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In Vitro Evaluation of Antihyperglycemic Activity of Polysaccharide Fractions Derived from Sesame Seeds Hulls

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ABSTRACT

This study aimed to investigate the influence of polysaccharides extracted from sesame seed hulls using ultrasound-assisted alkaline extraction techniques on the inhibition of α -amylase and α -glucosidase activities. Polysaccharides from sesame hulls, with a yield of 6.49%, were separated into three distinct fractions using column chromatography. These fractions were then analyzed for their primary components and their potential to lower blood sugar levels. The major fraction, Pol-1, accounting for 1.74% of the total, contained components such as rhamnose (9.3%), glucose (9.2%), glucuronic acid (14.1%), and galacturonic acid (50.4%), along with other constituents. Our results revealed that varying concentrations of these polysaccharide fractions significantly influenced the reduction of glucose diffusion and the inhibition of both α -amylase and α -glucosidase activities. Notably, Pol-1 exhibited the most substantial inhibitory effects on both enzyme activities, as well as a notable decrease in glucose diffusion rate. These effects have the potential to extend the blood glucose response and regulate postprandial glucose levels. Consequently, sesame seed hull polysaccharide fractions hold promise for improving blood glucose regulation and could find applications in functional foods.

Keywords: Sesame seeds hulls, Polysaccharide, α -glucosidase, α -amylase, Glucose diffusion



INTRODUCTION

Sesamum indicum L. holds a prominent position as an oil crop in Asian countries like India, China, Japan, and Burma (Tenyang *et al.*, 2017). Approximately 40–58% of sesame seeds consist of oil, while protein constitutes 20–25%, and fiber accounts for 13.5% of their composition. Kernel and hull make up a sesame seed. As part of the pre-processing of the seeds of sesame, the hull is eliminated and discarded since it includes oxalic acid and fiber, both of which are hard for people to digest (Cano-Medina *et al.*, 2011). The predominant use of the sesame kernel is for extracting oil, usually via organic solvent extraction and mechanical pressing techniques. Unsaturated fatty acids like oleic and linoleic acids are abundant in sesame oil (Uzun *et al.*, 2008).

Sesame meal, a by-product of oil production, has a protein content of around 50% and a polysaccharide content of about 35%. Because it contains a lot of protein, it is frequently used as animal feed (Vinayashree and Vasu, 2021). Monogastric animals utilize only a portion of the nutrients in the sesame seeds because the breakdown of cell wall polysaccharides is restricted. The primary defense against deterioration may be the cell wall (Ghosh *et al.*, 2004; Görgüç *et al.*, 2020). The nutritional value of by-products of manufacturing sesame used as animal meals must thus be improved, which requires an understanding of the microstructure and chemistry of cell wall polysaccharides.

A wide class of organic biopolymers are called polysaccharides (Yu *et al.*, 2018) They are made up of monosaccharide residues connected by glycosidic linkages. The prevalent glycosidic bonds observed include α -1,4, β -1,3, β -1,4, and α -1,6 linkages. Monosaccharide components within polysaccharides can form diverse structures through connections, leading to both branched and linear

configurations. The complexity and diversity of polysaccharide structures are extensive (Yang *et al.*, 2009). Galactan, fructan, Glucan, mannan, xylan and other single monosaccharides, as well as polymers of multiple monosaccharides (such as galactomannan and arabinogalactan), can be found in the major chains of polysaccharides (Hu and Goff, 2018 and Shi, 2016). Given the intricate array of polysaccharides within the cell wall, a solitary extraction method is insufficient for their complete removal. Thus, a more effective approach is sequential extraction, aligning with the cell wall's diverse polysaccharide composition (Yu *et al.*, 2022). Numerous research on sesame have focused on its phenolic compounds, protein and oil (Akca and Akpınar, 2021; Takemoto *et al.*, 2022 and Yang *et al.*, 2021). Research pertaining to the isolation, constitution, and configuration of polysaccharides within sesame kernel cell walls or hulls remains scarce, leaving intricate structural insights absent. Leveraging such knowledge could enhance the sesame meal's dietary value. Hence, the ongoing endeavor aims to devise an extraction technique for polysaccharides found in sesame seed hull or kernel and take the first steps in identifying these polysaccharides, thereby contributing potentially valuable insights.

