

Effect of Soybean Galactomannan on the Activities of -Amylase, Trypsin, Lipase and Starch Digestion

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Abstract: The present study was aimed to investigate the effect of galactomannan from soybean hulls on the activities of -amylase, trypsin, lipase and starch digestion. The galactomannan was incubated at different concentrations with enzyme for suitable period and starch digestion was studied using inverted sac technique at one concentration of galactomannan (200 mg). Data revealed that the pre-incubation of digestive enzymes with different concentrations of galactomannan led to inhibition of enzyme activity. Also, the galactomannan at concentration 200 mg inhibited the starch digestion in the mucosal side and inhibited glucose absorption into the serosal side. In conclusion, the galactomannan can be used in medical and pharmaceutical fields to decrease the activities of digestive enzymes and starch digestion.

Key words: Soybean, galactomannan, -amylase, trypsin, lipase, starch digestion

INTRODUCTION

The soybean, *Glycine max* (L.) Merrill, has been known since 3,000 BC to humans as food. The soybean has played an important role in the diet of oriental civilization for many years, as witnessed by the numerous rational soy-based foods found in the Orient. Soybean seeds, are known to contain galactomannans. The galactomannans from soybeans have been purified and soybean hulls have a high content of galactomannans^[28]. Galactomannans are neutral polysaccharides composed of linear main chains of -1→4 linked mannose units with -1→6 linked side chains of a single galactose unit. They differ in the ratio of mannose to galactose units, M/G. The more substituted of the commercial galactomannans is guar gum (M/G ~2:1); in tara gum, the M/G is ~3:1 while in locust bean gum is ~4:1^[24]. Galactomannans have attracted considerable academic and industrial attention because of their unique chemical and physical properties, in addition to their biological functions. The use of these polysaccharides (also known as gums) as substances for mummification can be traced back to 3000 BC in ancient Egypt and, hence, they are often called "Pharaoh's Polysaccharides". The importance of these polysaccharides can be seen in their wide use in industry, notably in food, pharmaceuticals, cosmetics, paper products, paints, plasters, well-drilling, explosives

and fire-fighting. However, there are few reports on the biological activities of isolated galactomannans^[16].

The effects of water-soluble non starch polysaccharides (sNSP) such as legume galactomannans and cereal -glucans can result in the formation of viscous solutions in the gastrointestinal tract and delay the absorption of nutrients, e.g. glucose, in the small intestine^[6,9,29]. The effects of sNSP on human metabolism are considered to be largely beneficial in that they decrease postprandial glycaemia and insulinaemia^[22,13] and lower the fasting level of plasma cholesterol, mainly the low-density lipoprotein fraction^[2]. One potential application of sNSP, therefore, is in the prevention and treatment of metabolic disorders. For example, guar gum, a galactomannan-rich legume flour, has received considerable attention as an oral antidiabetic agent in the treatment of mainly type 2 (noninsulin-dependent) diabetes^[8]. The mechanisms by which sNSP attenuate the postprandial rise in blood glucose are not well understood but their presence increases the viscosity of gastrointestinal contents, which affects physiological functions, e.g. gastric emptying and peristalsis. Increased viscosity and decreased water activity during hydrothermal treatment of starch could influence α -amylase action. guar galactomannan as a representative of sNSP has a direct noncompetitive inhibitory effect on α -amylase. the effects of sNSP in lowering postprandial glycaemia not only involve modifications of gut physiology, but also

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include direct inhibition of the first stage in the biochemical degradation of starch^[25].

The aim of this study was to investigate the inhibitory activity of soybean galactomannan on some digestive enzymes (-amylase, trypsin and lipase) and starch digestion. Although the effects were assayed *in vitro*, the results of this work should be relevant to the human body.

MATERIALS AND METHODS

Materials:

Plant Material: Hulls of soybean seeds of various varieties of *Glycine max* (L.) Merrill, were obtained from Soybean Factory, Food Technology Research Institute, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt.

