-enhancing (prebiotic) component. In this study the main idea was based on benefiting from flour containing prebiotic compounds in addition to being gluten-free to producing a new innovative product with a combination of flour ingredients that have never been combined together in one product that combines have two attributes prebiotic and gluten-free. As well as study the technological characteristics and sensory acceptance of the new innovative product, and emphasizing suitable consumption by celiac disease patients through biological experiment.

2. MATERIALS AND METHODS

2.1. Materials

Green banana fruit, tiger nut tubers, barley flour, flaxseeds as a source of prebiotic compounds **(Table 1)** and rice flour, oil, baking powder, sucrose, and maize starch were obtained from the local market in Cairo, Egypt. Sigma-Aldrich (St. Louis, MO, USA) supplied the gliadin. The plasma gliadin concentration, interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) enzyme-linked immunosorbent assay (ELISA) kits were acquired from Abcam, a company based in the United Kingdom. Other chemicals and all biochemical parameters include lipid profiles, liver enzymes, kidney functions, albumin, and total protein, according to Biodiagnostic, Egypt.

Table 1: Free gluten and prebiotic composite flour

Ingredients (g)	Type	Source of prebiotic
Rice Flour	(GF)	----------
Barley Flour	(P)	β-glucans water-soluble polysaccharides
Tiger Nut Flour	(P/GF)	dietary fiber polysaccharides
Green Banana Flour	(P/GF)	Resistant starch non-starch polysaccharides(lignin) fructooligosaccharides (fructans)
Flaxseeds	(P/GF)	lignans α-linolenic acid soluble dietary fibre

2.2. Methods

2.2.1. Value added flour preparation

The green banana fruits were manually peeled and promptly immersed in a citric acid solution with a concentration of 1 gram per liter for duration of 5 minutes **[36],** slice into pieces that are 2 millimeters in thickness, and rinsed again for one minute in the same solution before being dried at 40 °C and milled. Tiger nut tubers were cleaned dried at 60 °C, and ground. The green banana and tiger nut flours were passed through a 60-mesh screen to blend. The flour was packed and sealed off in polyethylene bags until used.

2.2.2. Physical properties of different flour samples

2.2.2.1. Flour Flowability

The determination of bulk density, tapped density, compressibility index (CI), and Hausner ratio (HR) was conducted for the value-added flours. The measurements of bulk density and tapped density were conducted **[37]**. A specimen weighing 50 grams was placed into a measuring cylinder with a capacity of 10 ml. The total volume was estimated to be equal to the volume occupied by the substance. The determination of bulk density entails the division of the mass of a given material by its corresponding bulk volume. On the other hand, the determination of tapped density involves dividing the mass of the material after tapping by the volume of the vessel. The compressibility index (CI) and Hausner ratio (HR) of various flour samples were determined using the formula proposed by **[38]**:

CI = 100 x (tapped density - bulk density / tapped density)

HR = tapped density / bulk density

2.2.2.2. Hydration Properties

The water holding capacity (WHC), swelling capacity (SC), hydration capacity, hydration index, oil holding capacity (OHC), and water binding capacity (WBC) of value-added flours were assessed. The determination of water holding capacity, swelling capacity, and water binding capacity followed the protocols outlined by **[39]. [40]** established and maintained the hydration capacity and hydration index, while the oil holding capacity was established utilizing the methodology outlined by **[41].**

2.2.3. Preparation of snack rolls

Various combinations of (rice, barley, tiger nut, green banana flours can be prepared as composite flour in prebiotic and prebiotic/gluten-free formulas, as seen in **Table 2. In the first recipe** composite flour, flaxseeds, and baking powder as dry ingredients were mixed in a bowl for three minutes, while, second one without adding barley flour and then vegetable shortening and water were poured. The dough was kneaded and rolled out into a thin sheet, cut into the desired shape over the perforated tray, and transferred into the convective oven at 160 °C. The snaked rolls were cooled, packed, and stored at room temperature.

Table 2: Recipe of prebiotic (P) and prebiotic/gluten-free (P/**GF)** snack roll samples

Ingredients (g)	Formulas	
	Prebiotic formula (P)	Prebiotic/gluten-free formula (P/GF)
Rice Flour	---	10
Barley Flour	30	---
Tiger Nut Flour	15	20
Green Banana Flour	5	20
Flaxseeds	2.5	2.5
Oil	5	5
Water	30	20
Baking Powder	1	1

2.2.4. Chemical composition of (P) and (P/GF) snack rolls samples

The chemical composition of two formulas (prebiotic and prebiotic/gluten-free formula) of snack rolls, including the moisture, fat, ash, fiber, and protein content, were estimated according to **[42]**. The minerals calcium and iron were quantified utilizing atomic absorption spectrometry (Pyeunicm Model 3300, PyeUnicam Ltd., Cambridge, England) at a wavelength of 422 nm, following the procedures outlined by **[43]**. The HPLC technique was employed to determine the concentrations of vitamins B1, B2, B9, and B12, following the methodology outlined by **[42].**

2.2.5. Baking Quality of (P) and (P/GF) snack rolls samples

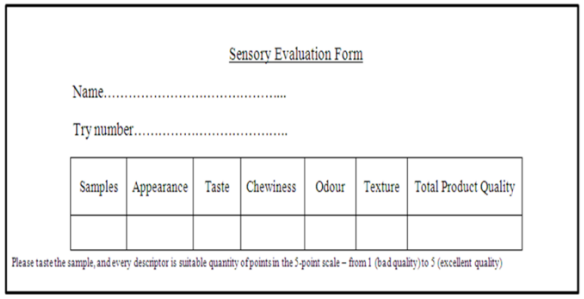
The baking quality attributes for (**P)** and (P/**GF)** snack rolls were evaluated after cooling at room temperature. Triplicates from different baking sets were analyzed and averaged for six sample pieces in each formula. **[44]** conducted measurements on the snack rolls, including weight, height, roundness, and volume for each snack formula**.** The changes in the diameter before and after baking were determined as percentage shrinkage **[45]**. The specific volume of the roll was calculated by dividing its volume by its weight. Baking time and bake loss after baking were determined according to **[46]** by the following formula:

$$Bakingloss\ \% = (Wbb – Wab)/\ WbbX\ 100$$

Where: *The variable "Wbb" represents the weight of the sample before the baking process*; *Wab* is the sample's weight after cooking and baking.

