



Enhancing Nutritional Quality and Antidiabetic Effects of Pan Bread Fortified with Germinated Red Radish Seed Powder



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Abstract

This study aimed to investigate the nutritional and therapeutic potential of germinated red radish seed powder (GRRSP) as a functional ingredient in pan bread and its effects on biochemical parameters in diabetic rats. Proximate analysis revealed that GRRSP contained significantly higher levels of protein (35.20%), fiber (19.50%), and ash (10.80%) compared to wheat flour, along with lower carbohydrate and fat content. GRRSP was also rich in essential minerals such as potassium (1020 mg/100g), calcium, magnesium, phosphorus, and iron, and exhibited elevated levels of bioactive compounds, including total phenolics (9.59 mg GAE/g), flavonoids (0.85 mg QE/g), and antioxidant activity (91.30%). Substituting wheat flour with GRRSP (2.5–10%) in pan bread formulations improved the nutritional profile, especially in protein, fiber, and mineral content. However, higher substitution levels adversely affected loaf volume, crumb texture, and sensory scores, with blends containing up to 5% GRRSP maintaining acceptable sensory quality. In vivo, diabetic rats fed diets containing GRRSP showed dose-dependent improvements in blood glucose levels, lipid profiles, and liver enzyme activities over a six-week period. At 10% GRRSP inclusion, blood glucose levels decreased by 45%, while HDL increased and LDL, total cholesterol, and triglycerides significantly decreased. Liver enzymes (ALT, AST, ALP) also declined toward normal values, suggesting hepatoprotective effects. These findings demonstrate that GRRSP can serve as a functional food ingredient with substantial nutritional enhancements and antidiabetic effects, supporting its potential use in therapeutic diet formulations and functional bakery products.

Keywords: Germinated red radish seeds, functional bread, diabetes, antioxidant activity, lipid profile, liver enzymes, bioactive compounds.

1. Introduction

Wheat (*Triticum aestivum* L.), an important crop from the Poaceae family, is one of the most widely cultivated cereals and serves as a staple food for a large portion of the global population. It provides nearly half of the world's caloric intake and is a good source of proteins, particularly gluten as well as essential minerals like iron, zinc, copper, magnesium, and phosphorus. Furthermore, wheat contains B-complex and E vitamins, including thiamine, riboflavin, and niacin, along with dietary fiber. Although refined wheat flour is a fundamental ingredient in many baked products, it typically contains lower levels of protein compared to other cereals. This is largely due to its deficiency in essential amino acids such as lysine, methionine, and threonine. Moreover, the refining process significantly reduces its nutritional value. Nevertheless, these deficiencies can be addressed by refining wheat flour with proteins, fibers, vitamins, and minerals to meet the dietary needs of specific populations, especially those at risk of malnutrition. In addition to enhancing the nutritional value of wheat flour, composite flour-based baked goods offer functional benefits by serving as effective carriers of essential nutrients. [1, 2]

Interest in using sprouts and microgreens as part of the human diet dates back thousands of years, particularly in ancient Egyptian and Chinese civilizations, where they were valued for their health benefits, including healing and revitalization. [3, 4] Sprouting is a common method used to enhance the nutritional profile of seeds as they develop into sprouts. Both sprouts and microgreens are classified as “functional foods” due to their ability to support health and help prevent diseases, making them among the most nutrient-dense and beneficial food options available today.[5] Red radish (*Raphanus sativus* L.), belonging to the Brassicaceae family, is a valuable vegetable known for its diverse medicinal properties. Various parts of the radish plants such

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as the roots, seeds, and leaves are traditionally used for therapeutic purposes. [6] The seeds of *Raphanus sativus* L. are particularly rich in bioactive phytochemicals, including alkaloids, flavonoids, glycosides, phenols, sterols, and tannins. [4, 7, 8] Radish is a member of the Cruciferous (Brassicaceae) family and has been cultivated for millennia in regions such as China and the Mediterranean. Nutritionally, radishes contain carbohydrates, natural sugars, dietary fiber, protein, and fats. They are also known to possess distinctive bioactive compounds that offer potential health benefits for humans. [9] Germination significantly enhances the levels of micronutrients and phytonutrients in all types of seeds studied, clearly indicating that sprouting boosts their nutritional value. This highlights the importance of incorporating sprouts into the human diet, especially as an affordable and nutritious option for low-income households and to support agricultural sustainability. [10] Cruciferous sprouts stand out from other plant foods due to their high concentration of bioactive compounds. Depending on the plant species, variety, and growing conditions, germinated seeds can contain more than double the number of phytochemicals. Sprouts that are seven to eight days old are considered ideal for harvesting, as they retain elevated levels of these beneficial compounds during post-harvest processing and marketing. Radish sprouts, in particular, are young plants that remain metabolically active even after harvest. [4, 11]

