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Health benefits and digestive properties of Ca²⁺-regulated sodium alginate from an endogenous method in buckwheat noodles

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Abstract

The present study investigated the impact of Ca^{2+} regulated sodium alginate gelation on buckwheat noodle digestion, as well as its physiological and biochemical effects on rat. An endogenous method was employed for noodle preparation, and the rate of starch hydrolysis was evaluated. Buckwheat noodles prepared using this approach exhibited significantly reduced rates of starch digestion compared to conventional methods. Increased concentrations of sodium alginate and Ca²⁺ led to the formation of dense gel networks that promoted weight gain in diabetic rats while simultaneously lowering postprandial blood glucose levels and improving glucose intolerance and abnormal insulin tolerance. Moreover, these gel networks enhanced liver glycogen synthesis by increasing SOD and CAT activities while reducing the levels of ALT and MDA, thereby mitigating morphological damage in the liver. Buckwheat noodles prepared using this endogenous method were found to potentially exhibit hypoglycemic effects and mitigate complications associated with type 2 diabetes. **Keywords:** buckwheat noodles, in vitro simulated digestion, sodium alginate, type 2

diabetes

Graphical Abstract



1. Introduction

Starch forms a major part of human diets and is hydrolyzed by amylase to produce glucose which can then be absorbed. However, they can easily undergo hydrolysis when entering the digestive system, thus accelerating the rate of starch digestion ^[1, 2]. This can result in disorders such as diabetes and obesity, and demonstrates the importance of eating a healthy diet ^[3].

Diabetes mellitus (DM) is characterized by disrupted glucose metabolism. Type 2 DM (T2DM) is the most common form of the disease ^[4]. T2DM is frequently accompanied by various complications, including hyperglycemia, hyperlipidemia, retinopathy, and cardiovascular disease ^[5]. The development of the disease is influenced not only by genetic and environmental factors but also by diet and lifestyle, including factors such as insufficient exercise, high-energy diets, and obesity ^[6]. T2DM is most frequently treated with drugs, together with diet and exercise recommendations ^[7]. Thus, the development of functional foods that are able to lower blood sugar levels and alleviate diabetic complications is extremely important. Polysaccharides are known to be effective for controlling glucose levels, as well as having antioxidant, anti-inflammatory, and immunomodulatory effects, and promoting the health of the gut microbiota ^[7, 8].

Buckwheat, indigenous to southwestern China, boasts a rich history of cultivation and extensive distribution in this region ^[9]. As a staple cereal commonly

incorporated into daily diets, buckwheat is distinguished by its high content of dietary fiber, polyphenols, and flavonoids. Studies have demonstrated that its consumption can serve as a preventive measure against various chronic diseases ^[10]. Therefore, buckwheat has attracted considerable attention within the food industry. Notably, buckwheat noodles, prized for their superior nutritional value, have gained significant popularity in both Asia and Europe.

Sodium alginate is a naturally occurring polysaccharide found in brown algae and consists of varying amounts of β -D-mannuronic acid and α -L-guluronic acid ^[11]. The compound is strongly hydrophilic and hygroscopic. The incorporation of divalent cations, particularly Ca²⁺ but not magnesium or mercury, results in the formation of an alginate gel with good tensile strength. Calcium ions selectively induce the formation of stable "egg-box" gel networks via specific interchain bridging. In contrast, magnesium ions demonstrate limited crosslinking capacity owing to their distinct hydration properties, while mercury ions predominantly result in nonspecific precipitation ^[11, 12]. These characteristics have enabled the wide use of sodium alginate in food production. In addition, many studies have shown that sodium alginate has antitumor and antihypertensive properties and is also effective for reducing blood lipid and glucose levels ^[13, 14]. It is also possible that it may regulate the intestinal microbiota. It is thus potentially useful for treating T2DM. For instance, a clinical trial by Paxman et al. showed that diets containing seaweed polysaccharides improved both lipid levels and inflammation in overweight participants^[15].