2.2.6. Sensory evaluation of (P) and (P/GF) snack rolls samples

A sensory evaluation of snack rolls was performed according to the full questionnaire for the fifteen participants used for the sensory evaluation (**Fig. 1**). Samples were evaluated for the degree of liking for the appearance, taste, chewiness, odour, texture, and overall product quality **[47, 48].** The experiment was conducted in accordance with established ethical guidelines, and all participants provided their full and informed consent. The study adheres to all regulatory requirements and provides confirmation of obtaining informed consent from all participants involved in this experiment. The present study received approval from the Ethical Committee of Medical Research at Egypt's National Research Centre (**approval no. 2495062022**).


Sensory Evaluation Form

Name.....

Try number.....

Samples	Appearance	Taste	Chewiness	Odour	Texture	Total Product Quality

Please taste the sample, and every descriptor is suitable quantity of points in the 5-point scale – from 1 (bad quality) to 5 (excellent quality)

Fig. 1. Sensory evaluation sheet

2.2.7. Evaluation of the (P) and (P/GF) snack rolls in different animal protocols to Study Gluten Sensitivity

2.2.7.1. Experimental Animals

This study employed two distinct protocols. The initial protocol involved the utilization of a cohort of eighteen male Sprague-Dawley rats, aged between one and two months, with a weight range of 150 to 200 grams. The Second Protocol involved the utilization of a cohort of eighteen Sprague-Dawley rat pups, aged seven days, which were specifically chosen for the purpose of inducing celiac disease. These rat pups were procured from the Animal House Colony at the National Research Center in Cairo, Egypt. In order to facilitate acclimatization and ensure optimal growth and behavior, the animals were provided with standard laboratory feed and water for duration of one week prior to the commencement of the experiment. The animals were housed in individual cages with solid bottoms in a controlled environment. The room was maintained at a temperature of 23 ± 10 °C, with a relative humidity of 40-60%. Additionally, the room was artificially illuminated with a 12-hour dark/light cycle. It is worth noting that no chemicals were used in this setup.

The utilization of all animal subjects in this investigation was granted approval by the Ethical Committee of Medical Research at Egypt's National Research Centre, under the approval number (**2495062022)**. The animal experiment was conducted in compliance with the Animals (Scientific Procedures) Act of 1986 in the United Kingdom, as well as the relevant guidelines outlined in EU Directive 2010/63/EU for animal experiments (Publication No. 85-23, revised in 1985)

2.2.7.2. Animals Diets

The composition of the synthetic base diet included casein (150 g/1 kg diet), unsaturated fat (100 g/1 kg diet), sucrose (220 g/1 kg diet), maize starch (440 g/1 kg diet), cellulose (40 g/1 kg diet), salt mixture (40 g/1 kg diet), and vitamin mixture (10 g/1

kg diet) **[49, 50]**. The formulation of the salt and vitamin mixtures was based on the AIN-93M diet, as outlined by **[51]**.

2.2.7.3. Celiac disease Induction

The gliadin was diluted in a 0.02 M acetic acid solution in order to obtain a solution with a concentration of 10%. The solution that had been prepared was administered via intragastric gavage at a dosage of 1.5 mg per gram of body weight. This administration occurred on days 7, 10, 13, 16, 19, and 22 postpartum until the rats reached the weaning stage **[32, 52]**.

2.2.7.4. Experimental Design

The first protocol had (18) rats were separated into three groups of six, as shown: **Normal Control:** Rats fed the basal synthetic diet. **Group (1):** Rats fed a prebiotic formula (P) (**Table 2**). **Group (2):** Rats fed a prebiotic/gluten-free formula (P/GF) (**Table 2**).

The second protocol had (18) rats rats were separated into three groups of six, as shown: **Normal Control:** Rats were fed the synthetic base diet and administered an intragastric solution containing acetic acid at a concentration of 0.02 M on days 7, 10, 13, 16, 19, and 22 postpartum. **Celiac Disease group:** celiac-induced animals fed the synthetic base diet. **Treatment group:** celiac-induced animals fed a prebiotic/gluten-free formula (P/GF) (**Table 2**).

2.2.7.5. Blood Samples and Organs Collection

The animals underwent a 12-hour fasting period and were then administered sodium pentobarbital (50 mg/kg, i.p.) as an anesthetic at the conclusion of the experimental period. The first protocol lasted four weeks, while the second protocol lasted seven weeks. The hematological tests were measured in whole blood collected by cardiac puncture in tubes with heparin sodium. The coagulated blood was left to clot at room temperature for 30 min, and then it was centrifuged for 15 min at 3600 g. Biochemical parameters were measured in blood samples collected from each animal. Serum and plasma were separated through centrifugation using a Sigma Laboratories, GMBH, West Germany, model 2-153360 osterode/Hertz centrifuge operating at 4000 revolutions per minute for duration of 15 minutes. Next, -20 °C was used to preserve the separated serum and plasma. Cytokine analysis was conducted on the proximal jejunum of each animal, specifically measuring the levels of IFN-γ.

2.2.7.6. Measurement of body weight change and food consumption

The basal synthetic diet was given to normal control in the first protocol and to normal control and celiac disease groups in the second protocol where the rolls of both formulas were given to group (1 &2) in first protocol and to treatment group in the second protocol. Each rat was feed about 20 gm/day where the rolls were crushed to be suitable for the rats to feed them. Water was giving daily for 24 hrs till the end of the experiment. The weekly body weight of the rats in both protocols was measured using a digital weighing balance in order to evaluate their weekly weight changes. On the other hand, the quantification and computation of food consumption were conducted utilizing metabolic cages and digital weighing balances.

2.2.7.7. Biochemical Parameters

The hematological parameters included red blood cells (RBC), white blood cells (WBC), platelets, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined.

Plasma gliadin conc., IFN-γ, tumor necrosis factor-α (TNF-α) and Interleukin-6 (IL-6) ELISA kits were obtained from Abcam, UK. Total cholesterol, HDL, LDL, triglycerides, and total lipids were assessed by the enzymatic colorimetric method **[53-57]**. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assessed by colorimetric techniques **[58, 59]**. Plasma total protein and plasma albumin (A) were determined by colorimetric methods **[60, 61]** as different indicators of liver function. Colorimetric methods evaluated creatinine and urea **[62, 63]**.

