

Evaluation of Chemical and Nutritional Changes in Thermally Processed and Frozen Grains and Their Effects on Blood Profile and Lipid Parameters in Male Rats

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Abstract

This study was conducted in the laboratories of the College of Agriculture at the University of Tikrit during the academic year 2024–2025. The aim of the study was to utilize wheat, oats, amber rice, potatoes, and white barley in three different treatments—unfrozen, frozen for one month, and frozen for two months—as supplementary components in the formulation of experimental diets for laboratory rats, in order to assess their effects on hemoglobin levels and various blood parameters. The results showed that wheat, under the one-month freezing treatment, produced the highest hemoglobin concentration, reaching $13.9 \pm 1.69a$. Amber rice, also subjected to one month of freezing, yielded the highest red blood cell (RBC) count, recorded at $7.42 \pm 1.29a$. As for the RBC count across treatments, unfrozen barley showed superiority, with a value of $5.0 \pm 0.27a$. In terms of biochemical parameters, unfrozen potatoes resulted in the highest triglyceride level among control treatments, reaching $159 \pm 7.22b$, and also led to the highest total cholesterol level, recorded at $166 \pm 4.69a$. Wheat subjected to one month of freezing showed the highest high-density lipoprotein (HDL) concentration at $72 \pm 2.28a$, while unfrozen potatoes yielded the highest low-density lipoprotein (LDL) level at $129 \pm 3.55a$. Additionally, unfrozen potatoes gave the highest aspartate aminotransferase (AST) level in the control group at $96.6 \pm 6.01a$, and barley under freezing treatment in the control group recorded the highest alanine aminotransferase (ALT) level at $172.9 \pm 7.51a$. Unfrozen potatoes also recorded the highest alkaline phosphatase (ALP) activity in the control group at $311.9 \pm 9.48a$. Regarding C-reactive protein (CRP), unfrozen amber rice demonstrated the highest level at $1.62 \pm 0.02a$ among all treatments.

Key words: Complementary foods, sprouted legumes, wheat germ .

.1 Introduction

In recent years, increasing attention has been directed toward understanding the impact of food processing and storage methods on metabolic health. Freezing, as a common preservation technique, may influence the nutritional and biochemical properties of food, thereby affecting physiological responses upon consumption. Certain staple foods such

as potatoes, barley, wheat, oats, and amber rice form a significant part of human and animal diets and are often subject to freezing during storage. However, the potential metabolic consequences of prolonged freezing on these foods remain underexplored. Cereals and their products constitute a fundamental source of human nutrition, providing a

substantial portion of daily requirements for energy, proteins, and carbohydrates. With advancements in food preservation technologies, freezing has gained increasing importance as an effective method for maintaining the quality of food products, including cereal-based items Ghassan [1.]

Recent studies indicate that frozen products can retain a significant portion of their nutritional value; however, prolonged storage may lead to chemical and physical alterations that negatively impact final product quality Kaur [2.]

These changes include reductions in vitamin and mineral content due to oxidation or chemical degradation, as well as deterioration in sensory attributes such as texture and flavor. Therefore, examining the effects of freezing and different storage durations is crucial to understanding the relationship between these changes and preservation methods, despite the well-established advantages of freezing Singh [3.]

Although certain processing methods applied to cereals may reduce the levels of several phytochemicals, other techniques such as sprouting and fermentation have been shown to enhance their concentration—particularly polyphenols and folic acid—or increase their bioavailability. Moreover, these processes are associated with improved antioxidant capacity Pradeep [4]. This study aims to investigate the effect of freezing duration of selected food items—including potatoes, barley, wheat, oats, and amber rice—on insulin levels in white rats, as well as their insulin and glucose sensitivity, in addition to evaluating associated blood parameters.

2 Materials and Methods

2.1 Sample sources

2.1.1 Wheat

Wheat samples were obtained from Tikrit Silo, and the seeds were of local origin.

2.1.2 White Barley

White barley was sourced from the local markets of Tikrit, and was locally grown.

2.1.3 Oats

Oats were acquired from the local markets of Tikrit, also of local origin.

2.1.4 Potatoes

Potatoes were obtained from the local markets of Tikrit.

2.1.5 Amber Rice

Amber rice was sourced from the local markets of Tikrit.