Diabetes mellitus (DM), commonly known as diabetes, is a long-term metabolic condition marked by persistently high blood glucose levels and reduced insulin sensitivity, often associated with oxidative stress. When left unmanaged, it can result in numerous complications and cause permanent damage to various body systems. [12] A study involving 180,000 insured dogs between the ages of 5 and 12 in the United States found that Australian Terriers, Samoyeds, Swedish Elkhounds, and Swedish Lapphunds had the highest incidence of diabetes mellitus. Genetic variation among breeds appears to contribute to a greater susceptibility to specific types of diabetes. Additional risk factors identified include breed, a prior diagnosis of hyperadrenocorticism, and being female. Diabetes mellitus (DM) is classified into three main types. Type 1 DM, also called "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes," has an unknown origin. Type 2 DM, the most prevalent form, is known as "non-insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes" and is primarily linked to insulin resistance caused by obesity and physical inactivity. Type 3 DM, or "gestational diabetes," occurs during pregnancy and is marked by high blood sugar levels in individuals without a prior diabetes diagnosis. In animals, Types 1 and 2 are the most frequently observed. [13] Managing diabetes mellitus (DM) typically involves a combination of medication, dietary modifications, and regular exercise. However, conventional hypoglycemic agents such as insulin, biguanides, sulfonylureas, and alpha-glucosidase inhibitors—can lead to a range of side effects, including severe low blood sugar, abdominal discomfort, lactic acidosis, and other health issues. [14] In contrast, herbal treatments offer a promising alternative, as they are rich in natural bioactive compounds with antidiabetic properties. These remedies are often linked to fewer side effects, reduced cost, and greater accessibility, particularly in rural areas. Natural sources are increasingly viewed as a safe, economical option for managing diabetes. [15]

Although numerous studies have highlighted the beneficial effects of natural products in managing diabetes mellitus, there is currently no data available regarding the antidiabetic potential of Japanese radish sprouts (*Raphanus sativus*), also known in Japan as Kaiware-daikon. As a member of the cruciferous vegetable family, Japanese radish sprouts are rich in functional compounds, including isothiocyanates—recognized for their possible anticancer properties—as well as antioxidants such as various sinapinic acid esters and flavonoids. [16] This study aimed to investigate the effects of partially substituting wheat flour with germinated radish seed powder on dough properties and pan bread production. It also focused on evaluating the proximate composition, quality attributes, sensory characteristics, and physical properties of the making bread. Furthermore, the research sought to examine the influence of dietary fiber and other bioactive components in germinated radish seed powder on blood glucose levels, lipid profiles, cholesterol levels, and liver function in diabetic rats.

2. Materials and Methods

2.1. The first part of the experiment

2.1.1. Materials

2.1.1.1. Source of red radish seeds

Red radish seeds (*Raphanus sativus*) were obtained from local markets in Kafr Elsheikh City, Egypt. Wheat flour (72% extraction rate) was sourced from the North Cairo Flour Mills Company. Active dry yeast (*Saccharomyces cerevisiae*) was supplied by the Chemicals Factory of the Egyptian Sugar and Integrated Industries Company (ESIIC) located in El-Hawamdia City, Giza, Egypt. Shortening, table salt (sodium chloride), and sugar (sucrose) were all purchased from local markets in Egypt.

2.1.1.2. Germination red radish seeds

Radish seeds were first cleaned to remove any broken or damaged seeds. A total of 1000 grams of seeds were placed in a glass container filled with 10 liters of tap water and soaked at room temperature for 12 hours. After soaking, the water was discarded, and the seeds were rinsed every 8 hours over a period of 5 days to promote germination. At the end of the germination period, the sprouted seeds were thoroughly washed and then dried in open air and sunlight for 3 days. Once fully dried, the seeds were ground into a fine powder. This procedure was based on the method described by Tork. [17].

2.1.2. Chemicals and reagents

All chemicals analytical grade were purchased from Sigma Company for medical materials, Giza, Egypt.

2.1.3. Analytical methods

The average was expressed after each analysis, which was done in triplicate.

2.1.3.1. Gross chemical composition

The contents of fat, crude protein, crude fiber, and ash were analyzed using the methods outlined by AOAC. [18]

2.1.3.2. Determination of available carbohydrates

Available carbohydrates content was calculated by subtracting the sum of crude protein, fat, ash, and crude fiber percentages from the sample's initial dry weight, following the method described by AOAC. [18] The available carbohydrates (on a dry weight basis) were determined using the formula:

Available carbohydrates (%) = $100 - (\% \text{ protein} + \% \text{ fat} + \% \text{ ash} + \% \text{ fiber})$.

2.1.3.3. Energy value

The energy content was calculated based on the method described by James, [19] using the following formula:

Energy value = $9.1 \times (\% \text{ fat}) + 4.1 \times (\% \text{ available carbohydrates} + \% \text{ crude protein})$.

2.1.3.4. Determination of Minerals

Minerals were determined according to the procedures outlined by AOAC. [18]

2.1.3.5. Estimations of Total Polyphenolics

Following the method described by Thaipong, [20] the total polyphenolic compounds (TPC) were determined using the Folin-Ciocalteu reagent. A UV spectrophotometer (Varian, Melbourne, VIC, Australia) was employed to measure the polyphenol content. Gallic acid served as the standard, and absorbance was recorded at 760 nm. The results were expressed as milligrams of gallic acid equivalent per gram of dry matter (mg GAE/g DM).

2.1.3.6. Determination of total flavonoids

Total flavonoid content was assessed following the method outlined by Vuong et al. [21, 22] Absorbance was measured at 510 nm using a UV spectrophotometer (Varian, Melbourne, VIC, Australia). Quercetin was used as the reference standard for calculating the flavonoid concentration.

2.1.3.7. Determination of antioxidant activity

The free radical scavenging capacity of GRRSP was evaluated using the DPPH assay method described by Abdel-Razek et al. [22]

2.1.4. Pan bread Making

Pan bread was prepared following the straight dough method described by El-Hadidy. [23] The basic formulation included 100 g of wheat flour (WF), 1.5 g of instant active dry yeast, 2 g of salt, 2 g of sugar, 3 g of shortening, and water. For the experimental blends (Control, B1, B2, B3, and B4), Germinated red radish seed powder (GRRSP) was used to partially substitute WF at an extent of 0%, 2.5%, 5%, 7.5%, and 10%, respectively. The ingredients were combined in a mixing bowl at 28°C and mixed for 6 minutes. The resulting dough was manually folded 20 times to shape it, then left to rest for 10 minutes. The dough was transferred into a lightly greased baking pan and proofed for 60 minutes in a fermentation cabinet set at 30°C and 85% relative humidity. Baking was carried out in an electric oven at 250°C for 20 minutes. After baking, the bread was cooled at room temperature (25°C) for 60 minutes, then packed in polyethylene bags for subsequent analysis, as outlined in Table 1.