Our earlier studies compared the effects of Ca^{2+} regulated sodium alginate gelation using both endogenous and exogenous preparation on the quality and digestibility of buckwheat noodles, finding that the rate of digestion was reduced in endogenously prepared noodles ^[16]. This work hypothesizes that the Ca^{2+} -regulated sodium alginate network has two effects: (1) Encapsulating starch granules to reduce enzymatic hydrolysis and postprandial glycemic spikes; (2) Serving as a prebiotic in the colon to promote beneficial microbiota and increase short-chain fatty acid (SCFA) production, which links gut health with glucose and lipid metabolism. Thus, noodles prepared using the same method were used here to explore the mechanism by which Ca^{2+} regulated sodium alginate inhibits the rate of starch digestion, as well as the *in vivo* modulation of the metabolism of diabetic rats after administration of a high-fat diet. The findings offer a theoretical foundation for the development and use of Ca^{2+} regulated sodium alginate in functional foods for the alleviation of hyperglycemia.

2. Materials and methods

2.1. Materials and chemical reagents

Pure buckwheat flour was obtained from Dalian Hongrun Whole Grain Food *Co. Ltd.* (Dalian, China). Sodium alginate (CAS No. 9005-38-3, Mw = 86,537 g/mol, viscosity = 200 ± 20 mPa·s, G/M ratio of 1, drying loss rate $\leq 15\%$) was purchased from Aladdin Science and Technology *Co. Ltd.* (Shanghai, China). Calcium carbonate (CAS No. 471-34-1, Mw = 100.09 g/mol), pepsin (1:3000), a-amylase (100000 U/mL), pancreatin (1:4000), and glucoamylase (15 U/mL) were obtained from Shanghai Yuanye Bio Biotechnology *Co. Ltd.* (Shanghai, China). The remaining reagents were purchased from the Beijing Chemical Reagent *Co. Ltd.* (Beijing, China). All reagents were of analytical grade.

2.2. Noodle preparation

In the control group, 90 g of buckwheat flour was mixed with 50 g of deionized water. For the comparison groups, the noodle dough was prepared by combining 90 g of buckwheat flour, 50 g of sodium alginate solution at varying concentrations (0.1%, 0.3%, 0.5%, and 0.7% w/w), and calcium carbonate at concentrations of 3%, 6%, or 9% (w/w), corresponding to 4.33 g, 8.94 g, and 13.85 g in its dry weight, respectively. In the control group, 90 g of buckwheat flour was mixed with 50 g of deionized water. The dough was mixed well for 15 min with a mixer (HMJ-A35M1, Guangdong, China) and allowed to stand for a further 15 min before sheeting with a noodle machine (MR-08, Guangdong). Noodles (6.0 mm width and 2.0 mm thickness) were cut and cooked in citric acid with a pH of 4.0. The specific cooking method is described in the previous article ^[16]. The reaction between citric acid and calcium carbonate produces endogenous Ca²⁺, which subsequently engages in cross-linking with sodium alginate present in the noodles.

Homemade feed 1: Noodles were prepared as above, using 90 g of buckwheat flour with 50 g of 0.7% sodium alginate solution. The noodles were cooked at 100 $^{\circ}$ C for 3 min and 40 s, as established in our previous research ^[16,17].

Homemade feed 2: The noodles were prepared using 90 g of buckwheat flour with $CaCO_3$ powder (9% of the dough) with the addition of 50 g of 0.7% sodium alginate solution. Noodles were prepared and cooked as for homemade feed 2.

These two types of homemade feed were given to experimental groups 1 and 2, respectively, in the animal experiments.

2.3. Animals and experimental design

Animals: Forty-five SD rats (male, 6–8 weeks old, 180–220 g) were acquired from the Hubei Provincial Laboratory Animal Research Center. After acclimatization for one week, the animals were fed high-fat diets together with the injection of 30 mg/kg streptozotocin (STZ) to induce T2DM. Fasting blood glucose (FBG) levels of over 11.1 mM/L on day 7 following the injection indicated the successful establishment of hyperglycemia.

Experimental Design. Four groups of rats were set up, namely, the blank, control, experimental 1, and experimental 2 groups. The rats were fed freely for three weeks, after which three animals were randomly chosen and analyzed. All rats used in the study were euthanized by anaesthesia (50 mg/kg pentobarbital sodium) and exsanguination.

The groups were fed as follows:

(i) Blank: Non-hyperglycemic rats, fed a normal diet (prepared according to the GB 14924.3-2010 standard).

(ii) Control: Hyperglycemic rats, fed a normal diet (prepared according to the GB 14924.3-2010 standard).