The hull makes up 17.0–19.0% by weight of the entire sesame seed. As a result, a significant number of sesame hulls are created as a manufacturing byproduct during the dehulling process. These hulls are now typically just thrown away and not properly utilized, despite the fact that they are rich in polysaccharides (42.7%) and nutritional fiber (25.6%) (Elleuch *et al.*, 2007). Furthermore, a linear homogalacturonan pectin exhibiting a yield of 9.72% and robust capacity for scavenging free radicals was identified within sesame hull (Liu *et al.*, 2021). These traits imply that sesame hulls may be used as a raw material for diets low in

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calories and function polysaccharides. Today, a wide variety of polysaccharides, including functional foods and bioactive substances, have already found uses in improving human health

Dawood *et al.*, 2021; Darwish *et al.*, 2022a,b; Khojah *et al.*, 2022 and Darwish, 2023). These polysaccharides often have great antioxidant qualities as well. Natural antioxidants in the diet reduce the risk of cancer and cardiovascular disease, according to epidemiological research (Ji *et al.*, 2023). Purifying sesame hull polysaccharides and identifying their fundamental structure were the goals of this study. Additionally, the impacts they could have on the digestive system were looked at using *in vitro* physiology. By determining how polysaccharides affect glucose transport and the activity of several digestive enzymes, the consequences were assessed. For its practical study, these studies could offer some relevant information on the structural characteristics of sesame husk polysaccharides.

MATERIALS AND METHODS

Materials

Chemical and reagents

Monosaccharide standards (D-galactose 99%, L-rhamnose 99%, D-fucose 98%, D-xylose 99%, D-arabinose 98%, D-fructose 99%, D-glucose 99.5%, D-mannose 99%, D-glucuronic acid 98%, and D-galacturonic acid 97%), as well as KOH, hydrochloric acids, sodium chloride, H₂SO₄, NaOH, NaAc, sodium phosphate buffer, dinitrosalicylic acid, Acarbose, α -amylase, α -glucosidase, pNPG, and Na₂CO₃, were procured from Sigma Chemical Co. (St. Louis, Mo, USA).

Plant materials

Sesame seeds were obtained from a local market in Mansoura city, Egypt, and were stored in a refrigerator until they were used.

Methods

Preparation of sesame seeds hulls

Sesame hulls were obtained following established procedures (Carbonell *et al.*, 2009; Zhang *et al.*, 2021). Initially, sesame seeds were soaked in an ample amount of distilled water at room temperature for 10 minutes to allow the skin to absorb water and swell. Subsequently, they were kneaded, causing the skins to separate from the seeds, revealing the white kernels. The kernels and skins were then collectively dried in an oven at 50°C for 6 hours. During this process, the crushed hulls contracted and reduced in size, enabling them to be sifted through a 30-mesh sieve and collected. The ratio of hull mass to seed mass fell between 17% and 19%, representing the yield of hulls. The dried hulls were subjected to oil extraction using the Soxhlet method and then placed in an oven at 50°C for an additional 6 hours to eliminate any excess solvent. Ultimately, the samples were stored in desiccators until they were ready for use. In addition, the protein content, which was found to be 4.52%, and the ash content, measuring 33.21%, of the defatted hulls were determined in accordance with the AOCS methods (AOCS, 1998)

Polysaccharide Isolation and Purification

According to Peng *et al.*, (2009) and Xu *et al.*, (2018), the following procedure was followed: Firstly, 25 mL of 5% KOH solution was combined with 1 g of dried defatted sesame hulls at 45°C subjected to ultrasonic extraction for a period of 40 min. Subsequently, the combination was moved to an incubator and underwent stirring at a temperature of