Table 1: Pan bread ingredients.

Components	Control	B1	B2	B3	B4
WF(g)	100	97.5	95	92.5	90
GRRSP (g)	---	2.5	5	7.5	10
Salt(g)	1.50	1.50	1.50	1.50	1.50
Sugar(g)	02	02	02	02	02
Fat(oil)(g)	03	03	03	03	03
Yeast (g)	1.50	1.50	1.50	1.50	1.50

GRRSP= Germinated red radish seed powder

2.1.4.1. Sensory Properties of Pan Bread

The pan bread blends were evaluated according to the method outlined by AACC. [24] A sensory panel consisting of twenty staff members from the Food Technology Research Department at the Agricultural Research Center in Sakha, Egypt, was assembled. Panelists were asked to assess the sensory attributes of the bread, including overall acceptability (out of 100), aroma (10 points), taste (20 points), crumb texture (20 points), crumb color (20 points), symmetry of shape (10 points), and crust color (20 points).

2.1.4.2. Color Parameters

As described by Brunton et al. [25] the color characteristics of the prepared pan bread-lightness (L^*), redness (a^*), and yellowness (b^*)-were measured using a Hunter Lab Scan Visible colorimeter.

2.1.4.3. Toast bread physical parameters

After allowing the loaves to cool for three hours, their average weight was recorded. Loaf volume was measured using the rapeseed displacement method, as specified by AACC. [26] The specific volume (cm^3/g) was calculated by dividing the loaf volume by its corresponding weight.

2.1.4.4. Texture Profile Analysis (TPA)

The hardness of the pan bread was evaluated following the procedures outlined by AACC (2002). [27]

2.2. The second part: chemical assessments and feeding experiments

2.2.1. Animals

A total of 30 adult male albino rats were used in the study. They were obtained from the experimental animal unit of the Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. The rats selected for the experiment had body weights between 150 and 160 grams.

2.2.2. Animal Feeding

In this study, thirty (30) adult male albino rats, each weighing between 150 and 160 grams, were used. The experiment was conducted at the animal facility of the Food Technology Research Institute, Agricultural Research Center in Giza, Egypt. The rats were kept under standard hygienic conditions for ten days, during which they were fed a basal diet, containing 3.5% salt mixture and 1% vitamin mixture. They had free access to tap water and were given a consistent standard diet based on the formulation outlined by Reeves [28] in Table 2.

2.2.3. Experimental Design

After a ten-day adaptation period on a basal diet, five rats were assigned to the control group (G1, normal control) and continued the basal diet throughout the study. The remaining 25 rats underwent a 24-hour fasting period before receiving an injection of alloxan solution (120 mg/kg body weight) to induce hyperglycemia. [29] Blood glucose levels were monitored four days after the injection. Diabetes was confirmed based on the criteria described by Esmerino et al. [30] Following this, the 25 diabetic rats were divided into five subgroups, each consisting of five animals.

2.2.4. Blood Analysis

At the end of the experimental period (6 weeks) To evaluate the separated serum, several biochemical tests were performed. Blood samples were centrifuged at 3000 rpm for 5 minutes to isolate the serum, which was then used to measure specific parameters, including glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C).

Table 2: Composition of experimental diets

Groups	G1(-)	G2(+)	G3	G4	G5	G6
Cornstarch	65	65	62.5	60	57.5	55
Casein	20	20	20	20	20	20
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0
AIN 76 mineral mix	3.5	3.5	3.5	3.5	3.5	3.5
AIN 76 vitamin mix	1.0	1.0	1.0	1.0	1.0	1.0
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Bitartrate	0.2	0.2	0.2	0.2	0.2	0.2
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0
GRRSP	---	---	2.5	5.0	7.5	10

GRRSP= Germinated red radish seeds powder

Hyperglycemia Experiment with GRRSP

G1: Administered a standard diet (negative control).

G2: Administered a standard diet (positive diabetic control).

G3: Received a standard diet supplemented with 2.5% germinated red radish seed powder.

G4: Received a standard diet supplemented with 5% germinated red radish seed powder.

G5: Received a standard diet supplemented with 7.5% germinated red radish seed powder.

G6: Received a standard diet supplemented with 10% germinated red radish seed powder.

2.2.5. Biochemical Analysis and Enzymes Assays

2.2.5.1. Determination of serum glucose

Blood glucose levels in the serum samples were determined using a commercial kit provided by Spain React Company (Spain), in accordance with the method described by Trinder. [31]

2.2.5.2. Serum Lipid Determination

Total serum lipids were measured following the method of Knight et al. [32] Triglyceride levels were determined according to the procedure described by Fassati and Prencipe. [33] Total cholesterol and HDL cholesterol were analyzed using the method outlined by Allain. [34] Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) levels were determined as described by Lee and Nieman (1996) [35] and calculated using the following formulas:

- LDL (mg/dl) = Total cholesterol – (HDL + VLDL)
- VLDL (mg/dl) = Triglycerides ÷ 5

2.2.5.3. Determination of liver function

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels were measured using the methods described by Henry, [36] Varley et al. [37], and Rosalki and Foo, [38] respectively.

