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Research Article

Effect of Substitution of β -Glucans on the Glycemic Response and Thermal Properties of Four Common Starches

Abstract

Diabetes Mellitus (DM) is a major public health concern worldwide which can lead to a series of disabling complications and diseases. β -glucans are non-starch polysaccharides that are being used as food additives for their numerous health benefits including the ability to lower the postprandial glucose response. The aim of this study was to determine the effect of substituting β -glucans on the glycemic response and thermal properties of four commonly consumed starches. Oat β -glucans were added at concentration levels of 0%, 10%, 20%, and 30% (w/w) to each of the four starch types. Composite starches were incubated with α -amylase followed by further incubation with amyloglucosidase. Glucose released was measured using the 3, 5-dinitrosalicylic acid (DNSA) method. Incremental Area under the Curve (iAUC) was used to represent the estimated glycemic response of the β -glucan/starch composites. Thermal analysis of the starch composite starches was conducted using a differential scanning calorimeter. An overall reduction in the amount of glucose released after the addition of β -glucans was observed ($p \leq 0.05$). Substituting starches with 20% and 30% β -glucans resulted in a significant reduction in the glucose release rate and thus improved the estimated glucose response of all starches. A marked increase in the enthalpy of gelatinization, ΔH , of all starches was observed. Substitution at 10% β -glucans caused a significant increase in ΔH of blank tapioca starch ($p \leq 0.05$). The 20% and 30% β -glucan samples also had significantly higher ΔH than the blank and the 10% β -glucan samples. The data from this study suggest the potential use of β -glucan as a suitable food ingredient in diabetic food products.

Introduction

Diabetes mellitus (DM) is a major public health concern which has become a primary global healthcare challenge. DM and uncontrolled long-term hyperglycemia are considered key causative factors in the development of irreversible secondary health complications such as diabetic retinopathy, nephropathy, cardiovascular diseases, myocardial infarctions, strokes, and many other chronic diseases that are disabling and often life threatening [1].

Whereas medicinal therapies are undoubtedly effective, studies have shown that nutrition and lifestyle approaches can be more effective in preventing and controlling the disease [2,3]. The important role of nutrition has been increasingly recognized with medical nutrition therapy (MNT) mainly focusing on normalizing blood glucose levels, blood pressure levels, and lipoprotein profile to improve the glycemic status and reduce associated complications [4]. Besides carbohydrate counting and glycemic load, another very important aspect in managing blood glucose levels is the Glycemic index (GI) of foods. The GI is a ranking system that represents how quickly a carbohydrate is broken down and how fast it raises postprandial blood glucose. Foods with a lower GI are broken down slowly by the body and thus enter the blood stream at a slower rate; this reduces both the glycemic response and the resultant insulin release [5]. On the other hand, foods with a high glycemic index because a rapid increase in blood glucose after a meal, followed by a sudden drop and potential hypoglycemia. This pattern of glycemic response has been reported to have a positive correlation with an increased incidence of type II diabetes [6].

Low glycemic index foods have been shown to increase the feeling of satiety and can accordingly help in appetite control and weight management [5]. Moreover, since insulin resistance and type II diabetes are commonly associated with excess body weight, the ability to manage weight gain via increased sense of fullness will help in reducing the risk and prevalence of type II diabetes [7].

β -glucans are non-starch polysaccharides composed of D-glucose units joined together in chains via β -glycosidic bonds [8]. They are naturally found in fungi, yeast, bacteria, and in cereal grains, such as barley and oats, and in fewer amounts in wheat and rye, where they are located in the cell wall of the endosperm [9,10]. Some β -glucans are soluble, such as the ones found in oats and barley, composed of linear (1,3)(1,4) β -glucans, while others are insoluble, such as those found in fungi composed of branched (1,3)(1,6) β -glucans [9]. Similarly, β -glucans vary in other physicochemical characteristics such as viscosity, molecular weight, and fermentation, which determine their potential health benefits in the body [7,10].

Numerous health benefits of β -glucans have been discovered, leading to the increased popularity and consumption of oats and oat products [10], in addition to their recent use as active food components [9]. Besides having negligible caloric value, beneficial roles of β -glucans include hypoglycemic, antihypercholesterolemic, antihypertensive, immunomodulatory, weight management, and wound healing properties, all of which are either risk factors or consequences of DM [10].