2.2 Experimental Animals

Male albino rats, aged between 10 to 12 weeks and weighing between 150 and 180 grams, were used in this study. A total of 45 male rats were utilized, distributed into three groups of 15 animals each, corresponding to the stages of pre-freezing, freezing for one month, and freezing for two months. The animals were obtained from the Animal House of the College of Veterinary Medicine – University of Tikrit. All rats underwent thorough medical examinations by a specialized veterinarian at the center to ensure they were healthy and free from any diseases prior to their use in the experiment.

2.3 Complete Blood Count (CBC)

The Complete Blood Count (CBC) test was conducted using a Spanish-made Hematology Analyzer. This device is an automated multi-parameter analyzer designed for use in diagnostic testing within the clinical laboratory. A blood sample of 0.5 ml was collected and placed in special tubes used for complete blood count tests, containing EDTA, an anticoagulant. The tube was then gently shaken and placed into the analyzer for testing. The device draws a specific amount of

blood and performs the analysis. The data was processed and read via a computer connected to the Hematology Analyzer. The CBC parameters included White Blood Cells (WBC) and Red Blood Cells (RBC).

2.4 Storage Conditions:

The food samples were stored at a temperature of -18°C in sealed plastic containers for durations of one and two months. After the storage period, the samples were administered to the experimental rats.

2.5 Determination of Total Serum Cholesterol Concentration

A Spanish-made BioSystem kit was used to measure the total cholesterol concentration in blood serum. The enzymatic method employed in this kit converts cholesterol and cholesterol esters into a red-colored quinone pigment through the following reactions:

Using a spectrophotometer, the absorbance of the resulting red complex was measured after calibrating the device at a wavelength of 500 nm. The total cholesterol concentration was then calculated using the appropriate equation [5]

2.6 High-Density Lipoprotein Cholesterol (HDL-C)

A Spanish-made BioSystem kit was used. The principle of the method is based on enzymatic reactions that involve the quantitative precipitation of low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and chylomicrons by the addition of phosphotungstic acid in the presence of magnesium ions (Mg^{2+}). The separation is carried out using a centrifuge, where the supernatant contains only HDL-C. The HDL-C concentration is then determined using a cholesterol enzymatic reagent.

The absorbance of the reaction product is measured using a spectrophotometer, calibrated at a wavelength of 500 nm. The

HDL-C concentration is then calculated Rifai [7]

2.7 Low-Density Lipoprotein Cholesterol (LDL-C)

The LDL-C concentration was calculated using the method described by Mustafa [6] as shown in the following equation:

$$\text{LDL mg / dl} = \text{Total cholesterol} - (\text{TG}/5) + \text{HDL}$$

Where:

- Total Cholesterol is the total serum cholesterol concentration,
- TG is the triglyceride concentration,
- HDL is the high-density lipoprotein cholesterol concentration.

2.8 Determination of Serum AST and ALT Enzyme Activities

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were determined using commercially available diagnostic kits provided by the Canadian company Randox. For each sample, two test tubes were used: the first tube received the blank reagent, while the second tube was designated for the sample to be analyzed for enzymatic activity. The procedure was carried out according to the concentrations and protocols recommended by the manufacturer (Goldberg).

2.9 Determination of C-Reactive Protein (CRP) in Laboratory Rats

The concentration of C-reactive protein (CRP) in rat serum was measured using an enzyme-linked immunosorbent assay (ELISA) kit specific for rat CRP, following the manufacturer's instructions. Blood samples were collected from the experimental rats, allowed to clot at room temperature, and then centrifuged at 3000 rpm for 10 minutes to separate the serum. The obtained serum was stored at -20°C until analysis. CRP levels

were quantified by measuring the optical density using a microplate reader at a wavelength of 450 nm. The concentrations were calculated based on a standard curve generated using known CRP standards provided with the kit.

3. Results and discussion

3.1 Measurement of Hematological Parameters in Laboratory Rats

Table (1) shows that the values of white blood cells (WBCs) increased in rats fed different types of carbohydrate-rich foods stored frozen for one month — including potato, amber rice, barley, oats, and wheat — with recorded values of (4.8 ± 0.15^a , 4.7 ± 0.12^a , 4.6 ± 0.15^b , 4.6 ± 0.14^b , and 4.5 ± 0.09^b), respectively. These were compared with the pre-freezing values (4.5 ± 0.19^b , 4.3 ± 0.07^b , 5.0 ± 0.27^a , 4.1 ± 0.05^c , and 4.7 ± 0.22^a), respectively.

Awad [8] reported a significant decrease in total white blood cell counts, particularly neutrophils, in malnourished animals compared to the control group, while lymphocyte counts were elevated in malnourished rats.