Chemicals: Benzoyl-DL-arginin-p-nitroanilide hydrochloride (BAPA), pancreatin (from porcine pancreas), crystalline porcine trypsin were purchased from Sigma Chemical Co. Glucose kit (for determination of glucose) was from Technogen Co., Giza, Egypt. All other chemicals were of analytical reagent grade.

Animals: Male Wistar white rats, for the experiment of starch digestion, were purchased from Experimental Animal House in Food Technology Research Institute, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt.

Methods:

Extraction of Galactomannan: Galactomannan was extracted from soybean hulls using the procedure reported by Whistler and Saarino^[28].

Assay of -amylase Inhibitory Activity: -amylase (1-4- -D-glucan glucanohydrolase, EC 3.2.1.1) inhibitory activity was assayed for galactomannan under investigation based on the method of Bernfeld^[11] with the modification of Chandrasekher *et al.*^[5].

Assay of Trypsin Inhibitory Activity: Trypsin (EC 3.4.21.4) inhibitory activity was assayed for polysaccharides under investigation based on the method of Hamerstrand *et al.*^[14].

Assay of Lipase Inhibitory Activity: The lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) inhibitory activity was assayed for polysaccharides under investigation based on the method of Mia *et al.*^[20].

Determination of Starch Digestibility: The digestibility of starch using the Inverted sac technique was determined using the method described by Madar and Shomer^[18].

Animals: Male Wistar white rats, weighing 450-500 g, were used for the experiment. The animals were housed in a controlled environment and fed, *ad libitum*, on a regular laboratory diet.

Krebs-henselheit Buffer (Khb): KHB possessed the following composition in mM: NaCl, 118.5; NaHCO₃, 25.0; KCl, 4.8; MgSO₄.7H₂O, 1.2; KH₂PO₄, 1.2. The pH was adjusted to 7.4.

Potato Starch Solution (2% W/v in Khb):

Preparation of the Inverted Sac: Rats were decapitated, and the intestine was removed by cutting of both the upper end of the duodenum and the lower end of the ileum. The entire intestine content was washed with cold saline solution (0.9% NaCl, w/v). The intestine was divided into 7-cm-long fragments and turned inside out. The inverted sac was lightly tied at one end, and 1 ml of Krebs-Henselheit buffer was introduced into the inverted sac, which was then ligated.

Procedure: The sac was placed in an Erlenmeyer flask (25 ml) containing 6.0 ml of potato starch solution with or without 200 mg of dry matter of galactomannan. One milliliter of pancreatin (10 mg/ml) was also included in the reaction mixture. The flasks were gassed with O₂:CO₂ (95:5) for 30 s, tightly capped, and incubated in a shaking bath at 37°C for 1 h. At the termination of the incubation period, the inverted sac was removed and the inner fluid was collected and the glucose liberated was measured in the serosal and mucosal sides.

Statistical Analysis: The data were analyzed by an analysis of variance (ANOVA) and the difference among means were tested for the least significant difference (LSD) at *P*<0.05. The results were processed by SAS computer program (1987).

RESULTS AND DISCUSSION

Effect of Galactomannan on Some Digestive Enzyme

Activities: The purpose of this study was to screen the effect of galactomannan on some digestive enzyme activities associated with carbohydrate, protein and lipid digestion. Pancreatic amylase, trypsin and lipase were used. In this study, the enzyme activities were assayed after pre-incubation of individual mucilage or gum at various concentrations with enzyme for suitable period. The obtained data (inhibitory units and inhibitory percentages of enzymes) were recorded in Tables (1, 2 and 3). The following headings described and discuss the effect of galactomannan on certain enzyme activities.

Table 1: Inhibitory activity of galactomannan (GM) on pancreatic amylase

<i>GM concentration(ppm)</i>	<i>Inhibition</i>	
	Unit×10 ⁻⁴	%
10	1.173 ^a ±0.50	52
20	1.346 ^a ±0.56	52.7
30	1.740 ^a ±0.66	57.4
40	2.590 ^a ±0.63	59.5
L.S.D	1.503	-

- Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05.