2.2.8. Statistical analysis

The mean values and standard deviations of parameters obtained from analyses of physical properties, chemical composition, baking quality, and in vivo studies were computed. Differences between the formulations were done using analysis of variance (ANOVA), with a significance level of $P < 0.05$. The statistical software package SPSS 20.0 (SPSS Inc., Chicago, USA) was utilized for these analyses. The data were subjected to a comparative analysis using Duncan's multiple range tests at a significance level of 5%

3. RESULT AND DISCUSSION

3.1. Physical properties of different flour samples

3.1.1. Flow properties

Flow properties including bulk density (BD), tapped density (TD), compressibility index (CI), and Hausner ratio (HR) for rice flour (RF), barley flour (BF), tiger nut flour (TF), and green banana flour (GBF) samples were assessed. The findings were displayed in **Table (3).**

3.1.1.1. Bulk density (BD)

There were statistically significant differences observed in the mean values of bulk density for the treatments RF, BF, TF, and GBF. The greatest values were recorded for GBF, with RF following closely behind (0.562 and 0.527 g/cm^3, respectively). The BF substance exhibited the lowest recorded values at 0.433 g/cm^3, while the TF substance followed closely behind at 0.416 g/cm^3. A high bulk density is essential and desirable because it can greatly lower expenses during packaging and transportation **[64].** The lower bulk density of BF and TF can be attributed to their large particle size, which results in more interparticle voids and a disparity in contact surface areas per unit volume **[65].**

3.1.1.2. Tapped density (TD)

The highest TD means value corresponds to GBF (0.667 g/cm^3). There is no significant difference between the TD of GBF and RF. Furthermore, the recorded measurements indicate that the lowest values were observed for BF, with a density of 0.588 g/cm^3, followed by TF, which had a density of 0.617 g/cm^3. Tapped density values are higher for more regularly shaped particles.

3.1.1.3. Compressibility index (CI)

Coefficient of Interparticle Contact (CI) and Hardness Ratio (HR) serve as reliable indicators of the compaction mechanism that arises when food powder materials are subjected to vibration or tapping during handling and processing. The CI for different flours is given in **Table (3).** The CI ranged from 32.542 to 15.739 % for all flours. The CI percentage of TF and BF was greater than that of RF and GBF (32.542, 26.270, and 20.168, 15.739 %), respectively ($p< 0.05$).

3.1.1.4. Hausner ratio (HR)

The RH of TF and BF were higher than all other flour samples. CI and HR are good conductors of the mechanical pressure that happens during the handling, processing, and storing food powder materials **[32].** Fair flowability could be shown in the RF's HR (1.252) and CI (20.168), and **[66]** reported comparable results. The results showed that the GBF sample had good flowability while the TF sample had poor flowability. The powders with increased surface area per unit are classified as having poor flowability **[67].**

Table 3: Flow properties of Rice, Barley, Tigernut and Green banana flour samples

Flour samples	Flour Flowability			
	Bulk density (g/ cm^3)	Tapped density (g/cm$^{3)}$	Compressibility index (%)	Hausner ratio
Rice flour (RF)	0.527± 0.008^b	0.659± 0.002^a	20.168± 0.92^c	1.253± 0.01^c
Barley flour (BF)	0.434± 0.004^c	0.588± 0.009^c	26.270± 0.64^b	1.356± 0.01^b
Tigernut flour (TF)	0.417± 0.001^d	0.618± 0.011^b	32.542±1.04^a	1.483± 0.02^a
Green banana flour (GBF)	0.562± 0.009^a	0.667± 0.013^a	15.739± 0.34^d	1.187± 0.004^d

Results are expressed as mean values ± SD of three replications. There is no significant difference ($p > 0.05$) observed between the means in a column with identical superscripts.

3.1.2. Hydration properties

The functional characteristics of flour, which are essential for the production of related products (**Table 4**), have an impact on the processing and quality of food products.

3.1.2.1. Water holding capacity (WHC)

The highest WHC of 1.562 g/g was observed in rice flour, and the lowest WHC of 1.050 g/g was observed in green banana flour **(Table 4).** The water-holding capacity (WHC) of barley and tiger nut flours did not show a significant difference. This conclusion agrees with those presented by **[68]**, who noted that the WHC of naked barley ranged from 1.43 to 1.46 g/g. Higher values of water holding capacity for rice and barley flour are related to the higher amount of starches and more gelatinized as well as the β-glucan that may absorb water **[69].**

3.1.2.2. Swelling index (SWI)

A higher swelling index indicates higher associative forces. The SWI ranged between 1.295 and 0.920 for all flours tasted. The SWI of rice flour was greater than that of the other flours tasted. A significant increase in SWI value for rice flour is related to starch granule swelling and an increase in particles during close packing.

3.1.2.3. Hydration capacity (HC) and hydration index (HI)

According to **Table (4)**, the highest HC value (2.280 g/ml) corresponded to rice flour, followed by green banana flour (2.120 g/ml), while the lowest value (.0945 g/ml) was obtained for tiger nut flour. There is a significant difference between the values of HC and HI in all the flours tasted. The observed disparity can be ascribed to variations in cellular microstructure and the individual's capacity for water hydration.

3.1.2.4. Water binding capacity (WBC)

The WBC of barley, rice, and green banana flour was greater than that of tiger nut flour (2.585, 2.563, and 2.482 g/g, respectively). Barley contains the soluble fiber β-D- glucan and pentosans like xylan. These viscous materials hydrate with water easily through hydrogen bonding at the percent of organic acid **[70].**

3.1.2.5. Oil holding capacity (OHC)

The OHC for four flours was significantly different ($p< 0.05$). Barley flour samples had a higher OHC (1.484 g/g) than other flours tasted. Prolamin is the dominant protein in barley, making up about 33–55% of total proteins **[71].** OHC percentages of rice, green banana, and tiger nut flour were found (1.392, 1.242, and 1.113 g/g, respectively). Tiger nut flour had lower oil absorption, which might be due to low hydrophobic proteins leading to the eminent binding of lipids **[72].**

Table 4: Hydration properties of Rice, Barley, Tigernut and Green banana flour samples