Al-Fahdawy, [9] indicated that the normal range of WBCs is between 4,000–11,000 cells/mm³ of blood. This count tends to decrease in cases of malnutrition, chronic illnesses, or exposure to toxic drugs. The marked increase in WBC count during inflammatory conditions is due to the presence of a plasma substance that stimulates the bone marrow to produce more defensive white blood cells. This substance is known as the leucocytosis-inducing factor Tobin [10] noted that WBCs play a defensive role and their numbers typically rise in bacterial, fungal, and viral infections, as well as in inflammatory conditions.

Table (1): Evaluation of blood profile parameters in laboratory rats fed different types of frozen carbohydrate-rich foods for two months

Hb	RBCs	WBCs	Freezing Treatment Duration	Type of Samples
mg/dl	×106/ml			
12.8±1.03a	6.12±1.10b	4.5±0.19b	zero	Potato
13.5±2.17a	7.30±0.34a	4.8±0.15a	S1	
12.4±1.31b	5.91±0.94b	4.6±0.07b	S2	
13.0±1.65a	6.35±0.91b	4.3±0.07b	zero	Amber rice
12.7±2.19b	6.22±1.22bc	4.7±0.12a	S1	
12.3±1.25b	5.86±1.16b	4.3±0.12b	S2	
12.6±1.18b	5.38±0.84b	5.0±0.27a	zero	Barley
12.8±2.03a	5.95±1.24b	4.6±0.15b	S1	
13.1±1.76a	6.14±1.03b	4.5±0.10b	S2	
12.2±2.22b	5.79±1.11c	4.1±0.05c	zero	Oats
12.4±2.45b	6.04±1.27b	4.6±0.14b	S1	
12.9±1.87a	6.33±0.83b	4.4±0.08b	S2	
13.3±1.92a	6.80±1.03b	4.7±0.22a	zero	Wheat
13.9±1.69a	7.42±1.29a	4.5±0.09b	S1	
12.8±1.03a	6.12±1.10b	4.5±0.19b	S2	

Zero: Indicates no freezing. S1: Indicates freezing for one month. S2: Indicates freezing for two months.

Different letters within the same column indicate statistically significant differences between the means at a significance level of $p \leq 0.05$.

= $\pm$ Standard Error (SE).

It was observed that RBC (Red Blood Cell) counts increased in rats fed various types of carbohydrate-rich foods frozen for two months — including potatoes, amber rice, barley, oats, and wheat — with values of (5.91 ± 0.94^b , 5.86 ± 1.16^b , 6.14 ± 1.03^b , 6.33 ± 0.83^b , and 6.12 ± 1.10^b), respectively. These values were compared to the pre-freezing group values of (3.7 ± 2.57^g , 3.6 ± 1.82^g , 5.5 ± 2.78^d , 5.7 ± 2.72^d , and 6.1 ± 2.06^c). This increase may be attributed to the body's need to maintain a specific normal RBC level, which varies from one organism to another, or it may be due to iron or vitamin B12 deficiency, or poor intestinal absorption of these nutrients.

Lopez [11] reported that feeding experimental animals with high-quality amino acids improved appetite and helped treat anemia. Similarly, Abdel Hamed. [12] found that vitamin C plays an important role in maintaining red blood cell vitality. Administering 200 mg of vitamin C to experimental animals contributed to protecting RBC membranes from damage.

The results also showed that hemoglobin (Hb) values increased in rats fed carbohydrate-rich foods frozen for two months — potatoes, amber rice, barley, oats, and wheat — with values of (12.4 ± 1.31^b , 12.3 ± 1.25^b , 13.1 ± 1.76^a , 12.9 ± 1.87^a , and 12.8 ± 1.03^a), respectively. These were compared with pre-freezing values of (12.8 ± 1.03^a , 13.0 ± 1.65^a , 12.6 ± 1.18^b , 12.2 ± 2.22^b , and 13.3 ± 1.92^a).

Nelson [13] indicated that an increase in Hb reflects a high-quality protein source in the

diet, which helps meet nutritional requirements. The elevation in hematological parameters may also be due to the presence of essential minerals in the proteins, especially iron, which plays a key role in stimulating red blood cell production and increasing hemoglobin concentration.

These findings are consistent with those of Awad [8] who induced malnutrition in rats over a three-week period. The Hb concentration in malnourished rats was reported to be 10.03 g/dL, compared to 11.1 g/dL in the control group.