(iii) Experimental group 1: Hyperglycemic rats, fed homemade feed 1.

(iv) Experimental group 2. Hyperglycemic rats, fed homemade feed 2.

The rats were weighed weekly. The feces were collected one day before euthanasia and were stored at -80° C; all rats were fasted for 12 h before euthanasia. Blood samples were collected and centrifuged (3000 g, 10 min), and the liver and pancreas were harvested and immediately frozen at -80° C.

2.4. Starch digestion in vitro

In vitro digestion was performed as described by Goh and Woolnough ^[18, 19] with modifications. After grinding, 2.5 g of the cooked noodles were added to 30 mL of distilled water in a 37 °C water bath with shaking. The experimental procedure involved simulations of oral, gastric, and intestinal digestion. For the oral simulation, samples were incubated with 100 μ L of a 10% α -amylase solution in water for 1 min, after which 800 μ L of 1 M HCl was used to halt the reaction. For the gastric simulation, samples were incubated with 1 mL of 10% pepsin in 0.05 M HCl for 30 min, and 2 mL of 1 M NaHCO₃ and 5 mL of 0.2 M maleate buffer, pH 6, were added to halt the reaction. A 1 mL aliquot was collected (time 0) and placed in 4 mL of ethanol to prevent further enzyme action. The inhibition of the end products of pancreatic α -amylase was prevented by the addition of 100 μ L of amyloglucosidase. For the intestinal simulation, the sample was added to 1 mL of 5% pancreatin in 0.2

M maleate buffer, pH 6. Aliquots were collected after 20, 30, 60, 80, 120, and 180 min, and were added to 4 mL of 100% ethanol, after which the reducing sugars were analyzed using the method of Ranawana and Henry ^[20]. The glucose content (Gt) was then assessed using the 3,5-dinitrosalicylic acid (DNS) method. The hydrolysis rate (%) was determined as follows:

Hydrolysis rate (%) =
$$\frac{G_t \times 0.9}{\text{starch content(mg)}} \times 100$$
 (1)

The RDS (rapidly digestible starch), SDS (slowly digestible starch), and RS (resistant starch) contents were assessed as follows:

RDS (%) =
$$\frac{(G_{20} - G_0) \times 0.9}{\text{TS}} \times 100$$
 (2)

SDS (%) =
$$\frac{(G_{120} - G_{20}) \times 0.9}{\text{TS}} \times 100$$
 (3)

RS (%) =
$$\frac{(TS - (RDS + SDS))}{TS} \times 100$$
 (4)

where G_0 is the content (mg) of free glucose, G_{20} is the amount (mg) of glucose released after 20 min, G_{120} is the amount (mg) of glucose released after 120 min, and TS is the amount (mg) of total starch in the sample.

2.5. X-ray diffraction (XRD)

After storage at -80 °C, the cooked noodles were lyophilized and ground (SCIENTZ 18N, Zhejiang Side Equipment Co. Ltd, Zhejiang, China), followed by sieving with a 60-mesh sieve. XRD was performed on an XRD-6000 diffractometer (Shimadzu, Japan), with the parameters of 40 kV operating voltage, 30 mA current, scan range (20) of 5 to 50°, and a scan speed of 2° /min.

2.6. Scanning electron microscopy (SEM)

Samples were lyophilized at -80 °C and sprayed with gold using a vacuum evaporator. Microstructural analysis was performed using SEM (S-4800, Hitachi, Japan) at an accelerating voltage of 5.0 kV and 1000 x magnification.

2.7. Body weights, blood glucose levels, and oral glucose tolerance (OGTT) and insulin tolerance (ITT) tests

Rats were weighed weekly and their weights were recorded. Blood samples were taken from the tail veins and glucose levels were assessed on days 0, 4, 8, 12, 16, and 20. Glucose levels were determined between 16:00 and 17:00.

OGTT: After fasting for 12 h, the rats received 4 mL/kg glucose solution by oral gavage. The blood glucose levels were assessed every 30 min thereafter using a glucose meter, and a glucose-tolerance curve was drawn after 2 h. The values were compared between the treatment groups using the area of integration under the curve (AUC), as follows:

$$AUC = 0.25 \times G_0 + 0.5 \times G_{30} + 0.75 \times G_{60} + 0.5 \times G_{120}$$

ITT: This was conducted three days after the OGTT. The rats fasted for 5 h after which they received intraperitoneal injections of 0.5 U/kg insulin. The blood glucose levels were examined at 30-min intervals for 2 h using a blood glucose meter. Changes in the glucose levels over this period were then plotted and the AUC was calculated.