Functional properties	Hydration properties					Oil holding capacity(g/g)
	Water holding capacity (g/g)	Swelling index	Hydration capacity (g/ml)	Hydration index (%)	Water binding capacity (%)	
Rice flour	1.562 ±0.008^{a}	1.295 ±0.007^{a}	2.280± 0.00^{a}	1.120 ±0.01^{a}	2.563 ±1.25^{a}	1.392 ± 0.085^{b}
Barley flour	1.387 ±0.039^{b}	0.920 ±0.014^{c}	2.145 ±0.035^{b}	1.015 ±0.05^{b}	2.585 ±1.72^{a}	1.484± 0.047^{a}
Tigernut flour	1.345 ±0.044^{b}	1.190 ±0.014^{b}	1.930 ±0.028^{c}	0.945 ±0.01^{c}	2.025 ± 3.89^{c}	1.113 ±0.082^{d}
Green banana flour	1.050 ±0.005^{c}	0.925 ±0.007^{c}	2.120 ±0.00^{b}	1.050 ±0.00^{ab}	2.482 ± 3.48^{b}	1.242 ±0.159^{c}

Results are expressed as mean values ± SD of three replications. The observed lack of significant differences ($p > 0.05$) between the means in a column with identical superscripts suggests that these means are not statistically distinguishable.

Based on flow and Hydration properties, the results showed that green banana flour had good flowability and functional uses in foods application because of its good industrial handling. Increased WHC of rice, Barley and tiger nut flours enhanced the function qualities of dough in bakery products. The Higher values of Water binding capacity for rice, barley, and green banana flours make it more suitable in various food products like dough and baked products. The oil holding capacity ranged between ‘’1.113 to 1.484’’ for all flours tasted. The results indicated that the value-added flour enhances the mouth feel and retains the flavour for the end products. The green banana and tiger nut flours could have functional uses in the food industry and be applied as good ingredients for healthy food products.

3.2. Proximate chemical composition

Proximate compositions of snack rolls for each formula (P and P/GF) are shown in **Table (5).** The findings indicated that there was no statistically significant disparity ($p > 0.05$) in the moisture content between the two formulas (P and P/GF). Formula P had the highest ash and protein contents (2.603 and 9.160 %, respectively) compared to the P/GF formula. At the same time, the highest fat (16.131%) and fiber (6.470%) contents were recorded in the P/GF formula. The results indicated that adding barley flour to snack rolls led to a significant ($P<0.05$) increase in their protein, ash, and total carbohydrate contents. At the same time, the highest fat and fiber content of the P/GF formula may be due to the high percentage of tiger nut and green banana flour. These findings agree with a previous study accomplished by **[73],** who reported that the mean value of fat and crude fiber content increased in biscuit samples as tiger nut flour addition progressed. **Table (5)** shows Iron and calcium content in the P and P/GF formulas as mg/kg. The obtained data showed that Fe content in the P formula is considerably higher than that in the P/GF formula, at 2.24 and 1.5 mg/kg, respectively, which may be

due to the high content of barley flour, which is considered A notable reservoir of numerous essential nutrients, including both soluble and insoluble dietary fibers **[74].** It is noticeable that the higher content of calcium (15.7 mg/kg) in the P/GF formula is due to the presence of tiger nut and rice flour as valuable sources of essential minerals such as Na, Ca, and K. These results are found to be comparable with the results in the literature **[75, 76].** In a similar study was obtained by **[77]** for produced rice and bean biscuits as a new gluten-free product, who reported that their biscuits have good mineral and fibers contents, these food items have the potential to provide various advantages to individuals adhering to gluten-restricted diets and those with celiac disease, specifically rice and beans. The higher content of vitamins B complex (B1, 2, 9, and 12) in the P formula is than P/GF formula **(Fig 2)**. Meanwhile, the P formula contains more vitamins B1 and B9, about 14.3% and 80.1%, than the P/GF formula, it could be due to the barley flour included in the second mixture, according to **[74]** who stated that Barley is rich in essential vitamins, including vitamin E and the B-complex vitamins.

Table 5: Chemical composition of (P) and (P/GF) snack rolls samples

Chemical composition (%)	P formula*	P/GF formula**
Moisture	3.958±0.08[a]	3.804±0.18[a]
Fat	12.906±0.28[b]	16.131±0.22[a]
Protein	9.160±0.33[a]	7.706±0.25[b]
Fiber	4.643±0.17[b]	6.470±0.10[a]
Ash	2.603±0.03[a]	2.260±0.10[b]
Total Carbohydrate	66.730±0.24[a]	63.629±2.11[b]
Mineral content (mg/Kg)		
Fe	2.244±0.01[a]	1.523±0.07[b]
Ca	8.783±0.18[b]	15.794±0.01[a]

Means in a row with same superscripts are not significantly different ($p > 0.05$);*P prebiotic;** P/GF prebiotic/gluten-free

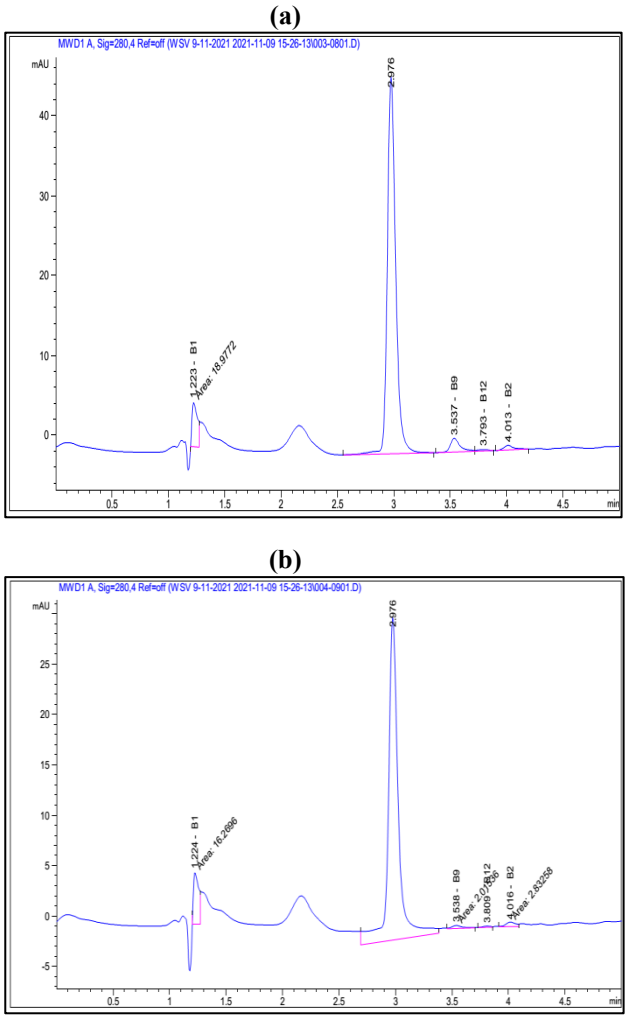


Fig. 2. HPLC chromatography for vitamins in snack rolls samples a) P formula and b) P/GF formula