45°C for 3.0 hours. After passing through a suction filter to extract the filtrates, hydrochloric acid (6 mol/L) was used to adjust the filtrates to pH 5.5. The combination was then subjected to centrifugation for 40 min at 4500g. A rotating vacuum evaporator was used to concentrate the liquid supernatant. The precipitate obtained after centrifugation was re-dissolved and dialyzed for several days using a dialysis bag, until no salt was detected in the dialysate. The solution was concentrated and lyophilized at -50 °C for 48 h. Subsequently, the sample underwent purification using column chromatography (DEAE-52, with dimensions of 300 mm × 55 mm), with progressive elution carried out at a flow rate of 2 mL/min using sodium chloride solutions. Elution curves were plotted to monitor the separation process. The fractions collected were subjected to dialysis and then lyophilized. Three distinct purified fractions, designated as Pol-1, Pol-2, and Pol-3, were obtained. The concentration of sugar of the resulted polysaccharides was determined according to Long *et al.* (2020).

Analysis of Monosaccharides

The monosaccharide composition was determined following the method described in a previous study (Peng *et al.*, 2009). In brief, samples weighing 5.00 ± 0.05 mg were mixed with 125 μ L of 72% H₂SO₄ and 1.35 mL of distilled water, then placed in a circulating hot air oven at 105°C for 3.0 hours. Subsequently, the hydrolysate was neutralized and diluted 50-fold with distilled water. The diluted solution was introduced into HPLC after being filtered via a filter (0.22 μ m). The rate of flow was set at 0.5 mL/min, and the temperature of column was kept at 30°C. At predetermined times, gradient elution was carried out using various concentrations of mobile phases A (0.2 mol/L NaAc, 0.1 mol/L NaOH) and B (0.1 mol/L NaOH). Monosaccharide standards were likewise analyzed to prepare a standard curve. The peak areas of the equivalent compounds in the unknown samples were then compared to the standard curve, allowing for the calculation of their concentrations.

Determination of the effect of polysaccharide fraction on α -amylase inhibition

Elbermawi *et al.* (2022a) has previously described a technique for calculating the rates of α -amylase inhibition. 200 mL of 20 mM sodium phosphate buffer (pH 7.0) was combined with 200 mL of various concentrations of polysaccharide fractions, and incubated at 37°C for 45 min. Then, 400 L of a 0.5% potato starch solution was added to each sample, and the combination was incubated at 37°C with continuous agitation at 100 rpm for 10 min. The addition of the dinitrosalicylic acid color reagent halted the enzymatic process. The test tubes were withdrawn from the 100°C water bath after 10 min and let to cool to the ambient temperature. Next, 3 mL of deionized water was used for diluting each sample. A spectrophotometer was used to measure the reaction solutions' absorbance at 540 nm. Both levels of α -amylase inhibition were compared and measured. Acarbose (5 g/mL) was used as a positive control.

Determination of the effect of polysaccharide fraction on α -glucosidase inhibition

The Jordan approach was used to determine the polysaccharide fraction's influence on α -glucosidase (Jordan *et al.*, 2013). Fifty μ L of each concentration of the polysaccharide fractions were made in test tubes together with 100 L of the α -glucosidase solution (0.35 U/mL). This mixture was then incubated at 37°C for 10 minutes. Subsequently, each tube was supplemented with A 100 mM

phosphate-buffered saline solution with a pH of 6.9 was used for dissolving 100 µL of 1.5 mM pNPG. These tubes were then incubated at 37°C for a duration of 20 minutes. Following the addition of 1 mL of 1 M Na₂CO₃ to cease the reaction, A 400 nm wavelength was used to measure the absorbance. The positive control consisted of acarbose, while the control samples lacked the test sample in the reaction mixture.

Analyzing the impact of polysaccharide fractions on the diffusion of glucose

With minor modifications, the methodology for determining the rate of glucose diffusion was adopted from Hu *et al.* (2013). In summary, the experimental design assessed how the polysaccharide fractions affected the rate of glucose diffusion using dialysis bags with molecular weight cutoffs ranging from 500 to 1000. In these dialysis bags, 10 milliliters of polysaccharide fractions (7.5 mg/mL) and a glucose solution (100 mM) were placed. An amount of 100 mL of deionized water was used as the dialysate throughout the dialysis procedure, which was carried out at a pH of 7.0 and a temperature of 37°C. After intervals of 20, 40, and 60 minutes, the glucose concentration was measured in 2 mL of the dialysate, following the procedure outlined by Miller (Miller, 1959).