2.8. Antioxidant levels and glycogen contents

One gram of liver tissue was homogenized (8000 rpm, 10 min) in 5 mL of cold saline followed by centrifugation (3000 g, 10 min). The supernatant was retained for measurement of the superoxide dismutase (SOD), alanine aminotransferase (ALT), catalase (CAT), and malondialdehyde (MDA) levels using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China).

2.9. Biochemical analyses

Triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDH-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured using kits (Nanjing Jiancheng Bioengineering Institute), according to the provided protocols.

2.10. Hematoxylin-eosin (H&E) staining

Liver tissues were fixed with 10% formaldehyde, followed by dehydration, paraffin-embedding, and sectioning (4 µm sections). Sections were stained with H&E using a kit (Nanjing Jiancheng Bioengineering Institute).

2.11. Statistical analysis

All experiments included three replicates. Data are presented as mean ± standard deviation and were analyzed using Origin 2021 for Windows. Differences between means were assessed by one-way analysis of variance (ANOVA) followed by Duncan's test.

3. Results and Discussion

3.1. In vitro digestibility of noodles

The influence of the gel network structure on starch digestibility was assessed in vitro. The hydrolysis rates of the noodle samples prepared by the endogenous method during digestion *in vitro* for 180 min are presented in Figure 1A. Similar hydrolysis trends were observed in all samples, with starch digestion gradually increasing with time. There was a rapid increase in the rate of hydrolysis during the first 20 min, followed by a decline between 20 and 120 min, after which it either decreased or stabilized. The results depicted in Figure 1A demonstrate that concentrations of sodium alginate between 0.1% and 0.5% had no significant influence on the rates of starch digestion, while a significant effect was observed at 0.7% sodium alginate at which markedly reduced digestion rates were found. Furthermore, the hydrolysis of noodles prepared by the endogenous combination of calcium ions and sodium alginate showed marked reductions at sodium alginate concentrations above 0.3%, while increasing sodium alginate and calcium ion concentrations were associated with reduced hydrolysis. As an example, at 0.7% sodium alginate, with the increase in calcium carbonate content, the hydrolysis rate of buckwheat noodles was reduced by 19.8-36.0% compared to samples without added calcium carbonate. This was likely due to the formation of a relatively dense network between the sodium alginate and calcium that did not allow the leaching of molecules from the interior. The incorporation of greater amounts of Ca^{2+} would enhance the density of the gel network, thus reducing the degree of hydrolysis. These results are consistent with those of an earlier report where Ca^{2+} was cross-linked with sodium alginate, forming a core shell of encapsulated calcium alginate ^[21].

The contents of RDS, SDS, and RS in the different samples were then determined (Figure 1B). The mere addition of sodium alginate to buckwheat noodles did not bring about significant changes in the contents of RDS, SDS, and RS, which is in correspondence with the previous data of starch hydrolysis rate. However, there are significant differences in the results of preparing buckwheat noodles by the endogenous method. Marked reductions in the RDS contents of the noodles were observed with increasing concentrations of calcium ions and sodium alginate, while the contents of RS increased by approximately 70% (buckwheat noodles contain 0.7% alginic acid sodium and 9% calcium carbonate compared to the control). This supports the observations of Jin et al. ^[22]. The explanation for the changes in the rate of starch digestion may be an increase in the RS content, since RS is a general term for undigested starch and its breakdown products, which are not normally broken down or absorbed in the small intestine and tend to be subject to amylase digestion, thus decreasing the overall starch digestion rate and increasing the risk of disorders such as obesity and cancer. The increase in RS content might be attributed to the gel network formed by sodium alginate and calcium ions in buckwheat noodles, which hindered the enzymatic digestion of starch.