3.2 Measurement of Blood Lipid Profile Parameters in Laboratory Rats

Table (2) shows that triglyceride levels decreased in rats fed different types of carbohydrate-based foods frozen for two months — including potato, amber rice, barley, oats, and wheat — with values of (145 ± 3.74^d , 141 ± 4.51^e , 139 ± 4.12^e , 145 ± 4.71^d , and 91 ± 4.01^g), respectively. These values were lower than the pre-freezing values of (159 ± 7.22^b , 162 ± 5.17^a , 152 ± 5.47^c , 151 ± 4.82^c , and 157 ± 8.53^b), respectively.

The elevated triglyceride levels in the control group may be attributed to reduced insulin secretion, which leads to decreased activity of lipoprotein lipase (LPL) — an enzyme that plays a crucial role in hydrolyzing triglycerides into fatty acids and glycerol, which are then absorbed by adipose tissue

Brown [14] Additionally, insulin deficiency may lead to enhanced lipolysis in tissues and a decreased rate of VLDL (Very Low-Density Lipoprotein) cholesterol utilization, both of which contribute to the increase in serum triglycerides Clarke [15]

Furthermore, it was observed that total cholesterol levels also decreased in rats fed frozen carbohydrate sources for two months — including potato, amber rice, barley, oats, and wheat — with values of (141 ± 3.45^e , 147 ± 4.55^d , 122 ± 3.44^i , 122 ± 4.78^i , and 121 ± 3.88^i), respectively. These were significantly lower than the pre-freezing levels of (166 ± 4.69^a , 158 ± 4.25^b , 135 ± 3.50^f , 134 ± 3.37^f , and 137 ± 4.15^f), respectively.

These findings are consistent with those of [15] and the observed elevation in cholesterol in the control group may be due to increased activity of the enzyme cholesterol acyl-transferase, which facilitates the absorption of cholesterol in the intestines and is activated under low insulin conditions Kaur [16] Moreover, as reported by Nopparat [17] the elevation of cholesterol levels in diabetic conditions could be attributed to slowed metabolic processes, where there is an imbalance between catabolic and anabolic activities, favoring catabolism, which ultimately results in increased lipid accumulation and elevated serum cholesterol.

Table 2 Blood lipid profile parameters in laboratory rats fed with various types of frozen carbohydrate-based foods for two months

Low-Density Lipoproteins (LDL)	High-Density Lipoproteins (HDL)	Total Cholesterol	Triglycerides	Freezing Treatment Duration	Type of Samples
g/kg	g/kg	Mg/dl	(unit/mL)		
129 ± 3.55^a	37 ± 2.57^g	166 ± 4.69^a	159 ± 7.22^b	zero	Potato
92 ± 3.34^d	48 ± 2.95^c	140 ± 4.94^e	151 ± 4.52^c	S1	
85 ± 2.75^e	56 ± 2.37^d	141 ± 3.45^e	145 ± 3.74^d	S2	
122 ± 3.65^b	36 ± 1.82^g	158 ± 4.25^b	162 ± 5.17^a	zero	Amber rice
105 ± 3.74^c	46 ± 3.04^e	151 ± 5.22^c	152 ± 6.68^c	S1	
107 ± 4.21^c	40 ± 2.86^f	147 ± 4.55^d	141 ± 4.51^e	S2	
80 ± 3.85^f	55 ± 2.78^d	135 ± 3.50^f	152 ± 5.47^c	zero	Barley
65 ± 3.17^h	62 ± 2.25^c	127 ± 3.00^h	150 ± 4.91^c	S1	
60 ± 2.79^i	62 ± 2.13^c	122 ± 3.44^i	139 ± 4.12^e	S2	
77 ± 3.18^g	57 ± 2.72^d	134 ± 3.37^f	151 ± 4.82^c	zero	Oats
65 ± 2.61^h	65 ± 3.15^b	130 ± 3.17^g	146 ± 4.83^d	S1	
55 ± 3.65^i	67 ± 2.78^b	122 ± 4.78^i	145 ± 4.71^d	S2	
76 ± 4.10^g	61 ± 2.06^c	137 ± 4.15^f	157 ± 8.53^b	zero	Wheat
53 ± 3.02^l	72 ± 2.28^a	125 ± 4.79^h	95 ± 3.53^f	S1	
51 ± 2.60^k	70 ± 2.17^a	121 ± 3.88^i	91 ± 4.01^g	S2	

Zero: Indicates no freezing. S1: Indicates freezing for one month. S2: Indicates freezing for two months.