While several studies have been conducted on the effect of ingesting β -glucans on postprandial biomarkers of glycemic response,

little has been reported on *in vitro* studies on the effect of β -glucan levels on α -amylase activity in relation to glucose release and glycemic index. Although β -glucans have been well-known for their ability to enhance nutritional quality of foods, the majority of research studies focus on the health effect of ingesting food sources of β -glucans, such as oats and barley, while less have been done on the changes that β -glucans impose upon the foods to which they are added.

The aim of this study, therefore, was to evaluate the effect of β -glucan substitution on the glycemic response and changes in thermal properties of four commonly consumed starch sources, namely rice, potato, corn, and tapioca.

Materials and Methods

Materials

Potato starch, corn starch, and tapioca starch, and oats β -glucan were obtained from commercial sources. Rice starch was purchased from Sigma-Aldrich (EC 232-679-6, Belgium).

Sample Preparation

Oat β -glucans were added at four concentration levels of 0%, 10%, 20%, and 30% (w/w) to each of the starch types forming four composites of 50 g individually. The β -glucans concentration levels selected were based on results from preliminary optimization studies. All samples, except for blanks, were prepared in duplicates. Additionally, one β -glucan blank sample (50g β -glucans) was prepared for comparison purposes. β -glucan/starch slurries were prepared by dispersing the specified amounts of soluble β -glucans in distilled water, then adding the starch powders and stirring mildly for 10 minutes at room temperature to avoid lump formation. The latter was then freeze dried to ensure homogeneity of the samples.

In vitro Digestibility of starches

In vitro starch digestion of lyophilized samples was performed based on the procedure of Goni et al. [11], with slight modifications. Initially, two enzyme solutions for digestion were prepared as follows: (1) α -amylase solution was prepared by dispersing 25 mg α -amylase from *Aspergillus oryzae* (EC 232-588-1, Sigma-Aldrich) to 10 ml of 0.2 M phosphate buffer (pH 6.9), and (2) amyloglucosidase solution was prepared by suspending 3 mg of amyloglucosidase from *Aspergillus niger* (70U/mg, EC 232-877-2, Sigma-Aldrich) in 50 ml 0.5 M sodium acetate buffer (pH 4.5). These enzyme solutions were freshly prepared for each digestion analysis.

Samples composing of 250 mg each of the freeze-dried β -glucan/starch composite were incubated with 10 ml of the freshly prepared α -amylase solution (pH 6.9) in a shaking water bath at 37 °C for 2 hrs. Aliquots (1 ml) were taken at baseline and every 30 minutes for 2 hours. Test tubes were shaken at each interval using a vortex, and aliquots were pipetted into fresh test tubes, and immediately immersed in a boiling water bath for 5 minutes to denature the enzyme. After the 120 minutes incubation period, aliquots were cooled to room temperature and then incubated again with 1 ml amyloglucosidase solution (pH 4.5) in a shaking water bath at 55 °C for 45 minutes to completely hydrolyze the digested starch. At the end of the incubation period, samples were placed in a boiling

water bath for another 5 minutes to denature the amyloglucosidase enzyme. Finally, samples were placed in two ml microfuge tubes and centrifuged at 13000 x g for 1 minute using a microfuge to precipitate the enzymes.

Determination of glucose content

The glucose content in the supernatant was measured colorimetrically using the 3,5-dinitrosalicylic acid (DNSA) method established by Miller [12]. The principle of this method is based on a redox reaction that takes place under alkaline conditions, upon heating, where reducing sugars (such as glucose, fructose, and maltose) reduce 3,5-dinitrosalicylic acid into 3-amino-5-nitrosalicylic acid which has a dark red color and is absorbed at 540 nm. The intensity of the color is directly proportional to the amount of reducing sugars present in an unknown sample [13]. This reaction is reported to be dependent on the type of reducing sugar, since different reducing sugars were reported to result in different color intensities [12].