3.2. XRD analysis

XRD was used to examine the crystal structures of the noodles. Figure 2 shows

the XRD results. The crystal structure of foods determines their properties and significantly influences their digestibility ^[23]. The diffraction patterns of pure buckwheat noodles resembled those of sodium alginate, with clear amorphous peaks observed at $2\theta = 20^{\circ}$. However, marked alterations in the starch morphology were seen after the inclusion of calcium ions. Sharp diffraction peaks appeared around $2\theta=30^{\circ}$, indicative of obvious crystallinity, and also indicating the presence of calcium carbonate in the starch ^[24]. The interaction between Ca²⁺ and starch components constitutes a critical structural determinant. Calcium ions demonstrate specific affinity for the hydroxyl groups of amylose via coordination bonding, thereby facilitating the reorganization of starch chains into crystalline domains^[25]. This ionic crosslinking synergizes with sodium alginate's gel network to form a composite matrix wherein calcium ions bridge starch-alginate hydrogen bonds ^[26]. This suggested that the crystallinity of the starch was altered by calcium ion cross-linking with sodium alginate after endogenous preparation, which may influence the noodle quality^[27].

3.3. SEM analysis

To understand the possible reasons underlying the changes in the sodium alginate gel structure produced by calcium ions, SEM was used for the microstructural evaluation of buckwheat noodle cross sections (Figure 3). Figure 3 (a) shows the presence of multiple hollows and voids on the surfaces of the control noodles, while these were less obvious, having either been reduced or disappeared,

after calcium incorporation, resulting in a denser, more compact structure, as shown in Figure 3 (b)-(e). In the absence of calcium ions and sodium alginate, the noodles did not form a continuous or tight network, resulting in the appearance of pores and voids, as seen in Figure 3 (a). However, the addition of calcium ions increased the number of interactions between the calcium and sodium alginate, resulting in a denser noodle structure with smaller holes. Ca²⁺ coordinates with amylose hydroxyl groups during heating (C6-OH > C2-OH), forming stable complexes that suppress excessive starch granule expansion. This reduces water absorption hysteresis and limits vapor bubble formation. Meanwhile, calcium-alginate "egg-box" networks increase matrix viscoelasticity during gelatinization, promoting bubble coalescence and collapse before structural fixation. Ca²⁺ bridges starch-alginate interfaces via COO⁻-Ca²⁺-OH hydrogen bonds, creating a reinforced hybrid gel that resists bubble expansion during cooling ^[28]. Hence, when 0.7% sodium alginate was used, a tight coating of the starch particles could be observed, indicating that endogenous preparation of noodles influences the calcium-sodium alginate interaction and leads to the formation of a starch gel network. Thus, the improved microstructures of noodles containing sodium alginate with calcium ion incorporation may be related to the formation of a significantly tighter network resulting from cross-linking between the sodium alginate and calcium ions in the formation of alginate gels. These observations are consistent with those of Lubowa et al. who investigated the combination of pregelatinized high amylost cornstarch and sodium alginate in rice noodles, with immersion of the

noodles in calcium chloride solutions to produce a denser structure ^[28]. This work shows that Ca²⁺-induced alginate cross-linking in rice noodles reduces porosity and enhances structural integrity. SEM results confirm that Ca²⁺-alginate interactions create denser matrices by bridging starch and alginate phases.

3.4. Effect of Ca^{2+} modulation of sodium alginate on body weight and blood sugar in rats

To investigate the effects of sodium alginate with incorporated Ca^{2+} on rats, buckwheat noodles containing sodium alginate and Ca^{2+} were fed to rats fed on high-fat diets. Significant differences in both body weights and blood glucose between the noodle-fed and the other groups were found. Figure 4A shows that the body weights of rats in the blank group differed significantly from those of the diabetic rats, with weights in the blank group increasing and then stabilizing after four weeks while those of the diabetic model rats increased after week 5. At the completion of the experiment, the average weights of the rats in the blank, control, experimental 1, and experimental 2 groups had increased by 49.84, 39.22, 39.84, and 40.55%, respectively. These results indicate that the treatment regimen applied to experimental group 2 has a positive impact on the weight gain of diabetic rats.

Changes in the postprandial blood glucose leves of the rats are shown in Figure 4B. The blood glucose levels of the normal rats in the blank group remained relatively stable after three weeks of intervention, fluctuating within the range of 4.8 to 6.1 mmol/L. In contrast, rats in the control group exhibited higher blood glucose levels,

ranging from 27.8 to 32.8 mmol/L, possibly due to continuous increases in blood glucose in diabetic rats fed a normal diet. However, the average blood glucose level in experimental group 1 decreased to 24.7 mmol/L after three weeks, with the most significant reduction seen in experimental group 2 where the final blood glucose value reached 22.3 mmol/L. These results indicated that the treatment in experimental group 2 had a greater effect on reducing elevated blood sugar levels.