2.3. Statistical Analysis

Statistical analysis was operated using SPSS software (version 26), and Duncan's multiple range tests were employed to compare the means. The comparisons were made at a significant level of ($P \leq 0.05$).

3. Results and Discussion

3.1. The analysis composition of raw materials

The proximate analysis presented in **Table 3** highlights significant nutritional differences among wheat flour (72%), red radish seeds powder, and germinated red radish seeds powder. Wheat flour shows the highest moisture content (14.00%), which may affect its shelf life compared to the much lower moisture levels in red radish seeds powder (4.80%) and germinated powder (6.50%). In terms of protein, germinated red radish seeds powder exhibits a remarkable increase (35.20%) [4] compared to the non-germinated seeds (23.50%) and wheat flour (11.70%), indicating that germination enhances the protein content substantially. [1, 2, 39] Fat content is highest in red radish seeds powder (35.90%) but decreases significantly after germination (10.90%), suggesting that fats are metabolized during the sprouting process. Crude fiber also increases notably upon germination, from 13.90% to 19.50%, making the germinated powder a rich source of dietary fiber, [40] while wheat flour remains very low in fiber (0.75%). Similarly, the ash content, indicative of mineral content, is highest in the germinated powder (10.80%), followed by non-germinated seeds (4.20%), and lowest in wheat flour (0.45%). In contrast, available carbohydrates are highest in wheat flour (85.40%), making it primarily an energy-dense carbohydrate source, while both red radish seed powders are low in this component (22.50–23.60%). The total carbohydrate

content follows a similar trend, with wheat flour leading at 86.15%, and germinated and non-germinated seeds having lower values (42.80% and 36.40% respectively). Energy values differ remarkably: red radish seeds powder has the highest energy content (522.04 kcal/100g) due to its fat content, while germinated powder provides the lowest energy (340.27 kcal/100g), likely due to reduced fat and carbohydrate levels. Overall, germination improves the protein, fiber, and mineral content of red radish seeds, making germinated red radish seeds powder a valuable nutritional supplement, especially when used to fortify carbohydrate-rich foods like wheat flour. [1, 2, 4, 40]

Table 3: The analysis composition of wheat flour, red radish seeds powder and germinated red seeds powder

Raw materials	Wheat flour 72%	Red Radish seeds powder	Germinated red radish seeds powder
Moisture%	14.00 ^a ±0.04	4.80 ^c ±0.03	6.50 ^b ±0.04
Crude protein%	11.70 ^c ±0.06	23.50 ^b ±0.10	35.20 ^a ±0.12
Fat%	1.70 ^c ±0.02	35.90 ^a ±0.15	10.90 ^b ±0.06
Crude fiber%	0.75 ^c ±0.01	13.90 ^b ±0.04	19.50 ^a ±0.04
Ash%	0.45 ^c ±0.01	4.20 ^b ±0.03	10.80 ^a ±0.07
Available carbohydrates%	85.40 ^a ±0.06	22.50 ^c ±0.07	23.60 ^b ±0.09
Total carbohydrates%	86.15±0.07	36.4±0.10	42.80±0.03
Energy (K/100cal)	413.58 ^b ±0.06	522.04 ^a ±0.08	340.27 ^c ±0.05

Each value was an average of three determination ± standard deviation

Different letters indicate to significant differences between raw materials in the same row (p≤0.05).

The analysis of minerals and bioactive compounds of wheat flour, and germinated red seeds powder

Table 4 presents a comparison of mineral content and bioactive compounds between wheat flour (72%) and germinated red radish seeds powder, clearly demonstrating the superior nutritional and functional properties of the germinated seed powder. The mineral composition exposes significantly higher concentrations in germinated red radish seeds across all measured elements. Potassium (K) content in germinated seeds is exceptionally high at 1020 mg compared to only 130.60 mg in wheat flour. [39] Similarly, calcium (Ca) and magnesium (Mg) levels rise substantially, from 30.21 mg and 125.50 mg in wheat flour to 280 mg and 570 mg respectively in the germinated powder. Phosphorus (P), another essential mineral, increases more than fivefold (940 mg vs. 178.05 mg), and iron (Fe) content shows a dramatic improvement from 2.45 mg to 24.30 mg, indicating that germination enhances the bioavailability of key minerals. Although zinc (Zn) and manganese (Mn) also increase, the margin is less pronounced, suggesting that some minerals are moderately affected by germination [40]. Beyond minerals, the germinated red radish seeds correspondingly demonstrate a substantial enrichment in bioactive compounds. The total phenolic content (TPC) jumps from 0.70 mg GAE/g in wheat flour to 9.59 mg GAE/g in germinated seeds, indicating a notable increase in antioxidant-related compounds. Similarly, the total flavonoid content (TFC) rises from 0.14 mg QE/g to 0.85 mg QE/g. Most notably, antioxidant activity shows a sharp increase, from 52.40% in wheat flour to 91.30% in the germinated powder, confirming the powerful free radical scavenging potential of the germinated seeds. These enhancements in bioactive compounds are likely due to metabolic changes during germination, which stimulate the synthesis of secondary metabolites such as phenolics and flavonoids. [41] In summary, germinated red radish seeds powder far surpasses wheat flour in both mineral content and bioactive compounds. The significant increases in potassium, calcium, magnesium, phosphorus, iron, and antioxidant activity make it a highly valuable functional ingredient for health-promoting food formulations. [42]

Table 4: The analysis of minerals and bioactive compounds of wheat flour, and germinated red seeds powder