For each composite sample, 0.25 ml of sample was pipetted into a test tube containing 4.75 ml distilled water and two ml DNSA reagent. The test tubes were then covered by aluminum foil and incubated in a boiling water bath for five minutes, until color changed to dark red, then immediately immersed in an iced water bath to rapidly cool down the mixture and stop the reaction. Another 13 ml of distilled water was added, diluting the solution to 20 ml. After cooling to room temperature, the absorbance of the supernatant was measured at 540nm using an Agilent UV-Vis-NIR (Cary 5000, USA) Spectrophotometer. A blank solution, containing no sample, was also prepared for the instrument's calibration purpose. The concentration of reducing sugars was finally determined using a standard curve obtained from a standard glucose solution.

Thermal properties

Thermal analysis of the starch composite samples was conducted using a differential scanning calorimeter (NETZSCH DSC 204F1 Phoenix) calibrated using indium and an empty sealed aluminum crucible as a reference. Duplicates of the β -glucan/starch samples, weighing 5 mg each, were placed in the aluminum crucibles, and sealed before heating in the DSC. Scans were performed from 25 to 300 °C at a controlled constant rate of 10 °C/min. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and enthalpy of gelatinization (ΔH), expressed as J/g dry starch, were automatically generated by the equipment software. The gelatinization temperature range (ΔT) was calculated as $T_c - T_o$.

Statistical analysis

IBM SPSS Statistics version 22.0 was used to analyze the data via Repeated Measures Analysis of Variances (ANOVA), in which the effect of different treatments on glycemic response and thermal properties was studied between and within starch types. Tukey's posthoc test was performed when treatment effects showed significance. An alpha level of ≤ 0.05 was considered statistically significant. Data was expressed as the mean value of duplicate measurements \pm standard deviation (SD).

Results

Treatment with different concentrations of β -glucans showed a significant effect on the iAUC after 120 minutes of *in-vitro* digestion

within all starch types ($p \leq 0.05$), as shown in **Figure 1**. The addition of β -glucans resulted in an overall lower glucose release in all of the starch types. Multiple comparison of the effect of substituting different β -glucan concentrations on the iAUC showed that, collectively, starch blanks and samples treated with 10% β -glucans were not significantly different ($p \leq 0.05$). Similarly 10%, 20%, and 30% treated samples were not significantly different. However, the effect of treatment was most evident and showed statistical significance between the blank starch samples and the samples substituted with 20% β -glucans ($p \leq 0.05$), as well as between the blank and the 30% β -glucan substituted starches. This indicates that substituting starches with 20% and 30% β -glucans resulted in a significant reduction in the glucose release rate and thus improved the estimated glucose response of all starches.

The effect of substituting different concentrations of β -glucans on potato starch showed that although the 10% and 20% β -glucans/starch samples were not significantly different in terms of glucose release, there was a clear difference between the potato blank and 30% β -glucan/starch sample; 30% β -glucan sample had a significantly lower iAUC than the potato blank ($p \leq 0.05$) (**Figure 2A**). Similarly, glucose release rate of the rice starch samples, (**Figure 2B**), exhibited a significantly lower glucose response for the 30% β -glucan substituted sample than the other three composites. The addition of different concentrations of β -glucans to corn starch also produced a significant effect ($p \leq 0.05$) (**Figure 2C**), representing significantly lower iAUC after the addition of 20% and 30% β -glucans as compared to the blank and the 10% β -glucan/starch samples. The calculated iAUC of tapioca starch (**Figure 2D**) showed that any amount (10%, 20%, or 30%) of added β -glucans had a highly significant effect on the rate of glucose release ($p \leq 0.001$).

Enzyme hydrolysis rate of each β -glucan/starch composite shown in **Figure 3** indicates a peak glucose release at 30 min for all starch types. At 30 minutes, blank potato starch showed the highest glucose release, followed by the 10% β -glucan/starch sample, and then the 20% and 30% β -glucan samples (**Figure 3A**). Final glucose release at 120 minutes showed a similar pattern marked by a significantly lower concentration of glucose for potato starch substituted with 30% β -glucans, and a significantly higher concentration released by the blank control as compared to the 10%, 20%, and 30% β -glucan samples respectively ($p \leq 0.05$). Rice starch (**Figure 3B**) also showed a significantly higher glucose release at 30 minutes for the blank sample

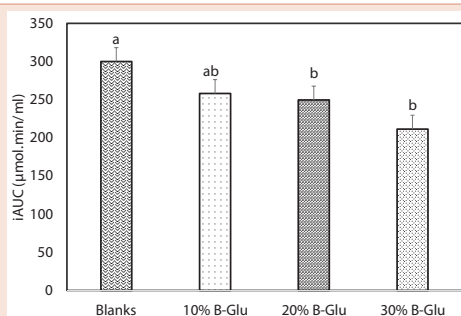


Figure 1: Pooled incremental area under the curve (iAUC) of all starch types substituted with different concentrations of β -glucans. Bars with different letters differ significantly ($p \leq 0.05$).