Different letters within the same column indicate statistically significant differences between the means at a significance level of $p \leq 0.05$.

= $\pm$ Standard Error (SE). (

It was observed that the levels of high-density lipoproteins (HDL-C) increased in rats fed carbohydrate-based diets frozen for two months, including potato, amber rice, barley, oats, and wheat, with values of (56 ± 2.37^d , 40 ± 2.86^f , 62 ± 2.13^c , 67 ± 2.78^b , and 70 ± 2.17^a), respectively, compared to pre-freezing levels of (37 ± 2.57^g , 36 ± 1.82^g , 55 ± 2.78^d , 57 ± 2.72^d , and 61 ± 2.06^c). Awad [8] reported that when rats were fed a protein-free diet, their cholesterol, triglycerides, HDL, and LDL levels were approximately 94.40, 145.80, 29.60, and 43.40 mg/dL, respectively. They noted elevated cholesterol, triglycerides, and LDL levels alongside a decrease in HDL. This was attributed to feeding the rats a high-fat, protein-free diet, which increased lipid levels in the animals and raised the level of "bad" cholesterol at the expense of "good" cholesterol due to impaired metabolic processes.

Similarly, low-density lipoproteins (LDL-C) levels increased in rats fed the frozen carbohydrate diets for two months, with values of (85 ± 2.75^e , 107 ± 4.21^c , 60 ± 2.79^i , 55 ± 3.65^l , and 51 ± 2.60^k), compared to pre-freezing values of (129 ± 3.55^a , 122 ± 3.65^b , 80 ± 3.85^f ,

77 ± 3.18^g , and 76 ± 4.10). Alamy and Bengelloun [18] pointed out that protein deficiency leads to lipid oxidation, which increases LDL levels at the expense of HDL due to oxidation. Furthermore, oxidation of cortical proteins occurs, where protein plays an important role as an antioxidant

3.3 Measurement of Liver Enzymes in the Blood of Laboratory Rats

As shown in Table (3), the levels of AST (aspartate aminotransferase) significantly decreased in the animals fed on carbohydrate-based foods—specifically potatoes, amber rice, barley, oats, and wheat—after two months of freezing. The recorded values were (64.1 ± 6.31^g , 55.3 ± 2.26^l , 58.4 ± 4.25^k , 61.8 ± 5.01^h , and 58.1 ± 2.59^k), respectively, compared to the pre-freezing levels which were (96.6 ± 6.01^a , 75.2 ± 3.57^d , 80.3 ± 3.71^c , 71.6 ± 3.37^e , and 73.2 ± 3.60^e), respectively. This reduction may indicate a decreased likelihood of conditions such as myocardial infarction, infectious hepatitis, acute pancreatitis, or inflammations of the lungs or brain (Buckley and Howe ([18]

Table 3: Liver Enzyme Parameters in the Blood of Laboratory Rats Fed on Various Types of Frozen Carbohydrate-Based Foods for Two Months

ALP (unit/L)	ALT (unit/L)	AST (unit/L)	Freezing Treatment Duration	Type Samples of
311.9±9.48a	171.4±7.26a	96.6±6.01a	zero	Potato
226.5±8.22c	150.8±5.51c	94.5±5.25b	S1	
125.4±5.11h	97.3±8.46k	64.1±6.31g	S2	
245.2±9.31b	111.3±5.11g	75.2±3.57d	zero	Amber rice
132.6±5.72g	80.5±6.30l	64.7±5.28g	S1	
85.8±6.84n	51.7±3.04m	55.3±2.26l	S2	
246.5±8.71b	172.9±7.51a	80.3±3.71c	zero	Barley
185.7±7.46f	162.5±8.22b	75.1±4.06d	S1	
103.2±9.81l	103.3±4.37h	58.4±4.25k	S2	
209.7±6.21e	145.5±5.27d	71.6±3.37e	zero	Oats
126.6±8.75h	126.4±4.80f	68.5±2.41f	S1	
97.5±4.73m	105.2±3.75h	61.8±5.01h	S2	
217.3±7.56d	159.7±6.33b	73.2±3.60e	zero	Wheat
173.2±6.88h	130.2±5.89e	68.4±4.27f	S1	
124.7±5.49h	112.5±5.74g	58.1±2.59k	S2	

Zero: Indicates no freezing. S1: Indicates freezing for one month. S2: Indicates freezing for two months.