3.3. Baking quality of snack rolls

The result of the baking quality of snack roll samples is shown in **Table (6).** The height of snack rolls prepared from the P and P/GF formulas was observed (24.7667 mm and 24.8717 mm, respectively). There were no statistically significant differences observed in terms of height between the two snack rolls, as indicated by a p-value greater than 0.05. The roundness of snack rolls prepared from the P formula was higher than other rolls prepared from the P/GF formula. The same trend was observed for the volume values of snack rolls. The rolls prepared from the P formula were greater than those prepared from the P/GF formula. This observation may be due to the increasing ratio of green banana flour in the P/GF formula. These results follow those found by **[78]**.

The weight of snack rolls produced from the P/GF formula (35.286 g) was considerably low compared to the sample P formula (36.245 g). The slight increase in the weight of snack rolls made from

the prebiotic formula could result from the high water-holding capacity of the composite flour used. These results were compatible with those obtained by **[79]**, who reported that the difference in biscuits' weight could be attributed to the different water-holding capacities of the flour blends. The findings presented in **Table (6)** indicate that there was no statistically significant difference in volume, as determined by a p-value greater than 0.05 between the snack rolls produced from the p and P/GF formulas. **[80]** reported that the low value of a specific volume of bakery products was connected with the weakening of the gluten network in dough and reduced gas retention in the dough.

Snack rolls made from the P formula had shrinkage of 5.6963%, whereas P/GF snack rolls had 7.0216% (**Table 5**). The baking loss was significantly higher (34.8983%) in P/GF snack rolls than in P snack rolls (**Table 5**). The baking loss in P snack rolls was found to be 31.0525%. The difference in shrinkage and baking loss percentages may be due to the WHC of composite flour **[37]**. According to their findings, the reduction in bread bake loss observed when substituting wheat flour with alternative flours can be attributed to the augmented protein content in their mixture. This higher protein content resulted in increased water retention during the baking process.

P formula snack rolls took 33 minutes to bake, while GF formula snack rolls had a baking time of 24.5 minutes **(Table 6).** Increasing time may be due to the increased polysaccharide levels in the composite flour (barley, tiger nut, and green banana flour). These polysaccharides are thought to resemble pectic material, resulting in enhanced water absorption capacity, viscosity, and an increase in baking time **[81].**

Table 6: Baking quality parameters of (P) and (P/GF) snack rolls samples

Baking Quality Parameters	P formula	P/GF formula
Height (mm)	24.7667±0.34^{a}	24.871± 0.59^{a}
Roundness (cm)	30.550±0.22^{a}	29.050±0.49^{b}
Volume (cm^3)	60.317±1.02^{a}	58.292±0.53^{b}
Weight (g)	36.246±0.76^{a}	35.286±1.82^{a}
specific volume (cm^3/g)	1.664±0.01^{a}	1.655±0.073^{a}
Shrinkage (%)	5.696±0.93^{b}	7.022±1.32^{a}
Baking loss (%)	31.025±0.39^{b}	34.898± 1.51^{a}
Baking time (min)	33.000±0.63^{a}	24.500±0.55^{b}

Means in a row with same superscripts are not significantly different ($p \leq 0.05$). Average value for 6 rolls for each property and formula

3.4. Sensory evaluation of snack rolls

Sensory evaluation of P and P/GF snack rolls is the assessment of the aspects of food experienced by the senses, including appearance, taste, odour, chewiness, texture, and total product quality. The mean sensory score of both snack rolls is presented in **Fig. (3).** The P/GF formula samples received higher ratings from panelists for appearance, odor, chewiness, and overall product quality than the P formula. Conversely, samples prepared from the P formula have a higher taste score, as seen in **Fig. (3).** Replacing barley flour with rice flour in the gluten-free formula showed high sensory attributes. Accordingly, this study can be among the recent trial to add a new product to the list of products for patients with celiac disorders. Other researchers have also achieved comparable outcomes, indicating that rice pasta fortified with alternative gluten-free flour received high ratings for its sensory attributes. This pasta has the potential to be a wholesome alternative to traditional pasta, providing individuals with digestive disorders with a diverse range of food options. Furthermore, the examination of the data indicated a statistically significant disparity in the taste and odor ratings of the P and P/GF snack rolls. In contrast, no significant difference was found in appearance, chewiness, textural or total product quality. Data on taste and odour in both formulas achieved high scores, whereas the use of tiger nut flour improved the product's organoleptic properties. **[82]** observed a similar trend, explaining that biscuit fortification with tiger nut flour application in biscuits has the potential to appeal to consumers while also providing health benefits to them. Aside from its nutritional value, banana flour had no negative effects on the characteristics and sensory qualities of the products. Green banana flour has no taste or odour, so the products retain their original flavour and aroma after adding GBF **[83].**

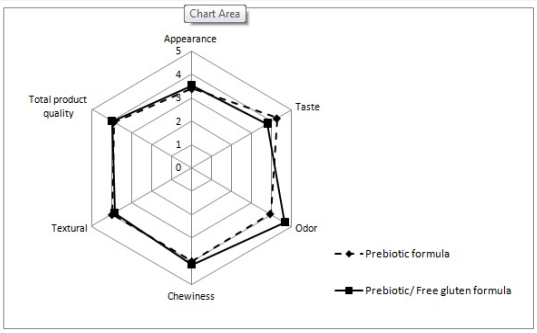


Fig. 3. Sensory evaluation of (P) and (P/GF) snack rolls samples

3.5. Effect of the (P) and (P/GF) snack rolls samples on the nutritional parameters of rats

As inferred from **Table (7)**, no significant variations in initial body weights, total food intake, or feed efficiency were detected when compared to (normal and celiac disease) groups in both protocols **[84, 85]**. In contrast, A notable decrease in final body weight and the rate of body weight gain was noticed in the celiac disease group when compared to normal animal control in the second protocol, while A notable enhancement was observed in the final body weight and body weight gain in the treatment group when compared to celiac disease group in the second protocol. The precise impact on final body weight and body gain remains uncertain; however, it is likely that this outcome is linked to the pronounced flavor profile of the ingredients employed in the formulations **[86-88]**. The aforementioned findings provide evidence in favor of incorporating gluten-free products as a supplementary measure to enhance the nutritional content of bakery products that are free from gluten.