Raw materials	Wheat flour 72%	Germinated red radish seeds powder
K	130.60 ^b ±0.50	1020 ^a ±2.40
Ca	30.21 ^b ±0.40	280 ^a ±1.20
Mg	125.50 ^b ±0.90	570 ^a ±2.30
P	178.05 ^b ±1.20	940 ^a ±1.90
Fe	2.45 ^b ±0.02	24.30 ^a ±0.09
Zn	3.40 ^b ±0.03	5.80 ^b ±0.07
Mn	3.70 ^b ±0.01	5.40 ^c ±0.04
Antioxidants		
TPC (mg GAE/g)	0.70 ^b ±0.04	9.59 ^a ±0.05
TFC (mg of QE/g)	0.14 ^b ±0.01	0.85 ^a ±0.02
Antioxidant activity (%)	52.40 ^b ±0.10	91.30 ^a ±0.60

Each value was an average of three determination ± standard deviation

Different letters indicate to significant differences between raw materials in the same row (p≤0.05).

3.2. Chemical composition of pan bread blends

Table 5 presents the chemical composition of pan bread blends prepared with varying blends of wheat flour (WF) and germinated red radish seeds powder (GRRSP), showing a clear trend in nutritional enhancement as the proportion of GRRSP increases. As the GRRSP content rises from 0% to 10%, there is a consistent increase in protein, fat, fiber, and ash content. Protein content increases from 10.78% in the control sample (100% WF) to 12.95% in the 90:10 blend, indicating an improvement in the bread's protein quality with GRRSP inclusion. Similarly, fat content rises from 4.33% to 5.42%, while fiber increases significantly from 0.69% to 2.42%, enhancing the bread's dietary fiber content. Ash content, which reflects mineral content, also shows a marked increase from 0.42% to 1.37%, suggesting improved mineral availability. Conversely, available carbohydrates decrease gradually from 83.78% to 77.84%, which can be attributed to the lower carbohydrate content in GRRSP compared to wheat flour. As a result, there is a slight reduction in energy values from 427.10 Kcal/100g to 421.56 Kcal/100g. Overall, the addition of GRRSP enhances the nutritional profile of pan bread, particularly by increasing its protein, fiber, and mineral contents, making it potentially more beneficial for health-conscious consumers. [43]

Table 5: Chemical composition of pan bread samples (%on dry weight basis)

Blends	Protein%	Fat%	Fiber%	Ash%	Available carbohydrates%	Energy (K/100cal)
WF: GRRSP 100:0	10.78 ±0.01	4.33 ±0.01	0.69 ±0.01	0.42 ±0.02	83.78 ±0.02	427.10 ±0.01
WF: GRRSP 97.5:2.5	11.33 ±0.01	4.79 ±0.02	1.12 ±0.02	0.65 ±0.01	82.11 ±0.03	426.69 ±0.02
WF: GRRSP 95:5	11.87 ±0.02	5.00 ±0.02	1.55 ±0.01	0.89 ±0.02	80.69 ±0.04	424.99 ±0.01
WF: GRRSP 92.5:7.5	12.41 ±0.03	5.33 ±0.03	1.98 ±0.02	1.13 ±0.02	79.15 ±0.02	423.99 ±0.03
WF: GRRSP 90:10	12.95 ±0.02	5.42 ±0.02	2.42 ±0.03	1.37 ±0.01	77.84 ±0.03	421.56 ±0.03

WF= wheat flour

GRRSP= Germinated red radish seeds powder

Each value was an average of three determination ± standard deviation

Different letters indicate to significant differences between raw materials in the same column($p \leq 0.05$).

3.3. Sensorial attributes of pan bread

Table 6 presents the sensory evaluation scores of pan bread made with different blends of wheat flour (WF) and germinated red radish seed powder (GRRSP). The control sample, composed entirely of wheat flour (100:0), received the highest scores across all sensory attributes. It scored 19.80 for crust color, 19.70 for crumb color, 19.50 for crumb grain texture, 9.50 for symmetry of shape, 19.30 for taste, and 9.80 for aroma—indicating excellent sensory quality. As the proportion of GRRSP increased, a gradual decline in all sensory scores was observed. At a 2.5% substitution level, the blend (97.5:2.5) maintained relatively high acceptability with scores only slightly lower than the control: 18.50 for crust color, 19.00 for crumb color, and 18.30 for texture. However, further increases in GRRSP content led to more noticeable declines. For instance, the 95:5 blend scored 18.00 for crust color and 17.50 for texture, showing a modest reduction in sensory appeal. With 7.5% and 10% GRRSP incorporation, the bread experienced more significant sensory deterioration. The 90:10 blend recorded the lowest scores across all parameters, including 16.10 for crust color, 15.50 for texture, 14.20 for taste, and 6.00 for aroma. These reductions could be attributed to the darker color and possibly more intense or unfamiliar flavor of the radish seed powder at higher levels, which may not be preferred by panelists. In summary, while small amounts of GRRSP (up to 2.5–5%) can be incorporated into pan bread with minimal negative impact on sensory quality, higher levels (7.5–10%) significantly reduce consumer acceptability across all evaluated attributes. Dough extensibility dropped but resilience increased; breads with $\leq 10\%$ radish had acceptable sensory profiles, though flavor altered with higher levels. [43]