($p \leq 0.05$) and at 60 minutes, the 30% β -glucan sample showed a significantly lower glucose release compared to the blank and 10% β -glucan samples. Glucose response curve for the 30% β -glucan treated corn starch (**Figure 3C**) showed the lowest concentration of glucose release at all-time points reaching a significant level at 90 min and 120 min ($p \leq 0.05$). The 20% β -glucan starch sample also had a significantly lower glucose release at 120 minutes than the blank and 10% β -glucan samples. Tapioca also showed a similar pattern in the sense that, the sample treated with 30% β -glucans showed the lowest final glucose release after 120 min (**Figure 3D**).

Thermal properties

Table 1 shows the thermal properties of the β -glucans-substituted starches. While the observed data for all starch composite types did not show clear patterns, it is evident that the enthalpy of gelatinization (ΔH) increased for rice and tapioca starches as they were substituted with β -glucans from the blank to the 30% level. Conversely, increasing β -glucan levels decreased ΔH for potato and corn starches.

DSC thermograms for each starch composite type are presented in **Figure 4**, and indicate shifts in the enthalpy of gelatinization and other thermal properties of the β -glucan substituted starches, albeit not in a consistent pattern. Peak (T_p) and onset (T_o) temperatures decreased with the addition of more β -glucans, while the conclusion temperature (T_c) increased. Results of the ΔT values (**Table 1**) of potato starch showed a similar trend as that found in all starches together, indicating that adding more β -glucans causes an increase in the gelatinization temperature range of the starch. Similar patterns were observed for the ΔH and ΔT of rice starch (**Figure 4B**). Although ΔH results for 10% and 30% corn starch did not differ, a marked reduction in ΔH occurred between the blank corn sample and the 20% and 30% substituted samples (**Figure 4C**, **Table 1**). Moreover, a clear increment of 32°C in the peak temperature (T_p) of corn starch was evident between the blank and 30% β -glucan samples indicating an elevation in T_p with the addition of β -glucans. ΔT of corn starch samples also showed an increased temperature range with a higher β -glucan concentration similar to that of rice and potato starch. Tapioca starch exhibited an increased ΔH with even the slightest amount of added β -glucans (**Figure 4D**). Substitution with only 10% β -glucans was enough to cause a significant increase in the ΔH of blank tapioca starch ($p \leq 0.05$). The 20% and 30% β -glucan samples also had significantly higher ΔH than the blank sample and the 10% β -glucan sample ($p \leq 0.05$).

Discussions

The addition of β -glucans resulted in an overall lower glucose response for all starch types as indicated by the significant reduction in total glucose released by the 20% and 30% β -glucan samples. Similar results were observed in a study using a different *in vitro* digestion method to determine the starch digestion rate of oat flours containing varying amounts of β -glucans. Results of this study showed a negative correlation between starch digestion and β -glucan concentration implying that the addition of β -glucans slowed down the rate of starch digestion [14]. Moreover, 20% and 30% β -glucan composites had significantly lower iAUC compared to blank starch samples ($p \leq 0.05$), while the 10% and blank starch samples were not

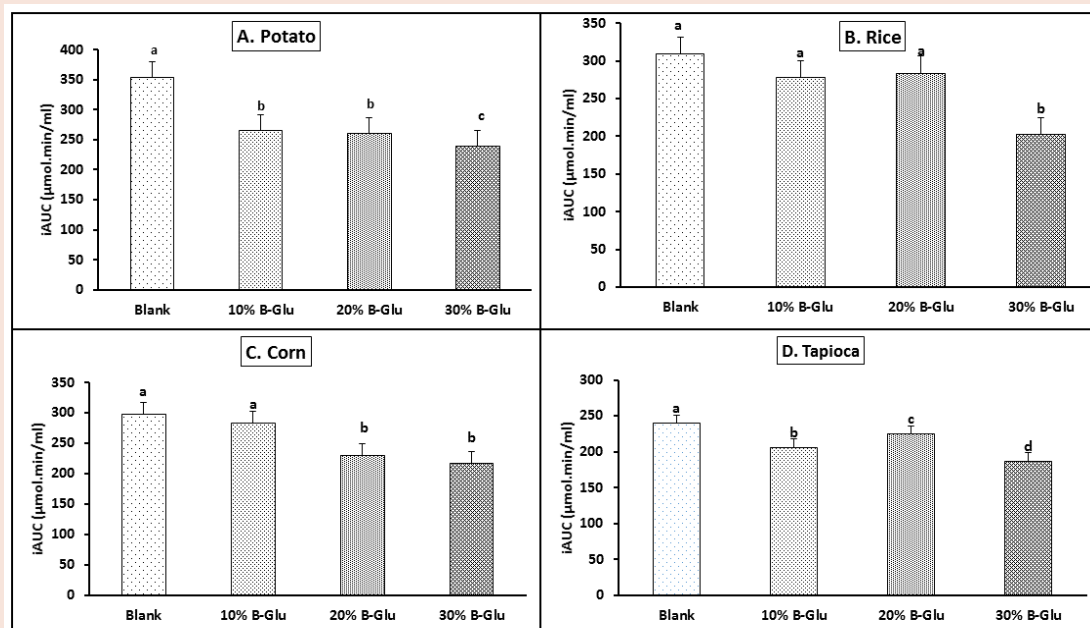


Figure 2: Incremental area under the curve (iAUC) of starches substituted with different concentrations of β-glucans. Bars in the same graph with different letters differ significantly ($p \leq 0.05$).

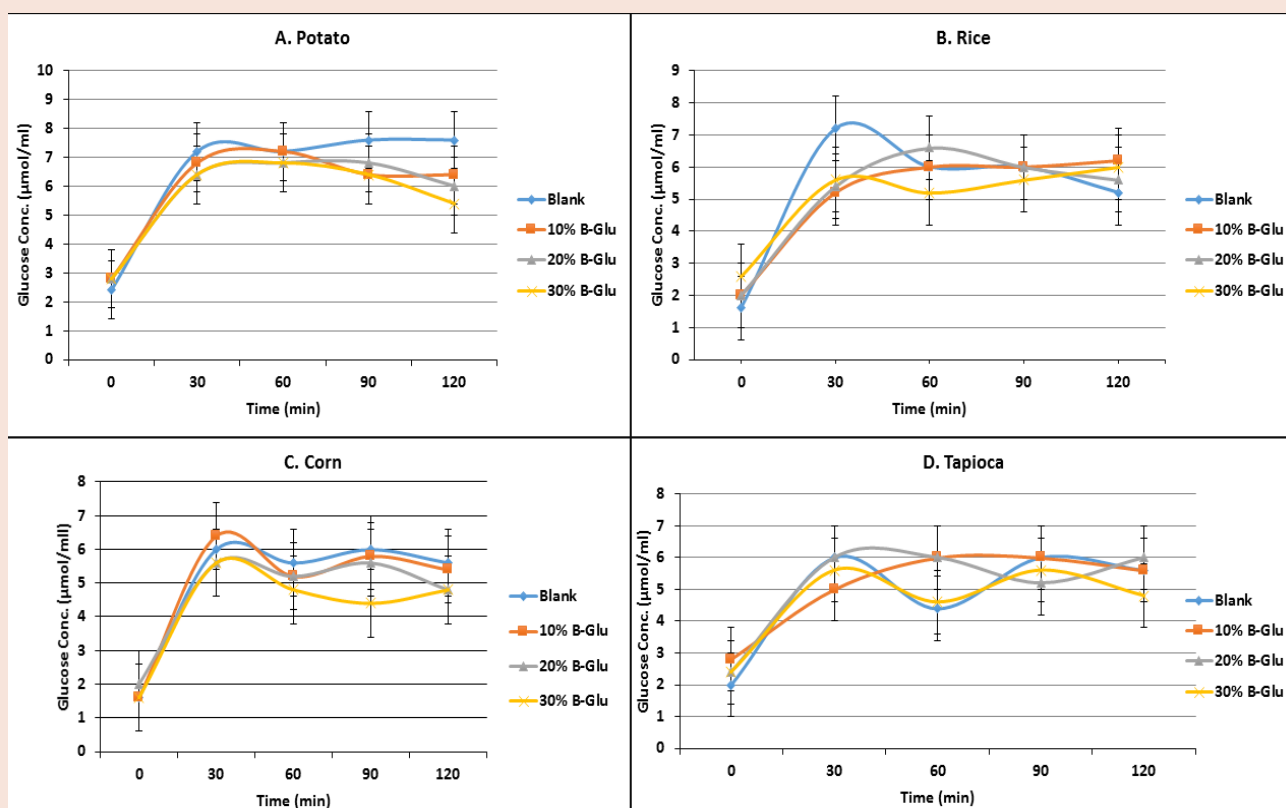


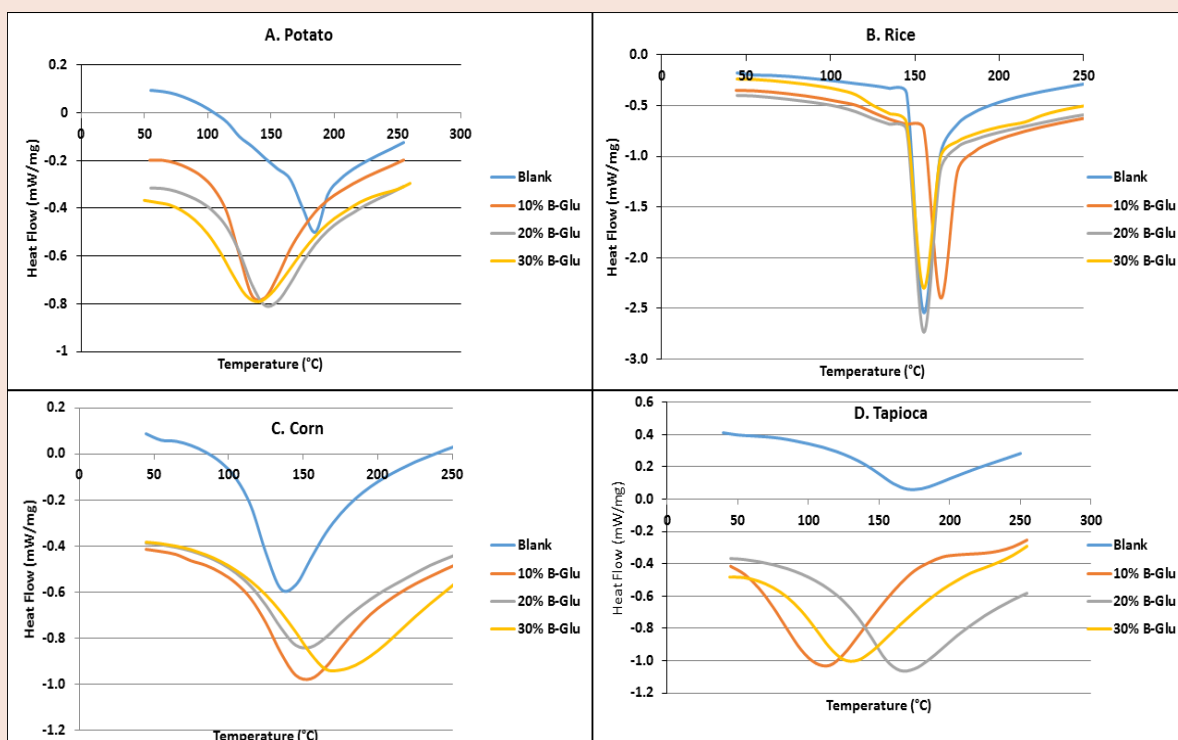
Figure 3: Rate of enzyme hydrolysis of starches substituted with β-glucan at different concentrations.

Table 1: Thermal properties of β -glucans-substituted starches.

	ΔH (J/g)	T_o ($^{\circ}C$)	T_p ($^{\circ}C$)	T_c ($^{\circ}C$)	ΔT ($^{\circ}C$)
Potato					
0% β -glucans (Blank)	241.31	171.2	172.5	177.2	6.0
10% β -glucans	234.56	109.8	140.1	181.5	71.7
20% β -glucans	211.99	80.1	147.9	155.5	75.4
30% β -glucans	199.5	96	138.8	229.9	133.9
Rice					
0% β -glucans (Blank)	240.06	153.6	155.1	162.2	8.6
10% β -glucans	260.65	162.1	163.9	170.4	8.3
20% β -glucans	304.9	152.9	154.0	158.7	5.8
30% β -glucans	314.54	151.1	152.3	156.9	5.8
Corn					
0% β -glucans (Blank)	259.12	107.9	138.6	179.9	72
10% β -glucans	244.12	104.9	151.9	195.5	90.6
20% β -glucans	205.55	108.9	151.2	241.2	132.3
30% β -glucans	244.29	110.8	170.1	239.0	128.2
Tapioca					
0% β -glucans (Blank)	90.76	139.0	175.0	226.2	87.2
10% β -glucans	200.98	73.3	112.2	173.2	99.9
20% β -glucans	272.19	118.1	168.3	223.9	115.8
30% β -glucans	292.6	87.3	129.5	149.8	62.5

significantly different (Figure 1). This suggests that the oat β -glucans are dose-effective in reducing the estimated blood glucose response, requiring a concentration of 20%-30% to exhibit more pronounced results. Such a dose response relationship has been reported by various studies investigating this aspect [14-16]. It was calculated, in a study done on sixteen non-insulin dependent diabetic patients, that approximately 4 units of glycemic index are reduced for every additional gram of β -glucans [17].

Starch hydrolysis rate (Figure 3) showed peak glucose release at 30 minutes for all β -glucan-starch composite samples followed by a plateau. This indicates that peak enzyme activity was at 30 minutes, meaning it took the α -amylase enzyme approximately 30 min to hydrolyze most of the starches in the samples into glucose. It is also evident from the curves that the blank samples, particularly those for potato, rice, and tapioca, elicited a sharper postprandial glycemic spike marked by a higher amount of glucose released at 30 min followed by a sudden fall below baseline, as compared to the β -glucans substituted samples. This is especially important since previous data suggests that a carbohydrate food might elicit a sharp glucose spike that disappears quickly and falls below baseline levels, yet at the same time have an iAUC as another carbohydrate food with a steadier rise and fall [18,19]. This implies that postprandial glucose spikes and fluctuations may be more indicative of the glycemic response of a food than the shape of the curve or the iAUC derived from it. Such observed pattern of glucose release is associated with high GI foods and could perhaps be reflective of reactive hyperglycemia, which is defined as the sudden rise in postprandial blood glucose preceded by a sudden drop below baseline levels [6,20].


Figure 4: DSC thermograms of β -glucan substituted starches.

From a nutritional point of view, the addition of β -glucans had a beneficial effect in reducing the rate of starch breakdown during *in vitro* digestion, and hence in reducing sugar release as shown by the lower iAUC in the samples with 20% and 30% β -glucans. This suggests a slower rate of starch breakdown and a gradual glucose release, reflecting an improved glucose response and a possibly lower GI in the body. This is of particular importance to diabetic patients since consumption of low GI foods has been associated with less episodes of hyperglycemia, which aids in the achievement of improved glycemic control as well as appetite control and weight management [5,20]. Such an effect of reduced sugar release rate shows a great potential for the use of β -glucans as local additives to improve the nutritional quality of commonly consumed foods; however, further studies are needed to determine the long-term glycemic control in healthy and diabetic patients.

Incremental area under the glucose curve (iAUC) data indicated that potato had the highest mean value and tapioca the least value. This implies that tapioca would be a better starch alternative due to its lower glycemic response reflecting a potentially lower GI, followed by corn, rice, and then potato. It must be noted, however, that consuming those starches as whole foods might elicit a different glycemic response, thus it is only safe to recommend these starches in their native forms or in the form of flour. This is because only a minimal amount of other nutrients are available in the form of flour that could interfere with its mode of action. Such recommendation should still be done with caution due to the fact that these results were obtained from *in vitro* observations that need to be further supported by *in vivo* studies.

Thermal properties

The addition of β -glucans resulted in a marked increase in the enthalpy of gelatinization ΔH of rice and tapioca starches, while a decrease was observed in potato and corn starches. Decrease in ΔH values with the addition of β -glucans to the potato and corn starches demonstrate that the blank starches required more energy to breakdown which decreased with the addition of more β -glucans. This finding in agreement with a previous study that showed a significant reduction in the degree of starch gelatinization after substitution of wheat starch with 5% barley β -glucans [21].

This observation suggests that the process of gelatinization involving the destruction of the starch's crystalline structure and loss of helical conformation was affected by the presence of β -glucans at the concentrations tested. This is likely to be due to the high intrinsic viscosity of β -glucans in solution that in turn restricts the amount of water available for diffusion into the starch granule, making it harder to penetrate and dissociate the granule's pseudocrystalline region [15]. Specifically, substituting starches with oat β -glucans made them more resistant to gelatinization. A study examining the effect of adding different kinds of β -glucans on the pasting properties of rice starch showed that the addition of β -glucans significantly increased the paste viscosity of the starch [22].

This can be interpreted as a potentially longer transit time and a slower digestion rate in the gut. This theory has been further illustrated in *in vivo* studies that have revealed a delay in the gastric

emptying as well as intestinal absorption of carbohydrates due to the elevated viscosity caused by β -glucans, which reduced postprandial hyperglycemia and insulin secretion. These actions, in turn, prolong the feeling of satiety and aid in weight control [23,24]. Another theory behind this mechanism of action is suggested to be the capability of β -glucans to alter the microstructure of food products [25].

The rate and degree of starch digestion is a major determinant to the rate of blood glucose rise. The observed results of reduced glucose release post the addition of 20% and 30% β -glucans might be explained as a slower starch breakdown rate elicited by the competition of β -glucans for water that prevented the starch from swelling, rendering it less susceptible to enzymic attacks and hence less available for digestion. A study on the effect of oat β -glucans on starch digestibility demonstrated similar results indicating that as the *in vitro* extract viscosity of β -glucans increased, the *in vitro* starch digestibility decreased as indicated by a lower iAUC as well as a reduced amount of RDS and an increased amount of SDS [26]. Another study by Kim and White [14], illustrating that a higher concentration of β -glucans resulted in a slower *in vitro* digestion rate also showed that the viscosity of oat flours increased with a higher concentration and molecular weight of β -glucans.

This evidence is consistent with previous research studies suggesting that the primary factor responsible for the low glycemic response produced by β -glucans is their viscosity. Wood et al. [27], showed that the viscosity of β -glucans accounted for 79%–96% of the changes in postprandial glucose and insulin response following the ingestion of a 50 g glucose drink. In addition to the concentration and total amount of β -glucans in food, the hypoglycemic effects of β -glucans resulting from their viscosity is suggested to be controlled by their solubility and molecular weight [15]. Soluble fibers appear to inhibit *in vitro* starch degradation and digestion to a larger extent than insoluble dietary fibers [28,22]. However, soluble fibers from the same source have been shown to exert differing levels of effect on starch digestibility, indicating that a fiber's ability to inhibit starch digestion may depend on the processing method as well as the product itself (Brennan 2005). Moreover, the effectiveness of β -glucans is also determined by its structure and the purity of the extract used [22].

The current data on thermal properties showed a rise in the temperature range (ΔT) with the addition of β -glucans in potato and corn starches (Table 1). This might have been caused by a shift from a homogeneous set of amylopectin crystallites with similar stability to a more heterogeneous set with varying stability, in this case due to the addition of β -glucans [29]. A study done by Biliaderis et al. [30] found similar results produced by the addition of β -glucans to maize starch. This was thought to be caused by a more stable granular structure of starches after the addition of β -glucans.

Conclusions

The current study demonstrated that substitution with 20% and 30% oat β -glucans improved the estimated glucose response of all four starches studied by reducing the rate of starch breakdown and glucose release during *in vitro* digestion. Results showed a dose-response relationship with a higher amount of β -glucans giving a more pronounced inhibitory effect on starch degradation. The

reduction in glucose release is of great relevance to human nutrition as it provides a way to regulate in vivo sugar release from traditionally high glycemic index foods thus enhancing their glycemic response. This suggests a great potential for the use of β -glucans as local food additives aiming to improve the nutritional quality of foods and expanding healthy food options for diabetics and individuals at risk of type II diabetes.

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