Table 7: Changes in nutritional parameters of experimental groups of rats

Group	Initial body weight (g)	Final body weight (g)	Body gain (g)	Total food intake (g)	Feed efficiency ratio
First protocol Normal control	200.5 ± 4.15	246.4 ± 5.68	45.9 ± 3.12	269.5 ± 2.29	0.017 ± 1.36
Group (1)	199.5 ± 4.33[a]	244.2 ± 5.87[a]	44.7 ± 5.4[a]	269.2 ± 3.08[a]	0.017 ± 1.78[a]
Group (2)	198.8 ± 4.35[a]	244.9 ± 6.77[a]	46.1 ± 2.08[a]	269 ± 1.61[a]	0.017 ± 1.29[a]
Second protocol Normal control	52.8 ± 4.92	95.2 ± 5.35	42.4 ± 0.43	269.2 ± 3.63	0.016 ± 0.01
Celiac disease Group	49.5 ± 3.03[a]	88.3 ± 3.17[a]	38.8 ± 0.64[a]	268.8 ± 2.54[a]	0.014 ± 0.25[a]
Treatment Group	51.2 ± 3.35[a]	92.3 ± 4.85[b]	41.1 ± 1.5[b]	268.5 ± 2.77[a]	0.015 ± 0.54[a]

The values are presented in the form of Mean ± SD (n= 6), where the use of identical letters within each column indicates a lack of statistical significance ($p>0.05$) among the different varieties. Conversely, the presence of distinct letters indicates a statistically significant difference at a significance level of $P \leq 0.05$. Feed Efficiency ratio= (Body Gain/ Total Food Intake)

3.6. Effect of the (P) and (P/GF) snack rolls samples on the relative organs weight of rats

According to the data presented in **Table (8)**, there were no statistically significant variations observed in the relative weight change of all organs across both protocols within the experimental groups of rats. However, a minor distinction was observed in the relative weight of the spleen in the second protocol. The group of celiac disease demonstrated a notable reduction in spleen weight in comparison to the normal control group. On the contrary, a modest yet statistically notable elevate in spleen weight was observed in the treatment group of the second cohort, as compared to the group diagnosed with celiac disease.

Table 8: Changes in the relative organs weight (g/100g BW) of experimental groups of rats

Group	Liver	Kidney	Brain	Tests	Heart	Lung	Spleen
First protocol Normal control	5.3 ± 0.15	0.87 ± 0.48	0.64± 0.03	1.5 ± 0.1	0.33 ± 1.36	0.74 ± 0.3	0.39 ± 0.03
Group (1)	5.5 ± 0.13[a]	0.86 ± 0.42[a]	0.63 ± 0.02[a]	1.4 ± 0.09[a]	0.31 ± 1.78[a]	0.72 ± 0.4[a]	0.37 ± 0.02[a]
Group (2)	5.4 ± 0.16[a]	0.86 ± 0.41[a]	0.63 ± 0.02[a]	1.4 ± 0.08[a]	0.32 ± 1.29[a]	0.73 ± 0.3[a]	0.36 ± 0.03[a]
Second protocol Normal control	4.4 ± 0.12	0.81 ± 0.45	0.66 ± 0.03	1.4 ± 0.1	0.31 ±0.01	0.72 ± 0.4	0.34 ± 0.03
celiac disease Group	4.7 ± 0.13[a]	0.73 ± 0.41[a]	0.6 ± 0.04[a]	1.1 ± 0.07[a]	0.28 ±0.25[a]	0.70 ± 0.3[a]	0.28 ± 0.02[a]
Treatment Group	4.5 ± 0.15[a]	0.8 ± 0.44[a]	0.65 ± 0.03[a]	1.3 ± 0.09[a]	0.30 ±0.54[a]	0.71 ± 0.4[a]	0.33 ± 0.03[b]

The values are presented in the form of Mean ± SD (n= 6), where the use of identical letters within each column indicates a lack of statistical significance ($p>0.05$) among the different varieties. Conversely, the presence of distinct letters indicates a statistically significant difference at a significance level of $P \leq 0.05$.

3.7. Effect of snack rolls samples on the Hematological Parameters of rats

Table (9) showed that the results revealed the rats' hematological profiles for both protocols. When comparing with the normal control group in the first protocol, no significant differences were observed in the blood counts (RBC, WBC, platelets, PCV, MCV, MCH, and MCHC) of the groups (1, 2). Gluten appeared not to influence blood parameters, which are important health indicators in animals, including humans. Similar counts of leukocytes, lymphocytes, neutrophils, and monocytes were found in gluten-free and gluten-fed people **[89]**. Gluten appeared not to influence blood parameters, which are important health indicators in animals, including humans. Similar counts of leukocytes, lymphocytes, neutrophils, and monocytes were found in gluten-free and gluten-fed people **[90]**.

The second protocol revealed a notable decrease in the blood counts of red blood cells (RBC) and white blood cells (WBC) in the group with celiac disease, in comparison to the normal control group. Simultaneously, a modest but noteworthy increase in red blood cell (RBC) count was noticed in the treatment group when comparing to the group with celiac disease. The utilization of the mean red blood cell (RBC) size has facilitated the enhanced characterization of anemia and the quantification of red blood cell dimensions within the circulatory system. Furthermore, it was observed that the treatment group exhibited a notable increase in white blood cell count (WBC) in comparison to the celiac disease group. This finding suggests that a gluten-free diet possesses more robust immune-protective properties than the control counterparts. The interaction between gluten and immune cells leads to the production of cytokines, which increases the susceptibility to white blood cell cancer **[91]**.

The complete blood count revealed a notable decrease in platelet count among group with celiac disease in comparison to the control group that tested negative for the condition. Furthermore, a notable increase was observed in the treatment group in comparison to the group diagnosed with celiac disease. Platelets are blood components that aid in clotting. The data collected on the rats were within their physiological norm, which was appropriate for their age. So, based on the findings of hematological investigations, we observed that prebiotic/gluten-free formula had a favorable influence on blood morphological indicators, assisting in the maintenance of general homeostasis in animals and confirming the biological safety of the examined supply.