The OGTT results (Figure 4C) indicated that the blood glucose levels initially rose and then declined after glucose administration in all groups, reaching peak values after 60 min. The lowest values were seen in the blank group, while those of the controls were the highest. The ranking of the AUCs was control group > experimental group 1 > experimental group 2 > blank group, clearly showing an acceleration of glucose elimination in experimental group 2. These findings strongly suggest that the structure of the gel network resulting from the endogenous incorporation of calcium ions with sodium alginate alleviated glucose intolerance and could mitigate the effects of impaired glucose tolerance in rats with diabetes.

As is well-known, insulin resistance or insufficient insulin secretion is likely to lead to disordered sugar, fat, and protein metabolism, thus inducing T2DM, and insulin resistance results in abnormal lipid metabolism in the body ^[29]. As shown in Figure 4D, the ITT results indicate that the blood glucose levels of rats after intraperitoneal injection of insulin showed clear reductions, reaching their lowest values at 60 min, after which they tended to increase gradually. Relative to the blank

group, the blood glucose contents of rats in the control group after feeding a high-fat diet were highest, while those in experimental groups 1 and 2 were markedly reduced (P< 0.01). The calculated AUC values also clearly confirmed the reduced levels, with accelerated elimination of insulin seen in experimental group 2. It is known that high-fat diets can induce resistance, and feeding with Ca²⁺-incorporated sodium alginate can alleviate this symptom and mitigate abnormal insulin tolerance.

3.5. Effect of Ca^{2+} modulation of sodium alginate on serum lipid levels in diabetic rats

Serum levels of TC, TG, LDL-C, and HDL-C are used to assess blood lipids. Diabetic patients commonly show dyslipidemia, manifested as increased levels of TG, TC, and LDL-C, and reduced levels of HDL-C (Figure 5A-D). In agreement with the findings of other studies ^[30], it was found that the diabetic rats in the control and experimental groups showed increased TG, TC, and LDL-C levels, together with reduced HDL-C levels, compared with their healthy counterparts in the blank group. However, these trends were reversed after three weeks of intervention in the experimental groups with a more marked effect seen in experimental group 2 compared with group 1.

The measurement of free fatty acids is often more effective in representing the status of lipid metabolism than the use of other lipid indices, and can also indicate insulin sensitivity in rats. Research has shown that free fatty acid levels can reflect the status of lipid metabolism as well as disease progression in patients with T2DM ^[31].

Here, following three weeks of intervention, the free fatty acid levels in the blank group were observed to be relatively low and stable (Figure 5E), while those in the control group increased gradually after week 3 to 2.1 mmol/L higher than those of the normal rats. This result suggested the presence of abnormal lipid metabolism which could result in oxidative stress and inflammation. The free fatty acid levels in the experimental groups, however, slowly declined, with a more marked effect seen in experimental group 2, suggesting that the calcium-sodium alginate gel assisted in the amelioration of abnormal lipid metabolism.

The observed lipid-lowering effects of calcium-sodium alginate gel may be attributed to its unique physicochemical properties. The cross-linked gel network likely acts as a physical barrier in the gastrointestinal tract, delaying fat absorption by binding to bile acids and cholesterol through electrostatic interactions, thereby promoting fecal excretion of lipids. Additionally, sodium alginate's ability to modulate gut microbiota composition may influence lipid metabolism, as reported in studies linking dietary fibers to hepatic cholesterol regulation ^[32]. The reduction in free fatty acids could be mediated through improved insulin sensitivity, as enhanced insulin signaling suppresses hormone-sensitive lipase (HSL) activity in adipose tissue, reducing lipolysis and subsequent free fatty acid release ^[33].