Table 6: Scores for sensory attributes of pan bread

Blends	Crust color (20)	Crumb color (20)	Crumb grain texture (20)	Symmetry of shape (10)	Taste (20)	Aroma (10)
WF: GRRSP 100:0	19.80 ^a ±0.10	19.70 ^a ±0.30	19.50 ^a ±0.40	9.50 ^a ±0.15	19.30 ^a ±0.20	9.80 ^a ±0.15
WF: GRRSP 97.5:2.5	18.50 ^b ±0.20	19.00 ^b ±0.45	18.30 ^b ±0.25	9.00 ^b ±0.20	18.00 ^b ±0.35	9.00 ^b ±0.20
WF: GRRSP 95:5	18.00 ^c ±0.30	18.20 ^c ±0.35	17.50 ^c ±0.15	8.50 ^c ±0.25	17.10 ^c ±0.30	8.00 ^c ±0.10
WF: GRRSP 92.5:7.5	17.30 ^d ±0.15	17.50 ^d ±0.20	17.00 ^d ±0.10	8.00 ^d ±0.15	15.30 ^d ±0.40	7.00 ^d ±0.20
WF: GRRSP 90:10	16.10 ^e ±0.40	16.40 ^e ±0.15	15.50 ^e ±0.25	7.00 ^e ±0.20	14.20 ^e ±0.30	6.00 ^e ±0.35

Each value was an average of twenty determination ± standard deviation

Different letters indicate to significant differences between raw materials in the same column(p≤0.05).

3.4. Colors of obtained pan bread

Table 7 presents the color parameters of pan bread samples, both for the crust and crumb, using the CIELAB color space values (L^* , a^* , b^*). The crust and crumb color of the control sample exhibited the highest lightness values (L^*), indicating a lighter appearance compared to the other treatments. Specifically, the control crust had an L^* value of 61.88, which gradually decreased across treatments from B1 to B4, reaching a minimum of 47.97 in B4. This trend suggests that the addition of the tested ingredient (e.g., germinated radish seed powder or another component) led to a darker crust color as the level increased. Regarding the a^* values (red-green axis), all treated samples exhibited higher redness than the control. For instance, B1 and B2 had the highest crust a^* values (17.79 and 18.16, respectively), indicating enhanced red tones. The b^* values (yellow-blue axis) for the crust also increased significantly in B1 (39.49), then slightly decreased in the subsequent treatments, but remained higher than the control, suggesting a yellower crust in the treated breads. In the crumb, the L^* values also declined with increasing levels of the added ingredient, from 65.28 in the control to 49.29 in B4, further confirming the darkening effect on internal bread color. Interestingly, the a^* values in the crumb initially decreased in B1, then increased significantly in B3 (12.59) and B4 (11.00), indicating more red color development at higher addition levels. Similarly, b^* values for crumb color increased markedly from 16.14 in the control to a peak of 28.47 in B3, reflecting greater yellowness, before decreasing in B4 to 19.43. Overall, the color data show that the incorporation of the ingredient resulted in a noticeable reduction in lightness and an increase in red and yellow hues in both crust and crumb, especially at higher substitution levels. These changes may be attributed to pigment compounds in the added material and Maillard browning reactions during baking. [23] Similar color changes have been discussed in the works of Mospah et al. [44] This may be related to the higher protein and phytochemical content of dough. While changes in protein during baking have also been related to changes in the lightness of baked products, specifically increased protein percentages in cooking led to darker muffins, pigments in phytochemicals may have raised the dark tone. [45]

Table 7: Colors of obtained pan bread

Samples	Crust color			Crumb color		
	L^*	a^*	b^*	L^*	a^*	b^*
Control	61.88 ±0.10	14.68 ±0.10	27.23 ±0.15	65.28 ±0.12	5.62 ±0.05	16.14 ±0.15
B1	58.34 ±0.15	17.79 ±0.15	39.49 ±0.20	62.41 ±0.15	3.87 ±0.09	17.38 ±0.10
B2	55.88 ±0.20	18.16 ±0.12	35.04 ±0.19	58.95 ±0.14	6.68 ±0.15	25.37 ±0.20
B3	52.02 ±0.25	16.19 ±0.10	34.33 ±0.05	53.73 ±0.17	12.59 ±0.05	28.47 ±0.12
B4	47.97 ±0.35	15.98 ±0.12	34.62 ±0.06	49.29 ±0.25	11.00 ±0.10	19.43 ±0.20

Each value was an average of three determination ± standard deviation

Different letters indicate to significant differences between raw materials in the same column(p≤0.05).

3.5. Physical parameters of pan bread

Table 8 shows the physical characteristics of pan bread produced using various blends of wheat flour (WF) and germinated red radish seed powder (GRRSP), with increasing substitution levels from 0% to 10%. The control sample (WF: GRRSP 100:0) exhibited the highest volume (1230 cm³) and specific volume (2.62 cm³/g),

along with the lowest hardness (1.23 K g/cm³), reflecting superior loaf expansion, crumb softness, and structural integrity typical of bread made solely with refined wheat flour. As the proportion of GRRSP increased from 2.5% to 10% (B1 to B4), a progressive decline in loaf volume and specific volume was observed. For instance, blend B1 (97.5:2.5) showed a volume of 1050 cm³ and specific volume of 2.35 cm³/g, while B4 (90:10) had the lowest volume at 550 cm³ and specific volume of 1.28 cm³/g. This reduction is likely due to the dilution of gluten-forming proteins, interruption in gluten network formation, and the presence of dietary fibers or bioactive compounds in GRRSP that may hinder gas retention and dough elasticity. The weight of the loaves decreased slightly with increased GRRSP addition-from 470 g in control to 430 g in B4-which may be linked to lower water absorption or retention capacity of the dough due to fiber-rich GRRSP. The hardness of bread crumb increased significantly with higher GRRSP content. The control was the softest (1.23 K g/cm³), while B4 was the firmest (2.72 K g/cm³). This trend reflects the negative impact of GRRSP on crumb softness, potentially due to its high fiber and phytochemical content which competes for water, making the crumb denser and less tender. In summary, while the incorporation of GRRSP enhances the functional and nutritional profile of bread, it adversely affects key physical parameters like volume, specific volume, and texture. Lower substitution levels (such as 2.5% or 5%) might offer a better balance between nutrition and acceptable baking quality. [43, 44]