Statistical analysis

The data was processed using SPSS Statistics 23.0. All the data were presented as mean values with accompanying standard deviations (SD). To assess variations among the means, Duncan's multiple range test was employed at a significant level of 0.05.

RESULTS AND DISCUSSIONS

Yield of purified polysaccharide fractions

The quantities of the three polysaccharide fractions obtained are presented in Table 1. The combined yield of Pol-1, Pol-2, and Pol-3 accounted for 4.84% of the original sesame hull amount. Specifically, the individual fractions yielded 1.74%, 1.31%, and 1.43% (w/w) based on the dry

weight of the sesame hull. It can be observed that Pol-1 had the highest yield among the three other polysaccharide fractions (Table 1). The polysaccharides yield from mulberry fruits was 3.13% in another study that used ultrasound as a tool to assist (Chen *et al.*, 2015). Similarly, 2.96% of polysaccharides were extracted using microwave assistance from leaves of *Moringa oleifera* Lam. (Chen *et al.*, 2017). Therefore, it can be concluded that the sesame hull has a relatively high polysaccharide content. The polysaccharide fractions Pol-1, Pol-2, and Pol-3 had respective total sugar contents of 95.68%, 97.41%, and 94.24%. The concentration of protein, determined using the Bradford procedure (Bradford, 1976), was found to be 0.94% for Pol-1, 1.57% for Pol-2, and 1.41% for Pol-3.

Table 1. Chemical composition of polysaccharides fraction obtained from sesame seeds hulls.

Samples	Yield	Total polysaccharide	Total Protein
Pol-1	1.74 ± 0.21	95.68 ± 0.25	0.94 ± 0.14
Pol-2	1.31 ± 0.13	97.41 ± 0.41	1.57 ± 0.22
Pol-3	1.43 ± 0.092	94.24 ± 0.32	1.41 ± .52

Note (n = 3; average ± standard deviation), Pol-1, Pol-2 and Pol-3 were three purified fractions of sesame seeds hulls.

Monosaccharides composition of purified polysaccharide fractions

The analysis of monosaccharides in the isolated fractions of polysaccharides is presented in Table 2. Pol-1 exhibited a high content of galacturonic acid (50.4%) and glucuronic acid (14.1%), along with glucose (9.2%), galactose (5.2%), arabinose (5.8%), mannose (1.4%), xylose (5.5%) and rhamnose (9.3%), and galacturonic acid was also detected in substantial concentrations in Pol-2 (66.2%), demonstrating that the Pol-1 and Pol-2 included polysaccharides of the pectic type (Dranca and Oroian, 2018; Zhang *et al.*, 2021). In contrast, Pol-3 primarily consisted of galactose (42.3%), xylose (30.4%), and arabinose (19.4%), with smaller units of galacturonic acid, glucuronic acid, and glucose.

Table 2. Monosaccharide compositions of sesame seeds hulls-derived purified polysaccharides.

Samples	Molar composition (mol%)							
	Glc	Gal	Ara	Man	Xyl	Rha	GalA	GlcA
Pol-1	9.2	5.2	5.8	1.4	5.5	9.3	50.4	14.1
Pol-2	n.d.	14.2	13.1	n.d.	11.2	n.d.	66.2	n.d.
Pol-3	2.1	42.3	19.4	n.d.	30.4	n.d.	1.9	2.4

The monosaccharides present in the polysaccharides of sesame hulls were arabinose (Ara), xylose (Xyl), galactose (Gal), ribose (Rib), glucose (Glc), fructose (Fuc), mannose (Man), rhamnose (Rha), galacturonic acid (GalA) and glucuronic acid (GlcA). Pol-1, Pol-2 and Pol-3 were three purified fractions of sesame seeds hulls.