3.6. Mitigation of oxidative stress, pathological morphology, and liver glycogen contents in diabetic rats by Ca^{2+} -sodium alginate networks

Oxidative damage plays a significant part in the pathogenesis of diabetes. In the liver, oxidative stress may lead to the development of diabetes as well as subsequent complications ^[34]. SOD and CAT activities were observed to be higher in the blank group and lower in the control group (Figure 6A and 6B), indicating disruption of these enzymes in the hepatocytes of diabetic rats. Despite similar initial levels in these enzyme activities in experimental groups 1 and 2, significant differences were observed after three weeks of treatment (P < 0.05), with marked increases in enzyme activity especially in experimental group 2. These findings indicate that both SOD and CAT activities were adversely affected by oxidative stress in the diabetic rats but were improved following intervention with Ca2+-sodium alginate, indicating protection against oxidative damage in the cells. Ca²⁺-sodium alginate gels may provide electrons via the carboxyl and hydroxyl groups in their polysaccharide structure, directly scavenging free radicals such as superoxide anions and hydroxyl radicals. This electron donation enhances the activity of SOD and CAT^[35].

ALT levels can be used as a measure of the severity of cell damage. ALT is a crucial marker of liver health and is widely used in clinical practice for the diagnosis of liver disease ^[36]. MDA accumulates after lipid peroxidation of cell membranes, and its levels are often used as a reflection of the degree of both lipid peroxidation and cell damage ^[37]. As shown in Figures 6C and 6D, both ALT activity and the MDA contents were markedly raised in the diabetic versus the normal rats, while the levels were significantly reduced in both experimental groups, especially in experimental

group 2. These findings demonstrate increases in intracellular ALT and MDA in diabetic rats and indicate that they can be reduced by intervention with the Ca^{2+} -sodium alginate gel.

The liver contains significant glycogen reserves which are responsible for the maintenance of glucose homeostasis in the body. The production and breakdown of glycogen in the liver are tightly regulated under normal circumstances. The results of the evaluation of liver glycogen concentrations in the diabetic rats is shown in Figure 6E. It was found that the concentrations in diabetic rats were markedly lower than those in the normal rats in the blank group while following three weeks of intervention, the glycogen concentrations in the livers of the control group decreased by 0.9 μ g/mgprot compared to those measured during week 1 of the experiment. The levels in experimental group 1 increased by 4.5 μ g/mgprot and those in experimental group 2 by 7.4 μ g/mgprot. This indicates that Ca²⁺-sodium alginate can effectively promote the synthesis of liver glycogen and thus improve liver function.

As dysfunctional glycogen metabolism is known to induce pathological changes in liver cells, the histopathology of liver sections from diabetic rats was examined with H&E staining. No abnormalities were observed in the normal rats (Figure 6F), seen in an intact liver with cells of normal size and structure. However, in the control group (Figure 6G), the livers showed the presence of steatosis with swollen hepatocytes and the accumulation of fat vacuoles, together with infiltration of inflammatory cells. The structures of the hepatic lobules in experimental group 1

(Figure 6H) appeared mildly disordered, with evidence of hepatocyte steatosis. However, in the rats belonging to experimental group 2 (Figure 6I), the hepatocytes had essentially returned to their normal size, and the fibrous septa between the hepatic lobules appeared relatively regular. Intervention with the Ca²⁺-sodium alginate gel effectively reduced these pathological changes, seen in decreased fat accumulation and the presence of fewer inflammatory cells ^[38]. These findings indicate that Ca^{2+} -sodium alginate significantly alleviated liver damage in diabetic rats.

 Ca^{2+} -sodium alginate gel may promote liver glycogen synthesis by modulating the insulin signaling pathway. Previous studies have demonstrated that sodium alginate can enhance insulin sensitivity, upregulate glucose transporter expression, and thereby facilitate glucose uptake and glycogen synthesis ^[39]. Furthermore, its antioxidant properties protect hepatic mitochondrial function, ensuring adequate ATP supply, which further supports glycogen synthesis. Sodium alginate may also alleviate the liver inflammatory response by inhibiting the NF- κ B signaling pathway and reducing the secretion of pro-inflammatory cytokines. Additionally, its gel-forming capacity enables it to absorb bile acids and cholesterol, thereby regulating lipid metabolism and mitigating hepatic steatosis ^[40].

4. Conclusions

In summary, we propose a method for the reduction of the starch digestion rate to mitigate elevated blood glucose levels after meals. This intervention involves the endogenous cross-linking of calcium ions with naturally occurring sodium alginate.