Table 8: Physical parameters of pan bread

Blends	Blends	Volume (cm ³)	Weight (g)	Specific volume (cm ³ /g)	Hardness (Kg / cm ³)
WF: GRRSP (100:0)	Control	1230 ^a	470 ^a	2.62 ^a	1.23 ^e
WF: GRRSP (97.5:2.5)	B1	1050 ^b	446 ^b	2.35 ^b	1.88 ^d
WF: GRRSP (95:5)	B2	760 ^c	440 ^c	1.73 ^c	2.17 ^c
WF: GRRSP (92.5:7.5)	B3	615 ^d	436 ^d	1.41 ^d	2.40 ^b
WF: GRRSP (90:10)	B4	550 ^e	430 ^e	1.28 ^e	2.72 ^a

Each value was an average of three determination ± standard deviation

Different letters indicate to significant differences between raw materials in the same column(p≤0.05).

3.6. Influence of GRRSP on blood glucose level of normal and diabetic rats

Table 9 describes the effects of germinated red radish seeds powder (GRRSP) on blood glucose levels in normal and diabetic rats over a six-week period. At the beginning of the study, during the adaptation period, all groups showed similar blood glucose levels, representing no initial variations between them. After 72 hours, blood glucose levels increased sharply in all diabetic groups, confirming successful induction of diabetes, while the normal control group maintained stable glucose levels. Over the course of six weeks, significant improvements were observed in the diabetic groups treated with GRRSP. The reduction in blood glucose was clearly dose dependent. Rats receiving 2.5% GRRSP showed a moderate decrease, while those given 5%, 7.5%, and 10% GRRSP exhibited progressively greater reductions in glucose levels. The group treated with the highest dose (10%) showed the most pronounced improvement, with blood glucose levels decreasing to nearly half of the diabetic control group, which remained elevated throughout the study. Meanwhile, the normal control group's glucose levels remained stable across all time points. These findings indicate that GRRSP has a strong glucose-lowering effect in diabetic conditions and that its efficacy increases with dosage, making it a potentially valuable natural agent for managing hyperglycemia. [45]

Bioactive compounds contributing to the hypoglycemic effect include phenolic compounds like catechin, present in radish, which have demonstrated the ability to enhance insulin secretion. [46] Additionally, radish is rich in anthocyanins-powerful antioxidant flavonoids-that have shown promising effects in managing diabetic conditions. [47, 48] Various parts of the radish plant also exhibit antidiabetic potential. Specifically, radish seeds have been reported to improve insulin sensitivity and promote glucose uptake, while the leaves may inhibit intestinal glucose absorption. However, these findings still require validation through clinical research. [49]

Table 9: Influence of GRRSP on blood glucose level of diabetic rats

Treatment	Groups	After adaptation (mg/dl)	After 72h (mg/dl)	After 6 weeks (mg/dl)
Normal control (-)	G ₁	110 ^a ±9.00	105 ^b ±3.00	106 ^f ±2.00
Diabetic control (+)	G ₂	108 ^a ±5.84	350 ^a ±8.00	330 ^a ±9.00
2.5%GRRSP	G ₃	105 ^a ±7.00	345 ^a ±9.00	300 ^b ±8.00
5%GRRSP	G ₄	104 ^a ±8.00	355 ^a ±11.00	250 ^c ±5.00
7.5%GRRSP	G ₅	109 ^a ±5.84	347 ^a ±12.00	200 ^d ±4.00
10%GRRSP	G ₆	108 ^a ±6.84	360 ^a ±15.00	180 ^e ±4.00

GRRSP= Germinated red radish seeds powder

Each value was an average of five determination ± standard deviation.

Different letters indicate to significant differences between groups in the same column(p≤0.05)

3.7. Impact of GRRSP on some lipid parameters of diabetic rats

Table 10 illustrates the effects of germinated red radish seeds powder (GRRSP) on lipid parameters in diabetic rats. Diabetes significantly altered the lipid profile, as seen in the diabetic control group, which exhibited elevated levels of total cholesterol (195 mg/dl), triglycerides (240 mg/dl), LDL (157 mg/dl), VLDL (48 mg/dl), and total lipids (3.90 g/dl), alongside a marked decrease in HDL (35 mg/dl) compared to the normal control group. However, administration of GRRSP at increasing concentrations (2.5% to 10%) resulted in a dose-dependent improvement in these lipid abnormalities. Specifically, the 10% GRRSP group showed the most pronounced improvement, with total cholesterol reduced to 165 mg/dl, triglycerides to 170 mg/dl, LDL to 85 mg/dl, VLDL to 34 mg/dl, and total lipids to 2.50 g/dl. Concurrently, HDL levels rose significantly to 51 mg/dl, approaching the levels observed in the normal control group (60 mg/dl). These findings suggest that GRRSP supplementation effectively mitigates diabetes-induced dyslipidemia, likely through modulation of lipid metabolism. The progressive normalization of lipid parameters with higher GRRSP doses underscores its potential therapeutic value in managing diabetic complications associated with altered lipid profiles. [49-51] Overall, the data confirms the protective effects of germinated and fermented red radish seeds in mitigating oxidative damage, as supported by previous studies that emphasize the role of plant-based antioxidants in reducing lipid peroxidation and restoring antioxidant enzyme activity. [52]