The inhibitory activity of polysaccharide fractions against α-amylase

The impacts of the three polysaccharide fractions on amylase activity were presented in Figure 1. Both polysaccharide fractions at concentrations of 5 and 7.5 mg/ml exhibited significant reductions in activity of α-amylase compared with the control (Figure 1). When Pol-2 was present, amylase activity dropped at a significantly faster pace than when Pol-1 or Pol-3 were present. The negative effect of polysaccharides on α-amylase activity may occur through one of two causes. First, Gourgue *et al.* (1992) demonstrated that the polysaccharides may contain a significant number of free carboxylic groups that might impede amylase function. α-amylase's activity may also be inhibited by a few insignificant substances like phytic acid and tannins on polysaccharides (Hu *et al.*, 2013).

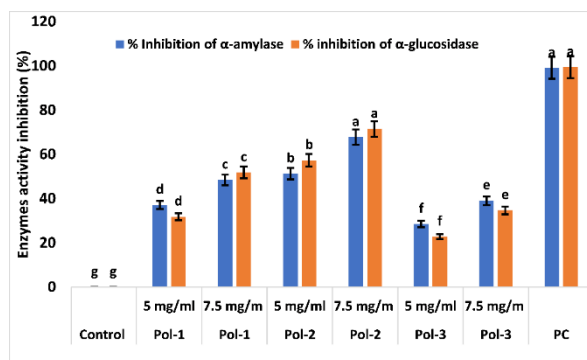


Figure 1. In vitro evaluation of the hypoglycemic impact of polysaccharide fractions derived from the hulls of sesame seeds. Significant changes (p < 0.05) between treatment conditions are denoted by lowercase letters. Data are presented as the means ± standard deviations.

In light of this, it was hypothesized that the polysaccharides from the sesame seed hull would include specific α -amylase inhibitory components or groups, directly affecting the reduction of activity of α -amylase. Second, in the opinion of Annison and Topping (1994), starch may adsorb polysaccharides and obstruct α -amylase's ability to hydrolyze starch. As a result, polysaccharide fractions from sesame seed hull may likewise be adsorbed to starch and have a subtly decreasing influence on the activity of the enzyme α -amylase. Further research is still required to determine if these polysaccharides are effective α -amylase inhibitors or merely serve as a barrier between the starch and α -amylase.

Inhibition of α -glucosidase activity

Intestinal activity of α -glucosidase may be controlled to prevent the production of glucose, which slows the increase of postprandial blood glucose levels by reducing uptake of glucose within small intestine (Rybka *et al.*, 1999 and Inzucchi, 2002). Acarbose and other inhibitors of α -glucosidase are commonly prescribed as part of a popular therapeutic approach for treating T2DM.

The outcomes indicated the inhibitory impact of all polysaccharide fractions on α -glucosidase, as shown in Figure 1. The inhibitory effect of α -glucosidase by Pol-1, Pol-2, and Pol-3 augmented with the rise in the concentration of the samples. Among the three fractions, Pol-2 displayed the most potent α -glucosidase inhibition effect. Indeed, research has revealed that several extracts or individual compounds isolated from plants or fruits exhibit inhibitory properties against α -glucosidase, causing minimal adverse reactions (Kim *et al.*, 2000; Grover *et al.*, 2002 and McCue *et al.*, 2005). Despite having less of an inhibitory impact than the medication acarbose, the data suggested that Pol-1, Pol-2, and Pol-3 could still be potential inhibitors of α -glucosidase.

Effects of polysaccharide fractions on glucose diffusion

A useful technique to assess the hyperglycemic impact of polysaccharides is glucose dialysis retardation (López *et al.*, 1996; Mishra and Jha, 2011 and Elbermawi *et al.*, 2022a,b). The impact of the fractions of polysaccharide fractions on the speed of sugar diffusion into an external solution was examined (Figure 2).

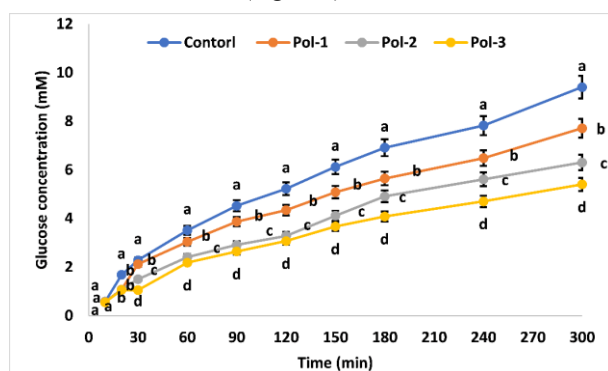


Figure 2. Polysaccharide fractional effects on glucose diffusion rate. Significant changes ($p < 0.05$) between treatment conditions are denoted by lowercase letters. Data are presented as the means \pm standard deviations.