Table 9: Changes in the relative blood picture of the experimental groups of rats

Group	RBC count ($\times 10^6$L)	WBC count ($\times 10^3$L)	Platelet count ($\times 10^3$L)	PCV (g/dl)	MCV (fL/cell)	MCH (pg/cell)	MCHC (gm/dl)
First protocol Normal control	5.5 ± 0.2	8110 ± 281	275 ± 18.1	49 ± 2.56	85 ± 2.35	27.5 ± 1.5	32.3 ± 1.53
Group (1)	5 ± 0.4[a]	8050 ± 460[a]	273 ± 13.8[a]	47 ± 2.48[a]	91 ± 2.42[a]	26.9 ± 1.2[a]	31.4 ± 2.3[a]
Group (2)	5.2 ± 0.21[a]	8100 ± 277[a]	274 ± 17.4[a]	48 ± 2.19[a]	93 ± 2.13[a]	27.1 ± 1.3[a]	31.6 ± 2.5[a]
Second protocol Normal control	5.3 ± 0.2	8100 ± 275	281 ± 11.8	48 ± 2.23	88 ± 2.34	27.9 ± 1.4	31.5 ± 1.4
celiac disease Group	4.2 ± 0.4[a]	7600 ± 85[a]	265 ± 13.3[a]	43 ± 3.12[a]	83 ± 3.3[a]	25.9 ± 0.9[a]	27.5 ± 2.6[a]
Treatment Group	5.1 ± 0.2[b]	7900 ± 381[b]	279 ± 9.4[b]	47 ± 2.44[a]	86 ± 2.14[a]	27.2 ± 1.3[a]	30.5 ± 1.7[a]

RBC= red blood cell, WBC= weight blood cell, PCV= packed cell volume, MCV= mean corpuscular volume, MCH = mean cell hemoglobin, MCHC= mean cell hemoglobin concentration. The values are presented in the form of Mean ± SD (n= 6), where the use of identical letters within each column indicates a lack of statistical significance ($p>0.05$) among the different varieties. Conversely, the presence of distinct letters indicates a statistically significant difference at a significance level of $P \leq 0.05$.

The results from the first protocol showed no significant change in the Hb level in groups (1, 2), which were 15.1 and 15.1 g/dl, respectively, when comparing to the normal control group, which was 15.3 g/dl. The results from the second protocol showed a significant reduction in the celiac disease group (13.7 g/dl) when comparing to the normal control group (15.4 g/dl) and a significant elevation in the treatment group (14.9 g/dl) when comparing to the celiac disease group (**Fig. 4**).

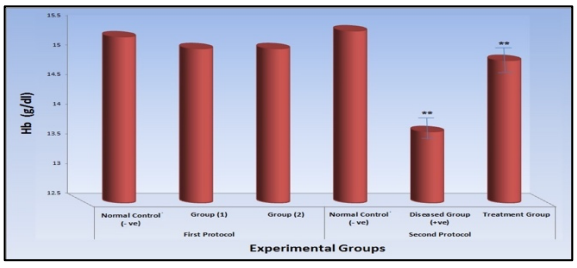


Fig. 4. Effect of prebiotic formula and prebiotic/gluten-free formula on Hb of the experimental groups of rats

3.3.8. Effect of snack rolls samples on the Biochemical Parameters of rats

Table (10) presents data indicating that there were no statistically significant variations observed in plasma gliadin concentration, as well as levels of IFN-γ, TNF-α, and IL-6, between groups 1 and 2 when comparing to the normal control group. In contrast, the findings from the second protocol demonstrated a notable decrease in plasma gliadin concentration, as well as in the levels of IFN-γ, TNF-α, and IL-6, within the celiac disease group when comparing to the normal control group. Conversely, the experimental group demonstrated a notable rise in plasma gliadin concentration, as well as increased levels of IFN-γ, TNF-α, and IL-6, in comparison to the group diagnosed with celiac disease. Currently, it has been observed that there exist gliadin peptides that are resistant to the enzymes found in the gastrointestinal tract. These peptides are able to traverse the submucosa of the small intestine and subsequently interact with T cells through antigen-presenting cells. Upon activation, T cells secrete interferon-gamma (IFN-γ), a cytokine that plays a crucial role in driving the adaptive immune response. This immune response subsequently leads to mucosal atrophy [27, 92, 93]. The exclusive therapeutic approach for celiac disease is the adoption of a gluten-free diet. In order to explore the potential impact of prebiotics or gluten-free formula on celiac disease, we conducted an analysis of IFN-γ levels within jejunal tissue [94-98]. The induction of gliadin led to the suppression of TNF-α and IL-6 levels. The aforementioned suppression was observed consequent to the recurrent intragastric administration of gliadin, leading to morphological modifications in the jejunum. The modifications encompassed the reduction in length of villi, the proliferation of crypts, and the infiltration of lymphocytes. The alterations observed were in line with the mucosal modifications commonly seen in individuals diagnosed with celiac disease.

Table 10: Changes in the plasma gliadin conc., IFN-γ, TNF-α and IL-6 of the experimental groups of rats

Group	Plasma Gliadin Conc. (ng/ml)	IFN-γ (pg/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)
First protocol Normal control	25.5 ± 4.52	577.7 ± 25.56	90.8 ± 3.41	46.43 ± 5.12
Group (1)	24.9 ± 3.46[a]	572.1 ± 23.7[a]	89.7 ± 3.16[a]	45.18 ± 4.21[a]
Group (2)	25.2 ± 3.65[a]	575.6 ± 24.35[a]	89.9 ± 3.34[a]	45.24 ± 5.04[a]
Second protocol Normal control	26.2 ± 4.34	562.1 ± 23.52	91.23 ± 2.87	45.17 ± 4.12
Celiac disease Group	6.2 ± 4.14[a]	914.9 ± 35.9[a]	255.83 ± 6.87[a]	118.22 ± 7.67[a]
Treatment Group	21.3 ± 3.65[b]	630.8 ± 27.15[b]	113.2 ± 3.34[b]	44.51 ± 5.39[b]

The values are presented in the form of Mean ± SD (n= 6), where the use of identical letters within each column indicates a lack of statistical significance ($p>0.05$) among the different varieties. Conversely, the presence of distinct letters indicates a statistically significant difference at a significance level of $P \leq 0.05$.