Different letters within the same column indicate statistically significant differences between the means at a significance level of $p \leq 0.05$.

= ±Standard Error (SE).

Furthermore, the levels of ALT (alanine aminotransferase) also decreased in the animals fed on carbohydrate-based foods—potatoes, amber rice, barley, oats, and wheat—after two months of freezing. The recorded post-freezing values were (97.3±8.46k, 51.7±3.04m, 103.3±4.37h, 105.2±3.75h, and 112.5±5.74g), respectively, compared to the pre-freezing values which were (171.4±7.26a, 111.3±5.11g, 172.9±7.51a, 145.5±5.27d, and 159.7±6.33b), respectively. The elevated ALT levels observed in the control group are indicative of acute hepatitis or hepatic cell necrosis, which may result from malnutrition affecting liver function (Buckley and Howe [18]

In addition, ALP (alkaline phosphatase) levels also decreased in the animals fed on carbohydrate-based foods—potatoes, amber rice, barley, oats, and wheat—after two months of freezing. The recorded values were (125.4±5.11h, 85.8±6.84n, 103.2±9.81l, 97.5±4.73m, and 124.7±5.49h), respectively, compared to the pre-freezing values which were (311.9±9.48a, 245.2±9.31b, 246.5±8.71b, 209.7±6.21e, and 217.3±7.56d), respectively. The elevated ALP levels observed in the control group may be attributed to continuous tissue and cell growth, or may indicate conditions such as bone diseases or bile duct obstruction (Buckley and Howe [18]

3.4 Measurement of C-Reactive Protein (CRP) in the Blood of Laboratory Rats

Table (4) shows that the levels of C-reactive protein (CRP) decreased in animals fed on carbohydrate-based foods—potatoes, amber rice, barley, oats, and wheat—after two months of freezing. The post-freezing values were ($1.20 \pm 0.01f$, $1.35 \pm 0.01e$, $1.30 \pm 0.01e$, $1.40 \pm 0.01d$, and $1.43 \pm 0.01c$), respectively,

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compared to the pre-freezing values which were ($1.46 \pm 0.01c$, $1.62 \pm 0.02a$, $1.40 \pm 0.01d$, $1.54 \pm 0.02b$, and $1.45 \pm 0.01c$), respectively. This decrease in protein activity may be attributed to the depletion of low-density lipoprotein (LDL) levels and LDL particles, as observed in statin treatment (Buckley and Howe[18

Table 4: Levels of C-Reactive Protein (CRP) in the Blood of Laboratory Rats Fed on Various Types of Frozen Carbohydrate-Based Foods for Two Months

C-reactive protein (CRP) u/I	Freezing Treatment Duration	Type of Samples
$1.46 \pm 0.01c$	zero	Potato
$1.38 \pm 0.01d$	S1	
$1.20 \pm 0.01f$	S2	
$1.62 \pm 0.02a$	zero	Amber rice
$1.30 \pm 0.01e$	S1	
$1.35 \pm 0.01e$	S2	
$1.40 \pm 0.01d$	zero	Barley
$1.33 \pm 0.01e$	S1	
$1.30 \pm 0.01e$	S2	
$1.54 \pm 0.02b$	zero	Oats
$1.44 \pm 0.01c$	S1	
$1.40 \pm 0.01d$	S2	
$1.45 \pm 0.01c$	zero	Wheat
$1.43 \pm 0.01c$	S1	
$1.43 \pm 0.01c$	S2	

Zero: Indicates no freezing, S1: Indicates freezing for one month, S2: Indicates freezing for two months.

Different letters within the same column indicate statistically significant differences between the means at a significance level of $p \leq 0.05$.

= $\pm$ Standard Error (SE).

4. Conclusions

1. The results of this study demonstrated the potential of using potatoes, amber rice, oats, barley, and wheat in the preparation of more effective diets to elevate blood glucose levels in diabetic rats.

2. It was found that the two-month freezing process contributed to an increase in certain pathological indicators, such as insulin sensitivity and C-reactive protein (CRP) levels.

3. Additionally, the two-month freezing period led to a reduction in serum triglyceride and total cholesterol levels, as well as a decrease in

.5Recommendations

.1 Use other types of grains and legumes to prepare dietary blends for rats that help reduce the risk of developing diabetes.

liver enzyme activity in the blood of laboratory rats.

.2 Explore longer freezing periods to better understand the potential benefits and drawbacks of extended freezing.

.3 Use different ingredients to create a variety of pleasant and appealing flavors.

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