When compared to the results obtained for the control, all fractions at a concentration of 5 and 7 mg/mL significantly ($p < 0.05$) decreased the glucose diffusion rates recorded after 20, 40, and 60 min (Figure 1). The glucose concentration of

the external fluids increased rapidly from 0 to 30 minutes, but gradually decreased between 30 and 150 minutes for all subjects.

CONCLUSIONS

Polysaccharides are abundant in the hulls of sesame seeds, which are a major by-product of sesame seed processing. This study extracted polysaccharides from sesame hulls (referred to as Pol) using ultrasound-assisted alkali extraction, resulting in a yield of 6.49%. The process involved column chromatography to obtain three distinct polysaccharide fractions. These fractions were then analyzed for their main constituents and their potential antihyperglycemic effects. The most prominent fraction, Pol-1, yielded 1.74% and was characterized by its content of rhamnose (9.3%), glucose (9.2%), glucuronic acid (14.1%), galacturonic acid (50.4%), and other components. The study revealed that different concentrations of these polysaccharide fractions had a significant impact on slowing down the diffusion of glucose and inhibiting the activities of both α -amylase and α -glucosidase. Notably, Pol-1 displayed the strongest inhibitory effects on both α -amylase and α -glucosidase activities, while also reducing the rate of glucose diffusion. These effects could potentially lead to the regulation of postprandial glucose levels by extending the blood glucose response. These findings highlight the potential of sesame seed hull polysaccharide fractions to enhance intestinal function in humans, making them promising candidates for incorporation into functional food products.

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التقييم المعملّي للنشاط الخافض لمستوى سكر الدم للسكريات العديدة المفصولة من قشور بذور السمسم

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الملخص

تتواجد السكريات العديدة بكثرة في قشور بذور السمسم، وهي منتج ثانوي لمعالجة بذور السمسم. وتبلغ نسبة السكريات العديدة المفصولة 6.49٪، تم استخلاص السكريات العديدة من قشور السمسم في هذه الدراسة باستخدام تقنيات استخلاص القلويات بمساعدة الموجات فوق الصوتية. وتم استخدام تقنية كروماتوجرافيا العمود للتقنية، تم إنتاج ثلاثة أجزاء نقية من السكريات العديدة. تم فحص مكوناتها الأساسية ونشاطها الخافض لسكر الدم. وبلغت نسبة Pol-1 1.74٪، وهي الجزء الأكبر. كان يحتوي على الرامنوز (9.3٪)، الجلوكوز (9.2٪)، حمض الجلوكتورونيك (14.1٪)، حمض الجالاكتورونيك (50.4٪)، ومكونات أخرى. أشارت النتائج إلى أن التركيزات المختلفة لأجزاء السكر العديدة كان لها تأثير كبير على تثبيط انتشار الجلوكوز وتثبيط نشاط كل من α -amylase و α -glucosidase. أظهر Pol-1 التأثير المثبط الأقوى على أنشطة α -amylase و α -glucosidase، بالإضافة إلى تقليل معدل انتشار الجلوكوز. قد يساعد ذلك في تحسين استجابة الجلوكوز في الدم وبالتالي تنظيم مستويات الجلوكوز بعد الأكل. أظهرت هذه النتائج أن أجزاء السكريات العديدة الموجودة في بذور قشور السمسم قد تكون مفيدة لتعزيز مستويات الجلوكوز في الدم ويمكن استخدامها كعنصر محتمل في التطبيقات الغذائية الوظيفية.

الكلمات الدالة: قشور السمسم، السكريات العديدة، ألفا جلوكوزيداز، ألفا أميليز، انتشار الجلوكوز