The results of the first and second protocols are presented in **Table (11).** They showed no significance in total cholesterol, HDL, or LDL in the serum of rats fed on both prebiotic formula and prebiotic/gluten-free formula when comparing with the normal control group in the first protocol and the celiac disease group in the second protocol. Triglyceride and total lipid levels in rats' blood fed with prebiotic formula and prebiotic/gluten-free formula were unchanged compared to the normal control group in the first protocol and the diseased group in the second protocol. **[89]** also discovered no variations in the lipid metabolism between experimental groups on gluten and non-gluten diets. As a result of the lipid profile, we discovered that both prebiotic formula and prebiotic/gluten-free formula had a good influence on the lipid profile of rats and had no negative effects on the rats' health.

Table 11: Changes in total cholesterol, HDL, LDL, Triglycerides and total lipids of the experimental groups of rats

Group	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)	Total Lipids (mg/dl)
First protocol Normal control	84.5± 4.31	41.3 ± 3.16	25.18 ± 4.21	77.32 ± 2.54	341.9 ± 4.35
Group (1)	82.1 ± 3.16[a]	40.2 ± 2.37[a]	24.7 ± 4.26[a]	75.18 ± 2.31[a]	339.3 ± 4.31[a]
Group (2)	83.2 ± 3.35[a]	40.6 ± 2.45[a]	24.9 ± 4.29[a]	76.94 ± 2.24[a]	340.4 ± 4.64[a]
Second protocol Normal control	85.2± 4.11	42.5 ± 3.62	25.43 ± 3.87	79.47 ± 2.42	344.7 ± 4.42
Celiac disease Group	76.2 ± 6.14[a]	40.9 ± 0.97[a]	26.2 ± 5.19[a]	69.29 ± 2.35[a]	335.9 ± 6.45[a]
Treatment Group	78.4 ± 3.45[a]	41.8 ± 0.75[a]	24.2 ± 2.28[a]	68.41 ± 2.31[a]	336.2 ± 3.31[a]

The values are presented in the form of Mean ± SD (n= 6), where the use of identical letters within each column indicates a lack of statistical significance (p>0.05) among the different varieties. Conversely, the presence of distinct letters indicates a statistically significant difference at a significance level of $P \leq 0.05$.

Based on the data presented in **Table (12)**, there were no statistically significant variations observed in AST and ALT levels among the experimental groups in either of the protocols. There was no observed statistically significant difference in urea levels among the experimental groups in either of the protocols. The second protocol in the celiac disease group exhibited a notable reduction in creatinine levels when compared to the normal control group. However, there were no statistically significant variations in the levels of creatinine observed among the remaining experimental groups. There were no statistically significant variations observed in the levels of total protein and albumin among the experimental groups in both protocols. The results presented in this study align with the findings reported by **[99]**, wherein they observed that gluten-free rats exhibited total protein and albumin levels that fell within the range of normal values.

Related to our results, both sensory evaluation and *in vivo* study indicated that the value-added flour enhances the mouth feel and retains the flavor for the end products. The green banana and tiger nut flours could have functional uses in the food industry and be applied as good ingredients for healthy and celiac disease food products.

Table 12: Changes in Liver enzymes, Kidney enzymes, and biochemical parameters of the experimental groups of rats

Group	AST (U/L)	ALT (U/L)	Creatinine (mg/dl)	Urea (mg/dl)	Albumin (g/dl)	Total Protein (g/dl)
First protocol Normal control	57.5± 2.31	54.3 ± 3.55	2.35 ± 0.07	56.23± 1.4	2.89± 0.04	5.34± .035
Group (1)	56.4 ± 2.26[a]	51.3 ± 2.37[a]	2.34 ± 0.06[a]	54.12 ± 1.8[a]	2.77 ± 0.03[a]	5.28 ± .024[a]
Group (2)	57.2 ± 2.46[a]	53.5 ± 2.22[a]	2.34 ± 0.05[a]	55.43 ± 1.6[a]	2.74 ± 0.04[a]	5.32 ± 0.31[a]
Second protocol Normal control	58.7 ± 2.25	56.3 ± 3.18	2.38± 0.07	57.22 ± 1.5	2.91± 0.03	5.39± .039
celiac disease Group	52.3± 2.38[a]	55.1± 2.83[a]	2.23 ± 0.02[a]	53.32 ± 1.9[a]	3.04 ± 0.04[a]	5.37 ± 0.11[a]
Treatment Group	54.2 ± 2.47[a]	55.7 ± 2.27[a]	2.34 ± 0.07[b]	54.2 ± 1.8[a]	2.89 ± 0.03[a]	5.38 ± 0.13[a]

The values are presented in the form of Mean ± SD (n= 6), where the use of identical letters within each column indicates a lack of statistical significance (p>0.05) among the different varieties. Conversely, the presence of distinct letters indicates a statistically significant difference at a significance level of $P \leq 0.05$.

3. CONCLUSION

Replacing the commonly used gluten-free flour with innovative ingredients with high nutritional value is one of the newest ways to enhance the nutritional and technological value of gluten-free bakery products. Tiger nuts have been cultivated since ancient Egypt, during which tubers were found in sarcophagi and tombs of the initial dynasties. The resulting flour derived from these tubers is devoid of gluten and can be utilized for diverse technological applications. However, it is worth noting that their potential remains largely unexplored. In order to offer a food product that meets the needs of celiac consumers, we propose a new formulation of gluten-free snack rolls based on tigernut with green banana and rice flour to improve the nutritional profile of the

proposed gluten-free product. The composite flour is widely regarded as a highly nutritious alternative due to its rich content of essential nutrients, including dietary fiber, vitamin B complex, and various minerals. This nutrient profile helps to address the nutritional deficiencies that may arise from the absence of gluten. These functional properties help determine the blends of different flours for various products. The biological evaluation of rats in this study concluded that both the prebiotic formula and the prebiotic/gluten-free formula improved the rats' health, particularly the prebiotic/gluten-free formula, which had an obvious improvement in the treatment group comparing to the diseased group in the second protocol, and that neither formula caused any serious side effects in all experimental groups of both. The results of our investigation lead us to the conclusion that the tigernut, green banana, and barley snack rolls examined in this research exhibit qualities of an inventive gluten-free food item. Furthermore, these snack rolls are considered to be both safe and beneficial for individuals diagnosed with celiac disease.

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