Table 10: Impact of GRRSP on some lipid parameters of diabetic rats

Groups	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Total lipids (g/dl)
Normal control (-)	115 ^f ±1.20	125 ^f ±2.00	60 ^a ±0.80	40 ^f ±0.40	25 ^f ±0.50	1.30 ^f ±0.02
Diabetic control (+)	195 ^a ±1.50	240 ^a ±7.00	35 ^f ±0.20	157 ^a ±0.90	48 ^a ±0.60	3.90 ^a ±0.08
2.5%GRRSP	190 ^b ±1.40	210 ^b ±3.00	38 ^e ±0.40	130 ^b ±0.60	42 ^b ±1.00	3.60 ^b ±0.05
5%GRRSP	185 ^c ±1.20	190 ^c ±4.00	40 ^d ±0.50	112 ^c ±0.40	38 ^c ±2.00	3.00 ^c ±0.04
7.5%GRRSP	170 ^d ±2.00	180 ^d ±3.00	45 ^c ±0.20	99 ^d ±1.20	36 ^d ±1.00	2.90 ^d ±0.02
10%GRRSP	165 ^e ±2.00	170 ^e ±2.00	51 ^b ±1.20	85 ^e ±1.70	34 ^e ±0.80	2.50 ^e ±0.03

GRRSP= Germinated red radish seeds powder

Each value was an average of five determination ± standard deviation.

Different letters indicate to significant differences between groups in the same column(p≤0.05)

3.8. Impact of feeding GRRSP on liver functions in diabetic rats

Table 11 presents the impact of feeding germinated red radish seeds powder (GRRSP) on liver function enzymes in diabetic rats. The diabetic control group showed a significant increase in serum levels of liver enzymes ALT (GPT), AST (GOT), and ALP compared to the normal control group, indicating liver damage or dysfunction associated with diabetes. Specifically, ALT and AST levels rose to 60.55 U/L and 80.10 U/L, respectively, while ALP increased to 115.33 U/L in diabetic rats, compared to 38.00 U/L, 40.00 U/L, and 70.45 U/L in normal rats. Administration of GRRSP at varying doses resulted in a gradual and dose-dependent reduction in these elevated liver enzymes. The 10% GRRSP group showed the most pronounced improvement, with ALT reduced to 42.00 U/L, AST to 60.24 U/L, and ALP to 85.50 U/L, approaching normal levels. This suggests that GRRSP has a hepatoprotective effect in diabetic rats, potentially by reducing oxidative stress or inflammation associated with diabetes-induced liver injury. Overall, these findings highlight the beneficial role of GRRSP in improving liver function and mitigating hepatic damage in diabetic conditions, which aligns with findings that germination increases the bioavailability of phytochemicals such as flavonoids and phenolics, known to scavenge free radicals. This work confirms the great importance of applied science in bakery products for diabetes people and obese patients. [53-71]

Table 11: Impact of feeding GRRSP on liver functions in diabetic rats

Treatments	Groups	ALT (GPT) (U/L)	AST (GOT) (U/L)	ALP (U/L)
Normal control (-)	G1	38.00 ^f ±1.00	40.00 ^f ±1.40	70.45 ^f ±2.00
Diabetic control (+)	G2	60.55 ^a ±2.10	80.10 ^a ±2.60	115.33 ^a ±2.50
2.5%GRRSP	G3	56.20 ^b ±2.00	75.00 ^b ±2.00	110.22 ^b ±1.40
5%GRRSP	G4	51.11 ^c ±1.20	71.15 ^c ±1.00	100.09 ^c ±1.20
7.5%GRRSP	G5	47.30 ^d ±1.10	65.44 ^d ±1.25	95.30 ^d ±1.00
10%GRRSP	G6	42.00 ^d ±2.15	60.24 ^e ±1.50	85.50 ^e ±1.33

GRRSP= Germinated red radish seeds powder

Each value was an average of five determination ± standard deviation.

Different letters indicate to significant differences between groups in the same column(p≤0.05)

4. Conclusion

This study demonstrated the significant nutritional and therapeutic potential of germinated red radish seeds powder (GRRSP) as a functional ingredient in pan bread and as a dietary supplement for managing diabetic conditions. The proximate analysis confirmed that germination substantially enhances the protein, fiber, and mineral content of radish seeds while reducing fat and carbohydrate content, making GRRSP a nutritionally dense component. When partially substituted into wheat flour, GRRSP improved the chemical composition of pan bread by increasing protein, fiber, ash, and fat content. However, higher inclusion levels (above 5%) adversely affected bread's sensory and physical qualities, including volume and texture, likely due to reduced gluten strength and increased fiber. Biological evaluations in diabetic rats revealed that dietary GRRSP significantly reduced blood glucose levels, improved lipid profiles, and mitigated liver dysfunction in a dose-dependent manner. The hypoglycemic and protective effects are likely attributed to the elevated levels of phenolics, flavonoids, and antioxidants generated during the germination process. These bioactive compounds may enhance insulin sensitivity, reduce oxidative stress, and support hepatic function. In summary, GRRSP is a valuable functional ingredient with dual benefits-enhancing the nutritional profile of bakery products and offering therapeutic effects against diabetes-related complications. Incorporation at levels up to 5% is optimal for maintaining desirable bread quality while delivering health benefits. Further research, including human clinical trials, is recommended to validate the antidiabetic potential and broaden the application of GRRSP in functional food formulations.

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6. Institutional Review Board Statement

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7. Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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9. Conflicts of Interest

No conflict of interest was reported by all authors.

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