Buckwheat noodles prepared using this method showed markedly reduced starch digestion rates while increasing RS and decreasing RDS contents, as shown in in vitro simulations. Analysis of the noodle microstructure indicated the formation of dense gel networks, with the density increasing with increased sodium alginate concentrations. Notably, the effects of these gel networks on hyperglycemia and hyperlipidemia in diabetic rats induced by high-fat diets and STZ injections were investigated in vivo. The results indicated that gel network and starch complex formed by Ca²⁺-induced sodium alginate in buckwheat noodles contributes to weight increases in diabetic rats while reducing the levels of blood glucose after a meal, mitigating glucose intolerance and abnormal insulin tolerance. The Ca²⁺-induced sodium alginate gel network and starch complex also reduced hyperlipidemia by modulation of the TC, TG, LDL-C, HDL-C, and free fatty acid levels. Furthermore, the Ca²⁺-induced sodium alginate gel network and starch complex enhanced liver glycogen synthesis, while increasing SOD and CAT activities, and reducing ALT and MDA, together with the amelioration of morphological damage in the liver. These results provide a theoretical foundation for the use of sodium alginate to both prevent and treat diabetes.

However, this study has several limitations that warrant further consideration. Firstly, the findings are derived from a rodent model of chemically induced diabetes, which may not fully capture the multifaceted nature of human type 2 diabetes pathophysiology. Secondly, the long-term impacts of sodium alginate gel networks on

gastrointestinal function and nutrient absorption have yet to be elucidated. Moreover, the dose-dependent efficacy and potential interactions with other dietary components in humans necessitate more investigation. Future research should prioritize clinical trials to evaluate translational applicability and explore the molecular mechanisms responsible for the observed metabolic improvements, such as alterations in gut microbiota or regulation of signaling pathways.

Credit Author Statement

Lingyu Han: Methodology, Formal analysis, Investigation, Writing-original draft,

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Conflict of Interest

The authors declare no conflicts of interest.

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Figure captions

Figure 1. (A) Effects of Ca^{2+} -sodium alginate on starch hydrolysis of buckwheat noodles, (B) Effects of Ca^{2+} -sodium alginate on RS, SDS, and RDS.

Figure 2. (A) Effects of Ca²⁺-sodium alginate on starch crystal structure

Figure 3. Microstructure of noodles. (A) Control group; (B) 0.1% sodium alginate (9% CaCO₃); (C) 0.3% sodium alginate (9% CaCO₃); (D) 0.5% sodium alginate (9% CaCO₃); (E) 0.7% sodium alginate (9% CaCO₃).

Figure 4. (A) Changes in rat body weights after eight weeks of treatment, (B) Blood glucose levels, (C) Oral glucose tolerance test (OGTT) curves at the end of week 8 showing the area under the curve, (D) Insulin tolerance test (ITT) curves at the end of week 8, showing the area under the curve. Note: The different letters mean significant differences p < 0.05 within the same indicator.

Figure 5. Effects of Ca²⁺-sodium alginate on serum lipid levels in diabetic rats. (**A**) Total cholesterol (TC), (**B**) Triglycerides (TG), (**C**) High-density lipoprotein cholesterol (HDL-C), (**D**) Low-density lipoprotein cholesterol (LDL-C), (**E**) Free fatty acids. Note: The different letters mean significant differences p < 0.05 within the same indicator.

Figure 6. Effects of Ca²⁺-sodium alginate on oxidative damage and pathological morphology in the livers of diabetic rats. (A) SOD activity levels, (B) CAT activity levels, (C) ALT activity levels, (D) MDA contents, (E) Liver glycogen contents, (F-I)

H&E-stained liver tissues from the different groups. (F) Blank group; (G) Control group; (H) Experimental group 1; (I) Experimental group 2.







RDS SDS RS 100 31% 31% 32% Proportions of RS, SDS and RDS 80 60 52% 52% 52% 53% 40 20 17% 16% 16% 17% 0 0.1% Sodium 0.1% Sodium 0.1% Sodium 0.1% Sodium 0.1% Sodium alginate alginate/3% alginate/6% alginate/9% (Citrie acid) CaCO₃ (Citrie acid) CaCO₃ (Citrie acid)







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Figure 3













Declaration of Interest Statement

 \boxtimes The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

□ The author is an Editorial Board Member/Editor-in-Chief/Associate

Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article.

□ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: