Scientific Research Publishing

Purification of *β*-Glucan of Oyster Mushroom (*Pleurotus pulmonarius*) and Its Application in Model Food

Hulya Demir

Health Science Faculty, Yeditepe University, Istanbul, Türkiye
Email: hdemir40@gmail.com

Abstract

Mushrooms are recognized as sources of *β*-glucan. The aim of this study was to purify *β*-glucan from oyster mushrooms (*Pleurotus pulmonarius*) by alkaline extraction. The extracted *β*-glucan was added to a cracker recipe and the sensory, physical and estimated glycemic index of the final product were evaluated. Optimum extraction parameters were determined to be 80°C, 90 minutes and 30% KOH. The *β*-glucan crackers had a glycemic index ranging from 88.07 to 67.32, while glucose had a glycemic index of 100. Based on the sensory study, it can be concluded that crackers enriched with *β*-glucan and possessing functional qualities may meet consumer expectations.

Keywords

1. Introduction

Mushrooms are remarkably nutritious due to containing high-quality proteins, vitamins and minerals. Mushrooms which include low calorie and a little fat and non starchy with a little amount of sugar are very suitable and delightful for obese people with no starch and very little amount of sugar. Although Türkiye is rich in mushrooms, *β*-glucan is not produced in our country. *β*-glucan products are imported to be used as food supplements and as food additives in food production processes. *β*-glucan, which has attracted attention in recent years through its potential contributions to health, is also used as an additive in many foods due to its beneficial effects as well as its gelling and thickening features [1]. Using *β*-glucan as a food additive in a variety of food industries, including breakfast cereals, prebiotic sausage formulations, sports nutrition products, dairy products like yoghurt,

bakery products like cookies, bread, cakes, and ready-to-eat snacks, beverages, salad dressings, and fat substitutes, has a noticeable potential in the near future [2].

These products have some functionalities like a remarkable effect on physical and sensory properties, calorie and cholesterol-reducing effects, and other benefits [3].

The present study aims to purify β-glucan from oyster mushrooms via alkaline extraction. Then, the produced β-glucan was added for the first time to a bakery product, a cracker, and the properties of the final product were characterized. The availability of foods that contain sufficient β-glucan to promote health or provide overall health benefits for the consumer is limited. Therefore, the findings of this study may significantly impact the integration of β-glucan into food products.

2. Material and Method

The oyster mushrooms were *Pleurotus pulmonarius* cultivated in Kutahya and purchased from a local grocery store in Kutahya, Türkiye. Analytical-grade chemicals were obtained from either Sigma-Aldrich Co. or Merck. The moisture content of the fresh mushrooms was determined using an automatic moisture analyzer (Weighed moisture box. A&D company Ltd. N 92; P1011656; Japan) [4]. Crude protein was analyzed using the methods described by the American Association of Cereal Chemists (AACC, 2000) [5]. A modified version of the Folch *et al.* (1957) technique [6] was utilized to determine the overall lipid content. A mixture of 50 mL chloroform:methanol (2:1 v/v) and five grams of finely ground mushrooms were combined, stirred, and left undisturbed for three days. The solution was filtered using a table centrifuge and centrifuged at 1000 g. The top layer of methanol was extracted using a Pasteur pipette, and heating was applied to evaporate the chloroform. The residue was the crude lipid [7]. The sample was precisely weighed into a crucible at one gram. The crucible was positioned on a clay pipe triangle and subjected to a low flame until the entire sample was completely charred. It was then baked in a muffle furnace for nearly six hours at 600˚C [8]. The conventional carbohydrate technique, with slight modifications, was employed to determine the quantity of available carbohydrates. The total amount of carbohydrates was calculated using the phenol-sulfuric acid technique, as per Fox and Robyt (1991) [9]. Energy is obtained from fat, protein, or carbohydrates, and its total amount was determined using the equations presented below [10].

Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g lipid);

Energy (kJ) = 17 × (g protein + g carbohydrate) + 37 × (g lipid)

All calculations were obtained through triplicate (n = 3).

2.1. Extraction of β-Glucan from Mushroom

With slight modifications to the technique [11], the polysaccharide was ex-

tracted from Yap and NG. First, 400 g of fresh oyster mushroom fruiting bodies were cleaned and cut into small pieces. The entire mushroom samples were then heated with KOH after cooking in 80% ethanol reflux. Wei and colleagues utilized alkali to extract β-glucan [12]. Sliced and ground oyster mushrooms were soaked in 80% ethanol to induce reflux for 6 hours. Afterwards, the mushrooms were mixed with varying concentrations of KOH (10%, 20%, 30%, and 40%), and then incubated at incrementally higher temperatures (50°C, 60°C, 70°C, 80°C, 90°C), with each iteration being allowed to stand for incrementally longer periods of time (30, 60, 90, 120, and 150 minutes). The key variables under study are the KOH concentration, extraction temperature, and duration. The extract was filtered using Whatman Nr1 filter paper and concentrated in a rotary evaporator operating at 60°C under reduced pressure. Concurrently, the Sevag reagent (chloroform/butanol, 4:1) was employed to remove proteins from the extract, which was then subjected to a three-fold concentration increase through the addition of 95% ethanol. The resulting mixture was centrifuged at 1500 g for 10 minutes at 20°C, and the pH of the supernatant was adjusted to 10 using Na_2CO_3. Following centrifugation at 22,000 g for 20 minutes at 4°C, the desired supernatant was obtained. A mixture of 80% ethanol and supernatant in a 1:2 ratio was maintained for 15 minutes. Following centrifugation at 4°C and 3500 g, the resulting pellets were dried in a vacuum oven.

2.2. Production of Crackers

A cracker recipe was developed based on a thorough review of literature. The necessary ingredients for the cracker consist of refined wheat flour, industrial shortening, salt, sugar, and water (refer to Table 1 for details [13]).

After mixing the cracker ingredients for 15 minutes in the mixer, the dough was flattened with a rolling pin and cut into circular shapes of equal thickness using a mold. The crackers were then placed on trays and baked at 175°C for 15 - 20 minutes in an electric oven. After cooling at room temperature, the cracker samples were stored in zipped pouches for a storage stability study lasting 3 months in a dark room at room temperature.

Table 1. The ingredients of cracker.

Control	Extracted 5 g β-glucan	Extracted 10 g β-glucan	Extracted 15 g β-glucan	Extracted 20 g β-glucan	Extracted 25 g β-glucan	Extracted 30 g β-glucan
250 g rafined wheat flour	245 g rafined wheat flour	240 g rafined wheat flour	235 g rafined wheat flour	230 g rafined wheat flour	225 g rafined wheat flour	220 g rafined wheat flour
50 industrial shorts	50 g industrial shortening	50 g industrial shortening	50 g industrial shortening	50 g industrial shortening	50 g industrial shortening	50 g industrial shortening
5 g salts	5 g salts	5 g salts	5 g salts	5 g salts	5 g salts	5 g salts
112.5 g water	112.5 g water	112.5 g water	112.5 g water	112.5 g water	112.5 g water	112.5 g water

2.2.1. Chemical Analyses Performed in Crackers

Moisture Analysis Moisture content in the samples of crackers was determined using the OHAUS MB 45 rapid moisture analyzer (at 103°C with a 1 g sample) [14]. **Protein Analysis** The protein content in the cracker samples was determined using the Kjeldahl method in accordance with the AACC (2000) 46 - 12 method. The resulting values were multiplied by a factor of 6.25 and used to calculate the final protein content using a formula [15]. **Ash Analysis** The ash content in cracker samples was determined using AACC Method No: 08-01 [16]. 2.3.1.4.

2.2.2. Determination of Textural Properties of Cracker Samples

The fragility and hardness values of cracker samples produced with the addition of β-glucan were determined with the help of TA. Pretest velocity (1 mm/s), test velocity (2 mm/s), post-test velocity (10 mm/s), gap (7 mm), and trigger power in analyzes performed with the help of a 3-point bending probe (HDP/3PB) (10 g) is set. Results are expressed as hardness (hardness, g-force) and fracturability (g-force) [17].

2.2.3. Steps of Glycemic Responce Determination System

Determination of glycemic response was carried out after *in vitro* simulated three-stage digestion system called as mouth, stomach, and small intestine [18].

Step 1: Oral digestion (Digestion in mouth) For *in vitro* oral digestion in the current study, a coffee grinder was used instead of chewing. Two separate samples of shredded cracker were ground and homogenized in the coffee grinder for 0.5 to 1 minute at room temperature. **Step 2: Stomach digestion** A 100 mL magnetic stirrer combined 0.5 grams of guar gum with pepsin enzyme, which contained over 250 international units of enzyme. A small amount of 0.1 N hydrochloric acid (HCl) was added, and the mixture was agitated using the magnetic stirrer. Afterward, the volume was adjusted to 0.1 HCl. **Step 3: Small intestine solution** Intervase (25.43 U/mL), amyloglucosidase (13.4 U/mL), and pancreatin (136 mg/mL) were added to each sample. For each sample, 4 mL of deionized water and 680 mg of pancreatin were put into a 50 mL falcon tube, the mixture was vortexed and then centrifuged for 10 minutes at 8000 rpm. The volume was completed to 5 mL after the supernatant was removed. 67 IU of amyloglucosidase enzyme (AMG), and 127.15 U of invertase were added.

Weigh the 250 mL Erlenmeyer flask with the 1 g homogenized sample. The mixture was then incubated for 30 minutes at 37°C in a water bath, with 10 mL of enzyme solution 1 and 5 mL of deionized water added while being shaken. Before adding 5.0 mL of 0.5 M sodium acetate solution, the pH was adjusted to 5.2. To prepare the entire quantity, 5 mL of enzyme solution 2 were combined with 100 mL of deionized water. The solution was then incubated in a water bath at 37°C for shaking. Samples were collected at 20, 30, 60, 90, 120, and 180 minutes and 0.5 mL was transferred to a 10 mL glass tube. Enzyme denaturation was achieved by keeping the tube in a 100°C water bath for 5 minutes. Next, the

sample was combined with deionized water in a 15 mL plastic falcon tube and centrifuged at 8000 rpm for 5 minutes. A mixture of 3 mL of GOPOD enzyme solution and 0.1 mL of the sample was prepared in a 10 mL glass tube. The participants were required to remain in a water bath that was heated to 50°C for 20 minutes prior to the spectrophotometer taking a reading of their absorbance at 510 nm [18].

2.2.4. Hydrolysis Index (HI) Procedure and Expression of HI Value

The Hydrolyzed Index (HI) value for glucose was determined using an Excel application, based on Hydrolyzed Curves, at five different durations between 0 and 120 minutes, as a result of *in vitro* digestion of each β-glucan sample. The HI value of commercial glucose was also determined using the same methods [18]. The HI values were then used to estimate the glycemic index (pGI) for each sample.

$$\text{AUC} = C_\infty \left(t_f - t_0 \right) - \left(C_\infty / k \right) \left[1 - \exp \left[-k \left(t_f - t_0 \right) \right] \right]$$

C_∞ is the ratio of the test sample's area under the hydrolysis curve (AUC) between 0 and 120 minutes to the reference sample's area under the curve (glucose). T The formula used to get the glycemic index value was discovered by Goni and reads as follows: Goni {GI = [39.71 + (0.559 × HI)]}.

2.2.5. Sensory Analysis

A sensory analysis of β-glucan crackers was carried out following a literature review. We modified a tasting form for the study and utilized the 1 - 9 Hedonic scale. This method is frequently used in consumer testing with untrained panelists, and it is the most common way to measure consumers' perceptions of a product. With the hedonic scale, we were able to measure the opinions, preferences, and likes/dislikes of the panelists regarding the product. There are nine options in this scale, ranging from "Like Extremely" to "Do Not Like at All," and consumers are asked to select one of these options for the product being offered. Two types of 9-point hedonic scales were employed, including the unipolar hedonic scale that ranges from 1 to 9. The study utilized a control group and 20% β-glucan crackers [19].

2.3. Statistical Analysis

Several tests were conducted to observe statistical differences among experimental groups. One-way ANOVA was utilized to compare groups at varying temperatures (50°C, 60°C, 70°C, 80°C) and four intervals (30, 60, 90, 120, 150 minutes). This method determines whether the means of independent groups are equivalent. Pearson correlation analysis was employed to assess the relation between time and the amount of β-glucan recovered. This analysis examines the magnitude and direction of the linear association between two variables. The correlation coefficient (*r*) reveals the strength and direction of the relationship, while the p-value determines the statistical significance of the association. All

analyses were completed at a threshold of significance where the p-value was <0.05, indicating statistical significance of the findingsicant [20].

3. Results and Discussion

3.1. Moisture Content, Ash, Crude Fat, Crude Protein, CHO, Energy (kcal/100g)

Different mushroom types have different nutritional qualities in terms of dry matter, moisture, crude protein, ash, organic matter, and energy. Nutritional value and bioactive chemical content of mushrooms differ due to species, maturity level, substrate, and growth conditions. *P. pulmonarius* is known for its high energy, protein, and carbohydrate content [20]. This study identified the following nutritional values: 84.6% moisture, 40.6% kcal energy, 3.40% protein, 0.89% fat, 0.71% ash, and 4.75% CHO. Previous research has outlined that fresh mushrooms have 90% moisture and 10% dry substance [20] [21] [22] [23] [24], thus potentially increasing the consumption of protein-rich food items created from edible mushrooms while providing a delicious substitute for animal protein [25]. Additional factors influencing mushroom growth include variations in the physical and chemical composition of the growth environment, the nutritional content of the substrate used for culture, and the specific species of the mushroom. It is important to note that the nutritional composition of the substrate and growing conditions can significantly impact the fat content of the mushrooms. Typically, mushrooms contain a low amount of fat and are dominated by unsaturated fatty acids. *P. pulmonarius* has a crude fat content of 0.89%, while fat levels in Pleurotus spp. have been observed to range from 0.85% to 3.16% [26] [27] [28]. *P. pulmonarius* was estimated to have an energy value of 40.6 kcal. *Pleurotus pulmonarius* was estimated to have an energy value of 40.6 kcal. Upon comparison with data from other investigations [28] [29], it was found that *P. pulmonarius* had a similar energy value.

3.2. Extraction of *β*-Glucan from Mushroom

As the concentration of KOH increased, more N- and O-type carbohydrate-peptide connections were degraded, resulting in the release of more polysaccharide. However, when the KOH concentration was increased above 30%, the structure of the polysaccharide was damaged. High temperature caused protein denaturation and a decrease in extraction rate. Extraction time is another factor that affects polysaccharide extraction; prolonged extraction time leads to polysaccharide degradation. Therefore, an optimal extraction time of 90 minutes was determined. Alkaline extraction was selected as the preferred technique based on the rate of polysaccharide extraction. The procedure involved gradually increasing the KOH concentration (0%, 10%, 20%, 30%, and 40%) and incubation temperatures (50˚C, 60˚C, 70˚C, 80˚C, and 90˚C) for increasing durations of time (30 minutes, 60 minutes, 90 minutes, 120 minutes, and 150 minutes). The text is grammatically correct, precise, objective, and follows conventional

academic structure. Citations are properly formatted, and the language is formal and balanced. Meanwhile, we considered three variables: KOH concentration, extraction temperature, and extraction time. Each element was checked three times. In conclusion, the optimal extraction parameters were 30% (w/v) KOH concentration, 80˚C extraction temperature, and a 90-minute extraction time, with 90 minutes being the ideal period. It is only by isolating purified β-glucan from the plant matrix using various extraction methods that such an outcome is possible. Several pH levels, including acidic, neutral, and alkaline, can be utilized in the wet extraction process. Alkaline water processes yield the highest amount of extracted beta-glucan, although the process possesses unique properties [28] [29] [30]. Both neutral pH and notably alkaline pH coincided with a simultaneous increase in protein solubility [31] [32] [33].

3.3. Production of Crackers

The production of crackers utilized a modified formulation and baking process based on the American Association of Cereal Chemists (AACC 2017) [34] standard. The amount of wheat flour used in this formulation was reduced by 5, 10, 15, 20, 25, and 30 g, and β-glucan was used instead, and the other ingredients and their amounts in the formulation were not changed (Figure 1).

Chemical Analyses Performed in Crackers
Results of analysis are shown in Table 2.

Figure 1. Crackers with varying amounts of β-glucan.

Table 2. β-glucan amount (g), protein (%), ash (%), moisture (%).

Samples	β-Glucan amount(g)	Protein (%)	Ash (%)	Moisture (%)
1	0	9.40 ± 0.23	2.10 ± 0.06	4.15 ± 0.21
2	5	9.45 ± 0.23	2.08 ± 0.06	5.07 ± 0.21
3	10	9.58 ± 0.23	2.33 ± 0.07	5.27 ± 0.23
4	15	10.50 ± 0.26	2.24 ± 0.06	6.06 ± 0.23
5	20	10.67 ± 0.26	2.25 ± 0.06	6.21 ± 0.21
6	25	10.72 ± 0.26	2.74 ± 0.02	6.63 ± 0.22
7	30	10.93 ± 0.27	2.76 ± 072	7.74 ± 0.23

The protein value (%) decreases as the amount of β-glucan (g) approaches to 0 while the protein value (%) is the highest in those with a β-glucan amount (g) of 30. Increases in the moisture value (%) are greater when the amount of β-glucan (g) changes from 0 to 5, 10 to 15, and 25 to 30. Although a decrease is observed in the ash value (%) when the amount of β-glucan (g) changes from 0 to 5 and 10 to 15, the ash value (%) increases in all other changes of the amount of β-glucan (g). As the amount of β-glucan (g) increases from 0 to 30, the protein (%) and moisture (%) values increase proportionally. On the other hand, there is a decrease in Ash (%) values at 0 - 5 and 10 - 15 changes despite of a proportional increase in all other changes (Table 3).

The moisture content of crackers is sensitive to changes in temperature during the baking process. The moisture content primarily affects the shelf life of the cookie. The baking process converts the viscoelastic batter to a solid, baked good. This process establishes characteristics of the resulting cookie, such as cracker size (diameter and thickness), cookie weight, and moisture levels [35].

3.4. Textural Properties of Craker Samples

Texture properties, which are important parameters for consumer acceptance of food products, determine production capacity. Bakery product textures, for instance crackers, are typically characterized by their mechanical properties. Table 4 exhibits the hardness values of the doped crackers. Hydrocolloids negatively impact starch structure. Some studies suggest that the increase in dough softness

Table 3. Statistical analysis showing the relationship of β-glucan amount (g) with protein (%), ash (%) and moisture (%).

		β-Glucan amount (g)	Protein (%)	Ash (%)	Moisture (%)
Beta-Glucan amount (g)	r	1	0.946**	0.880**	0.980**
	p		0.001	0.009	0.000
	n	7	7	7	7
Protein (%)	r		1	0.738	0.921**
	p			0.058	0.003
	n		7	7	7
Ash (%)	r			1	0.842*
	p				0.017
	n			7	7
Moisture (%)	r				1
	p				
	n				7

*p < 0.05, *p < 0.01.

Table 4. Structure analysis of cracker samples produced with the addition in different amounts of β-glucan (g).

Sample	Dry Matter (%)	Hardness (g-force)	Fracturability (g-force)
Control	95.85	17126.45 ± 2345.32	17,255 ± 2432.12
5 g β-glucan	94.93	17852.47 ± 3437.58	13906.85 ± 2674.58
10 g β-glucan	94.73	17814.07 ± 4180.41	13082.46 ± 1811.05
15 g β-glucan	93.94	31169.05 ± 3797.95	13434.02 ± 2702.84
20 g β-glucan	93.79	34129.30 ± 4443.23	7584.98 ± 985.67
25 g β-glucan	93.37	16886.23 ± 3105.48	16538.52 ± 1878.92
30 g β-glucan	92.26	15409.50 ± 1306.27	10936.43 ± 1629.09
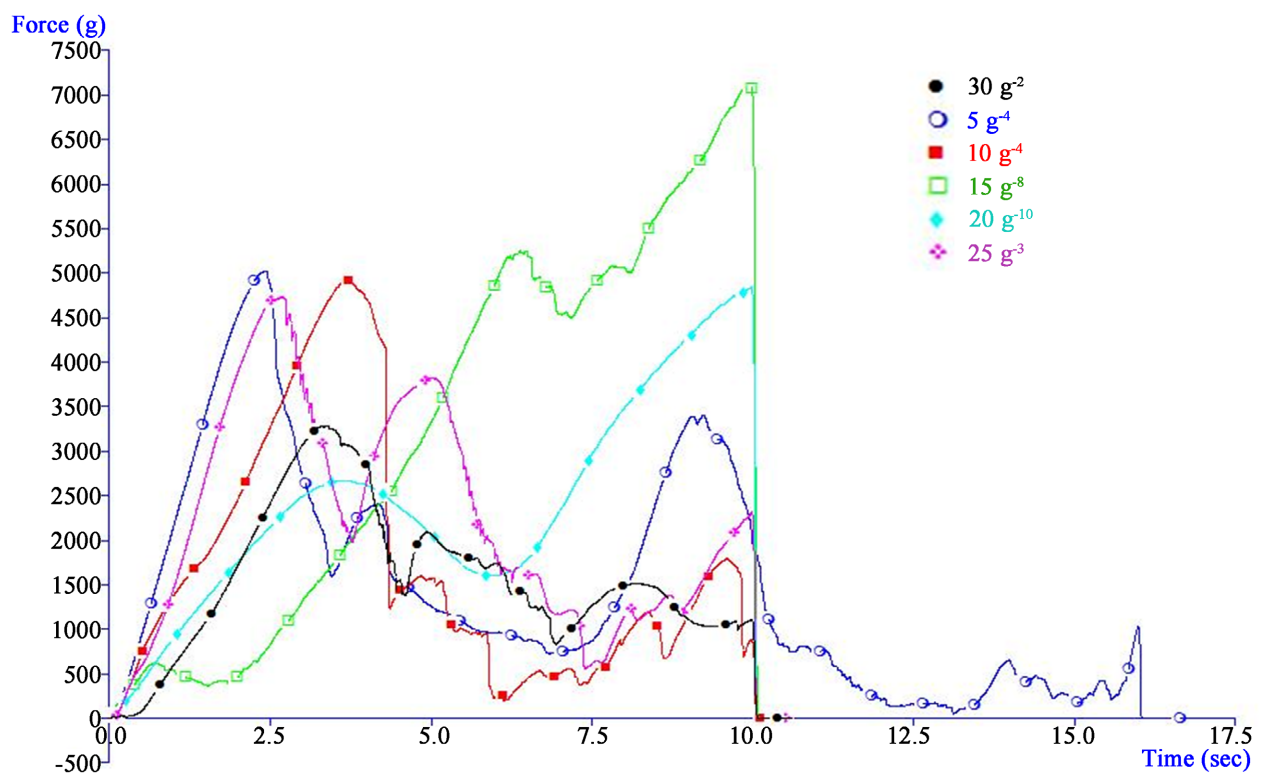
Figure 2. Graphic of the crackers hardness (g-force) and fracturability (g-force).

parallels the increase in hydrocolloid concentration. Hardness refers to the amount of force needed to compress a material. Fracturability, on the other hand, refers to how easily the material breaks.

As shown in Figure 2, the crackers with added β-glucan exhibited lower maximum breaking force values (N) as compared to the control. A decrease in hardness was observed with an increase in the β-glucan content added to the crackers. The addition of β-glucan may dilute the protein gluten, leading to a decrease in the hardness of the crackers. This delay in the formation of the gluten matrix, caused by the absorption of water by β-glucan, may explain the ob-

served decrease. The ideal cracker should have a certain level of crispness and hardness.

The results of the correlation test performed to examine the relationship between Dry matter (%), Hardness (g-force) and Fracturability (g-force) are shown in Table 5. The analysis showed no statistically significant relationship between Dry Matter (%), Hardness, and Fracturability (g-force) ($p > 0.05$). Also, there is no statistically significant relationship between Hardness and Fracturability ($p > 0.05$).

The analysis reveals a variation between the Hardness (g-force) and Fracturability (g-force) of all samples, with each sample having a higher Hardness value. The sample with the most substantial difference is seen in 15 g β-glucan and 20 g β-glucan. An increase in hardness (strength) values was observed in 15% - 20% cracker samples prepared by adding β-glucan at different rates, and harder and more durable crackers were obtained. It is believed that the increased hardness is due to the increase in water absorption. The hardness value of crackers, as with other quality traits, is influenced by the dough content and production process. Furthermore, hardness is one of the most important quality criteria affecting consumer taste, and a cracker that is too hard or too soft is not preferred. Excessive crispy and delicate structure of crackers leads to crumbling during packaging, transportation, and marketing, resulting in economic losses. The cracker should have enough hardness to provide crispiness and softness to give an orally disintegrating texture in the mouth.

3.5. Hydrolysis Index and Estimated Glycemic Index

It is demonstrated that the rate of hydrolysis of β-glucan crackers can be easily tested using a simple *in vitro* approach. Estimating the glycemic response to β-glucan crackers could be beneficial. It is important that the *in vitro* approach allows us to mimic enzymatic digestion as closely as possible. The process utilized alpha-amylase to hydrolyze starch in this study, and pepsin was utilized to avoid protein-starch interactions. Amyloglucosidase was utilized to release glucose from glucose hydrolysis products, with the specific enzyme used varying across different approaches. While amylase was sometimes used independently,

Table 5. Statistical analysis showing the relationship of β-glucan amount (g) with hardness (g-force), fracturability (g-force).

		Dry Matter (%)	Hardness (g)	Fracturability (g)
Dry Matter (%)	r	1	−0.072	0.212
	p		0.878	0.649
Hardness (g)	r		1	−0.564
	p			0.187
Fracturability (g)	r			1
	p			

it was also combined with proteases [36]. It can be concluded that the *in vitro* method could aid in assessing GI. Additionally, a mathematical first-order equation was discovered to describe the hydrolysis properties of each food item. In heated cracker slurries, GI was shown to have an inverse correlation with *β*-glucan concentrations.

The hydrolysis index (HI) was determined by calculating the area under the hydrolysis curve of the sample (0 - 120 min) relative to the area of glucose (reference sample). The glycemic index (GI) was calculated utilizing the equation proposed by Goni *et al.* [37]. **Table 6** and **Table 7** illustrate the calculated HI and *in vitro* GI values for the *β*-glucan crackers. Additionally, **Figure 3** shows the hydrolysis curves for *β*-glucan crackers. The samples with the lowest GI were those containing the greatest quantity of *β*-glucan when compared to the other samples. It is assumed that the GI is influenced by the *β*-glucan content. The ratio of the area under the curve (AUC) in response to a test and reference meal consumed by an individual over many days and under predetermined conditions is used to calculate GI. The AUC is a measure of the blood glucose response [38]. High, medium, and low GI meals refer to meals with GI values over 70, 55 - 69, and below 55, respectively [38]. White wheat bread has a GI rating of 100 [39]. Those seeking to lower their blood glucose levels, such as diabetic patients, prefer the consumption of low glycemic index foods [40]. The graphs demonstrate that the hydrolysis rate increases during the initial 0 to 90 minute period, followed by the gradual attainment of a myxial plateau between 90 and 120 minutes.
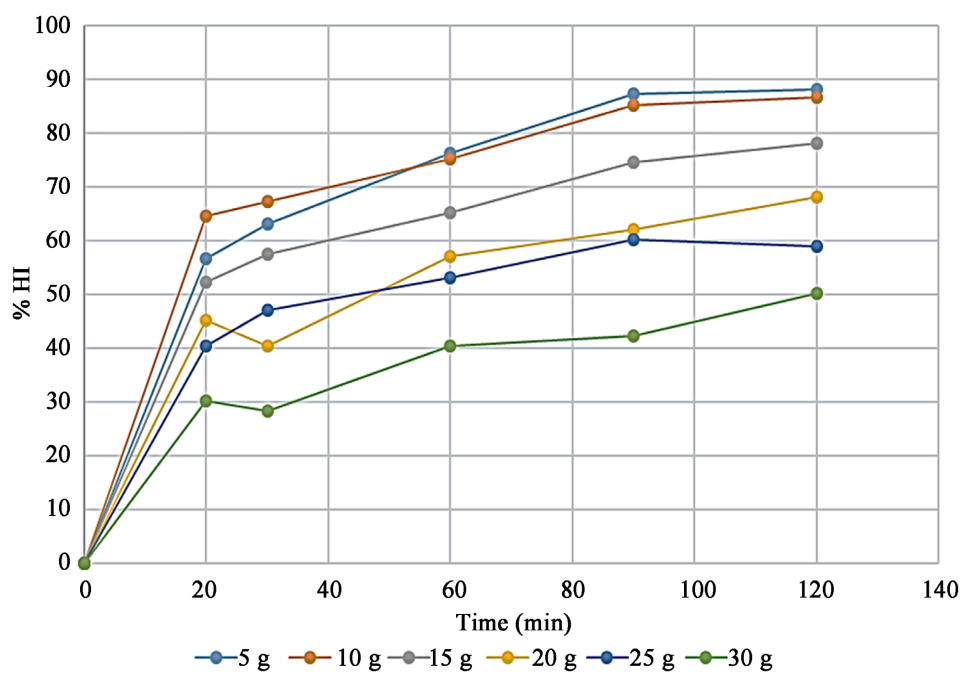
This table displays the Pearson correlation coefficients (r) and associated p-values, examining the relationship between varying amounts of *β*-glucan (5 g, 10 g, 15 g, 20 g, 25 g, and 30 g) and time. The sample size (n) is presented for each correlation coefficient. The results indicate a strong, statistically significant positive correlation with time, with r = 0.835 and p = 0.000. The time increases significantly with greater amounts of *β*-glucan. For the 10-gram *β*-glucan intervention, there was a strong positive correlation between *β*-glucan and time (r

Table 6. Hydrolysis index of 5 g, 10 g, 15 g, 20 g, 25 g, 30 g *β*-glucan-containing crackers and glucose between 0 and 120 minutes.

β-glucan	5 g	10 g	15 g	20 g	25 g	30 g
Time (min)	HI	HI	HI	HI	HI	HI
0	0	0	0	0	0	0
20	56.6	64.6	52.3	45.2	40.5	30.2
30	63.2	67.3	57.5	40.5	47	28.3
60	76.4	75.2	65.2	57.2	53.2	40.4
90	87.4	85.2	74.6	62.1	60.2	42.4
120	88.1	86.7	78.2	68.1	59	50.3

Table 7. β-glucan and HI correlation.

		Time
	r	0.835**
β-glucan (5 g)	p	0.000
	n	18
	r	0.776**
β-glucan (10 g)	p	0.000
	n	18
	r	0.815**
β-glucan (15 g)	p	0.000
	n	18
	r	0.848**
β-glucan (20 g)	p	0.000
	n	18
	r	0.805**
β-glucan (25 g)	p	0.000
	n	18
	r	0.877**
β-glucan (30 g)	p	0.000
	n	18

Figure 3. Comparison of GI of crackers containing β-glucan.

= 0.776, p = 0.000), indicating a statistically significant relationship. Likewise, the higher amounts of β-glucan (15 g, 20 g, 25 g, and 30 g) were also positively correlated with time, indicating a significant relationship. In all cases, the p-values were below 0.05, indicating statistical significance. Two asterisks (**), representing a high level of statistical significance with a p-value of less than 0.01, generally indicate the strength of the results. In summary, this table indicates that the increase in the amount of β-glucan results in a proportionate increase in time. This suggests that β-glucan may have various applications depending on the specific process it affects.

This table presents the Pearson correlation coefficients (r) and associated p-values between different amounts of β-glucan (5 g, 10 g, 15 g, 20 g, 25 g and 30 g) and time (Table 8). n represents the sample size for each correlation coefficient (41).

For β-glucan (5 g): r = 0.729, p = 0.001. This indicates a strong and statistically significant positive relationship between time and β-glucan. As the amount of beta-glucan increases, the time also increases. This correlation coefficient also shows that there is a strong and statistically significant positive relationship between time and β-glucan, with r = 0.690 and p = 0.002. There is a strong and statistically significant positive relationship between time and β-glucan for other amounts of β-glucan (15 g, 20 g, 25 g, and 30 g). These results are statistically significant as the p-value is much less than 0.05. Two asterisks (**) often indicate that the p-value is less than 0.01, which represents a very strong level of statistical significance. Therefore, as shown in this table, increasing amounts of β-glucan also increase the time (Table 9). This could suggest that an event or process impacted by β-glucan will have a longer duration as the concentration of β-glucan increases.

The study found the lowest values for HI (30.2%) and pG1 (56.38%) in 30 g of β-glucan. The results indicated that the quantity of β-glucan in cracker formulations was associated with lower HI, resulting in a decrease in the anticipated glycemic index (pGI) (Table 10). Several studies have shown that a low-GI diet

Table 8. Glycemic index of 5 g, 10 g, 15 g, 20 g, 25 g, 30 g β-glucan-containing crackers and glucose between 0 and 120 minutes.

β-glucan	5 gr	10 gr	15 gr	20 gr	25 gr	30 gr
Time (min)	GI	GI	GI	GI	GI	GI
0	0	0	0	0	0	0
20	70.78	75.18	68.42	64.52	61.94	56.28
30	74.4	76.65	63.16	61.94	65.51	55.24
60	80.96	80.99	74.46	71.11	68.91	61.72
90	87.69	86.48	80.66	73.8	72.75	62.98
120	88.07	87.31	82.64	77.09	72.1	67.32

Table 9. Correlation between β-glucan and GI.

		Time
β-glucan (5 g)	r	0.729**
	p	0.001
	n	18
β-glucan (10 g)	r	0.690**
	p	0.002
	n	18
β-glucan (15 g)	r	0.740**
	p	0.000
	n	18
β-glucan (20 g)	r	0.727**
	p	0.001
	n	18
β-glucan (25 g)	r	0.679**
	p	0.002
	n	18
β-glucan (30 g)	r	0.716**
	p	0.001
	n	18

Table 10. Hydrolysis index and Glycemic index correlation analysis with sample.

Correlations							
		GI 5 gr	GI 10 gr	GI 15 gr	GI 20 gr	GI 25 gr	GI 30 gr
HI 5 gr	r	0.982**					
	p	0.000					
HI 10 gr	r		0.991**				
	p		0.000				
HI 15 gr	r			0.987**			
	p			0.000			
HI 20 gr	r				0.972**		
	p				0.001		
HI 25 gr	r					0.982**	
	p					0.000	
HI 30 gr	r						0.955**
	p						0.003

improves the metabolic effects of insulin resistance and reduces insulin resistance [41]. Improvements in fibrinolytic activity may benefit the management of diabetes and cardiovascular disease, in conjunction with changes to glucose and lipid metabolism [41]. Slow-digesting starch refers to a starch fraction that is digested gradually but incompletely in the large intestine, while fast-digesting starch leads to a rapid increase in blood glucose levels following consumption [42]. Slow-digesting starch refers to a starch fraction that is digested gradually but incompletely in the large intestine, while fast-digesting starch leads to a rapid increase in blood glucose levels following consumption [43]. β-Glucan in crackers can be classified as a food with a low glycemic index due to its high-fiber content and abundance of soluble fiber. The consumption of high glycemic index meals can lead to significant fluctuations in blood glucose levels and glucose response demand as they are rapidly digested and absorbed. Conversely, low GI meals, such as those with slow digestion and absorption rates, have a gradual impact on blood sugar and insulin levels. In individuals with Type I and Type II diabetes, a low glycemic index diet may potentially enhance glucose and lipid levels, decrease insulin levels, and alleviate insulin resistance, as suggested by an increasing body of evidence [44].

3.6. Sensory Analysis

The following results from the hedonic test indicate that taste strongly correlated with the overall acceptance of β-glucan crackers. These findings can guide product manufacturers in implementing acceptable flavor enhancement methods to increase consumer acceptance of β-glucan crackers. To improve the sensory acceptability scores, future evaluations should include differently flavored or formulated crackers. Given the high consumption of bagels in Türkiye—approximately 2.5 million per day—enhancing the formulation and promotion of the product would increase β-glucan utilization. In sensory analyses, 20% of participants found the β-glucan samples to possess a slightly bitter taste, potentially limiting their consumption. Improving this taste would facilitate greater consumption of the crackers. Natural sweetening agents are preferred because they do not have any negative effect on health. Natural sweeteners are useful substitutes for diabetic patients. An example, vanilin, steviol glucosides. It is expected that reducing the amount of the bitter sample will eliminate this issue, and further research should be carried out on the nutritional properties of the enriched β-glucan crackers.

4. Conclusions

The yield of purified β-glucan from oyster mushrooms is high (the yield 5.50%), 30% (w/v) KOH concentration, 80°C, and a 90 min extraction time were the ideal extraction parameters.

Lastly, β-glucan was added to the cracker recipe, and the finished product's sensory and physical characteristics as well as its estimated glycemic index were

assessed. β-glucan is best to add to biscuits 30 g due to low GI (56.38%), hardness (g-force) 15409.50 ± 1306.27; fracturability (g-force) 10936.43 ± 1629.09. During the sensory analysis, participants reported that 20% of the samples containing β-glucan exhibited a subtle bitter taste. If this flavor profile were addressed, the crackers could become more palatable and suitable for consumption.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Zhu, F., Du, B. and Xu, B. (2016) A Critical Review on Production and Industrial Applications of β-Glucans. *Food Hydrocolloids*, **52**, 275-288. https://doi.org/10.1016/j.foodhyd.2015.07.003

[2] Lante, A., Canazza, E. and Tessari, P. (2023) β-Glucans of Cereals: Functional and Technological Properties. *Nutrients*, **15**, Article 2124. https://doi.org/10.3390/nu15092124

[3] Sengul, M. and Ufuk, S. (2022) Therapeutic and Functional Properties of Beta-Glucan, and Its Effects on Health. *Eurasian Journal of Food Science and Technology*, **6**, 29-41.

[4] Bradley, R.L. (2010) Moisture and Total Solids Analysis. In: Nielsen, S.S., Ed., *Food Analysis*, Springer, Boston, 85-104. https://doi.org/10.1007/978-1-4419-1478-1_6

[5] Chang, S.K.C. (2010) Protein Analysis. In: Nielsen, S.S., Ed., *Food Analysis*, Springer, Boston, 133-146. https://doi.org/10.1007/978-1-4419-1478-1_9

[6] Folch, J., Ascoli, I., Lees, M., Meath, J.A. and Lebaron, F.N. (1951) Preparatıon of Lipide Extracts from Brain Tıssue. *Journal of Biological Chemistry*, **191**, 833-841. https://doi.org/10.1016/S0021-9258(18)55987-1

[7] American Oil Chemists' Society (1989) A.O.C.S. Official Method Ce lb-89. Official Methods and Recommended Practices of the American Oil Chemists' Society. Champaign.

[8] Raghuramulu, N., Madhavan, N.K. and Kalyanasundaram, S. (2003) A Manual of Laboratory Techniques. Indian Council of Medical Research, New Delhi, 56-58.

[9] Fox, J.D. and Robyt, J.F. (1991) Miniaturization of Three Carbohydrate Analyses Using a Microsample Plate Reader. *Analytical Biochemistry*, **195**, 93-96. https://doi.org/10.1016/0003-2697(91)90300-I

[10] Council Directive 1990 (2013) Nutrition Labelling for Foodstuffs. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1990L0496:20081211:EN:PDF

[11] Yap, A.T. and Ng, M.L. (2001) Immunopotentiating Properties of Lentinan (1-3)-b-D-Glucan Extracted from Culinary-Medicinal Shiitake Mushroom *Lentinus edodes* (Berk.) Singer (Agaricomycetideae). *International Journal of Medicinal Mushrooms*, **3**, 6-19. https://doi.org/10.1615/IntJMedMushr.v3.i1.20

[12] Ahmad, A., Anjum, F., Zahoor, T., Nawaz, H. and Ahmed, Z. (2010) Extraction and Characterization of β-D-Glucan from Oat for Industrial Utilization. *International Journal of Biological Macromolecules*, **46**, 304-309. https://doi.org/10.1016/j.ijbiomac.2010.01.002

[13] Sedej, I., Sakac, M., Mandic, A., Nisan, A., Pestoric, M., Simurina, O. and Canadanovic-Brunet, J. (2011) Quality Assessment of Gluten-Free Crackers Based on Buckwheat Flour. *LWT—Food Science and Technology*, **44**, 694-699. https://doi.org/10.1016/j.lwt.2010.11.010

[14] Yeboah-Awudzi, M., Lutterodt, H.E., Kyereh, E., Reyes, V., Sathivel, S., Manful, J. and King, J.M. (2018) Effect of Bambara Groundnut Supplementation on the Physicochemical Properties of Rice Flour and Crackers. *Journal of Food Science and Technology*, **55**, 3556-3563. https://doi.org/10.1007/s13197-018-3281-0

[15] Straasø, L.A., Saleem, Q. and Hansen, M.R. (2016) A Toolbox of Solid-State NMR Experiments for the Characterization of Soft Organic Nanomaterials. *Annual Reports on NMR Spectroscopy*, **88**, 307-383. https://doi.org/10.1016/bs.arnmr.2015.12.001

[16] Cankurtaran Komurcu, T. (2023) Effect of Hazelnut Skin Addition on Quality Characteristics of Functional Crackers. *Journal of Agriculture and Nature*, **26**, 1368-1376. https://doi.org/10.18016/ksutarimdoga.vi.1222490

[17] Zbikowska, A., Kupiec, M., Szymanska, I., Osytek, K., Kowalska, M., Malciniak-Lukasiak, K. and Rutkowska, J. (2020) Microbial β-Glucan Incorporated into Muffins: Impact on Quality of the Batter and Baked Products. *Agriculture*, **10**, Article 126. https://doi.org/10.3390/agriculture10040126

[18] Goñi, I., Garcia-Alonso, A. and Saura-Calixto, F. (1997) A Starch Hydrolysis Procedure to Estimate Glycemic Index. *Nutrition Research*, **17**, 427-437. https://doi.org/10.1016/S0271-5317(97)00010-9

[19] Silva, A.N., Silva, R.C., Ferreira, M.A.M., Minim, V.P.R., Costa, T.M.T. and Perez, R. (2013) Performance of Hedonic Scales in Sensory Acceptability of Strawberry Yogurt. *Food Quality and Preference*, **30**, 9-21. https://doi.org/10.1016/j.foodqual.2013.04.001

[20] Manzi, P., Gambelli, L. and Marconi, S. (1999) Nutrients in Edible Mushrooms: An Inter Species Comparative Study. *Food Chemistry*, **65**, 477-482. https://doi.org/10.1016/S0308-8146(98)00212-X

[21] Kırbag, S. and Korkmaz, V. (2014) Effect on the Nutritional Value of Some Cultivated Mushroom Species of Various Agricultural Waste Materials. *Artvin Coruh University Journal of Forestry Faculty*, **15**, 126-131.

[22] Sardar, H., Ali, M.A., Anjum, M.A., Nawaz, F., Hussain, S., Naz, S. and Karimi, S.M. (2017) Agro-Industrial Residues Influence Mineral Elements Accumulation and Nutritional Composition of King Oyster Mushroom (*Pleurotus eryngii*). *Scientia Horticulturae*, **225**, 327-234. https://doi.org/10.1016/j.scienta.2017.07.010

[23] Masahito, Y., Mariko, S., Masayoshi, Y., Hirofumi, H., Shazwin, M.T., Shahabaldin, R., Md Din, M.F. and Mohd Hanafi, F.H. (2019) Cultivation of Oyster Mushroom (*Pleurotus ostreatus*) on Fermented Moso Bamboo Sawdust. *Journal of King Saud University—Science*, **31**, 490-494. https://doi.org/10.1016/j.jksus.2018.04.021

[24] Guillamón, E., García-Lafuente, A., Lozano, M., D'Arrigo, M., Rostagno, M.A., Villares, A. and Martínez, J.A. (2010) Edible Mushrooms: Role in the Prevention of Cardiovascular Diseases. *Fitoterapia*, **81**, 715-723. https://doi.org/10.1016/j.fitote.2010.06.005

[25] González, A., Nobre, C., Simões, L.S., Cruz, M., Loredo, A., Rodríguez-Jasso, R.M. and Belmares, R. (2021) Evaluation of Functional and Nutritional Potential of a Protein Concentrate from *Pleurotus ostreatus* Mushroom. *Food Chemistry*, **346**, Article ID: 128884. https://doi.org/10.1016/j.foodchem.2020.128884

[26] Oyetayo, F.L. and Akindahunsi, A.A. (2004) Nutrient Distribution in Wild and Cul-

tivated Edible Mushroom, Pleurotus sajor-caju. *Journal of Food, Agriculture and Environment*, **2**, 166-168.

[27] Rashad, M.M. and Abdou, H.M. (2002) Production and Evaluation of *Pleurotus ostreatus* Mushroom Cultivated on Some Food Processing Wastes. *Advances in Food Sciences*, **24**, 79-84.

[28] Furlani, R.P.Z. and Godoy, H.T. (2007) Valor Nutricional de Cogumelos Comestiveis Nutritional Value of Edible Mushrooms. *Ciência e Tecnologia de Alimentos*, **27**, 154-157. https://doi.org/10.1590/S0101-20612007000100027

[29] Bach, F., Helm, C.V., Bellettini, M.B., Maciel, G.M. and Haminiuk, C.W.I. (2017) Edible Mushrooms: A Potential Source of Essential Amino Acids, Glucans and Minerals. *International Journal of Food Science & Technology*, **52**, 2382-2392. https://doi.org/10.1111/ijfs.13522

[30] Bhatty, R.S. (1995) Laboratory and Pilot Plant Extraction and Purification of β-Glucans from Hull-Less Barley and Oat Brans. *Journal of Cereal Science*, **22**, 163-170. https://doi.org/10.1016/0733-5210(95)90046-2

[31] Harasym, J., Suchecka, D. and Gromadzka-Ostrowska, J. (2015) Effect of Size Reduction by Freezemilling on Processing Properties of β-Glucan Oat Bran. *Journal of Cereal Science*, **61**, 119-125. https://doi.org/10.1016/j.jcs.2014.10.010

[32] Guan, X. and Yao, H. (2008) Optimization of Viscozyme L-Assisted Extraction of Oat Bran Protein Using Response Surface Methodology. *Food Chemistry*, **106**, 345-351. https://doi.org/10.1016/j.foodchem.2007.05.041

[33] Jodayree, S., Smith, J.C. and Tsopmo, A. (2012) Use of Carbohydrase to Enhance Protein Extraction Efficiency and Antioxidative Properties of Oat Bran Protein Hydrolysates. *Food Research International*, **46**, 69-75. https://doi.org/10.1016/j.foodres.2011.12.004

[34] Anonim (2022) Approved Methods of the American Association of Cereal Chemists 2017. http://methods.aaccnet.org/toc.aspx

[35] Cronin, K. and Preis, C.A. (2000) Statistical Analysis of Biscuit Physical Properties as Affected by Baking. *Journal of Food Engineering*, **46**, 217-225. https://doi.org/10.1016/S0260-8774(00)00053-4

[36] Barak, S., Mudgil, D. and Khatkar, B.S. (2014) Effect of Flour Particle Size and Damaged Starch on the Quality of Cookies. *Journal of Food Science and Technology*, **51**, 1342-1348. https://doi.org/10.1007/s13197-012-0627-x

[37] Villemejane, C. and Roussel, P. (2013) Technological and Sensory Tools to Characterize the Consistency and Performance of Fibre-Enriched Biscuit Doughs. *Journal of Cereal Science*, **57**, 551-559. https://doi.org/10.1016/j.jcs.2013.03.005

[38] Warren, F.J., Zhang, B., Waltzer, G., Gidley, M.J. and Dhital, S. (2015) The Interplay of α-Amylase and Amyloglucosidase Activities on the Digestion of Starch in *in vitro* Enzymic Systems. *Carbohydrate Polymers*, **117**, 192-200. https://doi.org/10.1016/j.carbpol.2014.09.043

[39] Reka, P., Azeez, T.B. and Seethalakshmi, M. (2017) Elemental Composition of Selected Edible Seaweeds Using SEM Energy Dispersive Spectroscopic Analysis. *International Food Research Journal*, **24**, 600-606.

[40] Rahelić, D., Jenkins, A., Bozikov, V., Pavić, E., Jurić, K., Fairgrieve, C., Romić, D., Kokić, S. and Vuksan, V. (2011) Glycemic Index in Diabetes. *Collegium Antropologicum*, **35**, 1363-1368.

[41] Aktas, N. and Ozdogan, Y. (2016) Food and Nutrition Literacy. *Harran Journal of Agricultural and Food Science*, **20**, 146-153.

[42] Schneider, I., Heinemann, M. and Hahn, A. (2015) Comparison of Glycemic Index and Satiety of Cereals Containing Amaranth. *Journal of Human Nutrition and Food Science*, **3**, 1074.

[43] Del Prato, S., Leonetti, F., Simonson, D.C., Sheehan, P., Matsuda, M. and DeFronzo, R.A. (1994) Effect of Sustained Physiologic Hyperinsulinaemia and Hyperglycaemia on Insulin Secretion and Insulin Sensitivity in Man. *Diabetologia*, **37**, 1025-1035. https://doi.org/10.1007/BF00400466

[44] Brand, J., Colagiuri, S., Crossman, S., Allen, A., Roberts, D. and Truswell, S. (1991) Low-Glycemic Index Foods Improve Long Term Glycemic Control in NIDDM. *Diabetes Care*, **14**, 95-101. https://doi.org/10.2337/diacare.14.2.95