Table 2: Inhibitory activity of galactomannan (GM) on pancreatic trypsin

<i>GM concentration(ppm)</i>	<i>Inhibition</i>	
	Unit	%
20	8.260 ^b ±1.09	19.2
40	10.56 ^{ab} ±1.88	24.5
60	13.13 ^{ab} ±1.75	30.5
80	15.70 ^a ±2.31	36.4
L.S.D	5.091	-

- Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05.

Table 3: Inhibitory activity of galactomannan (GM) on pancreatic lipase

<i>GM concentration(ppm)</i>	<i>Inhibition</i>	
	Unit	%
50	5.08 ^c ±0.96	5.06
100	5.92 ^c ±0.12	5.92
150	10.95 ^b ±0.02	10.45
200	19.68 ^a ±0.09	19.68
L.S.D	1.580	-

- Values are means of three replicates ± SE. Numbers in the same column followed by the same letter are not significantly different at *P*<0.05.

Amylase: Data in Table (1) demonstrated that the pre-incubation of pancreatic amylase with galactomannan led to inhibition of enzyme activity. The inhibitory activity was 1.173×10⁻⁴ unit at the lowest concentration of galactomannan (10 ppm), while the highest concentration (40 ppm) was 2.590×10⁻⁴ unit. The percentages of inhibitory activity ranged between 52.0 and 59.5% for the lowest (10 ppm) and highest (40 ppm) concentrations, respectively. There were no significant differences in the inhibitory activity among all the different used concentrations.

Trypsin: Different concentrations (20, 40, 60 and 80 ppm) of galactomannan were employed to evaluate their potentials on pancreatic trypsin after pre-incubation with enzyme. The obtained data are given in Table (2). Results showed that galactomannan exhibits an inhibitory effect on trypsin activity. The inhibitory activity was increased by increasing the concentration of galactomannan. The inhibition percentages ranged between 19.2 to 36.4% for the

lowest (20 ppm) and the highest (80 ppm) concentrations, respectively. The data revealed that the highest concentration of galactomannan (80 ppm) significantly decreased the enzyme activity to 15.70 units. Otherwise, treatment with lowest concentration (20 ppm) reduced the activity to 8.26 units. Treatment with 40 and 60 ppm reduced the activity by 24.5 and 30.5%, i.e. to 10.56 and 13.13 units.

Lipase: The effect of galactomannan at different concentrations (50, 100, 150 and 200 ppm) on pancreatic lipase was studied after pre-incubation of galactomannan with enzyme. Inhibitory activity of galactomannan after pre-incubation with enzyme was recorded in Table (3). The obtained data showed that the reduction in lipase activity varied according to the used concentration. The inhibition percentages ranged between 5.06% for the lowest concentration (50 ppm) and 19.68% for the highest concentration (200 ppm). Moderate percentages (5.92 and 10.45%) were found in the case of used galactomannan at 100 and 150 ppm, respectively. The highest inhibitory activity (19.68 units) was noticed with galactomannan at concentration of 200 ppm.

From previous results, generally, it can be concluded the following:

- 1- Galactomannan under investigation possesses inhibitory activity against some pancreatic enzymes associated with digestion of starches (amylase), proteins (trypsin) and lipids (lipase).
- 2- Inhibitory activity of galactomannan is varied from enzyme to other.
- 3- There is a positive correlation between inhibitory activity of galactomannan and its concentrations in enzyme assay medium.

On the other hand, the inhibitory activity of galactomannan may be due to one or more of the following mechanisms:

- 1- Direct effect of galactomannan on enzyme.
- 2- Reduction the enzyme-substrate binding because of a relatively high viscosity of the reaction medium (galactomannan)
- 3- Altering the enzyme conformation.
- 4- Interaction between galactomannan and enzyme (as protein).
- 5- Interaction between galactomannan and enzyme substrate.

These possibilities in agreement with those of^[17,15,6,3,22,26,27,11,19]. Guar galactomannan as a representative of sNSP has a direct noncompetitive inhibitory effect on a-amylase. the effects of sNSP in

Table 4: Effect of galactomannan (GM) on potato starch digestion by using the inverted sac technique^a

GM concentration (mg)	Glucose (mg) liberated in		Total glucose(mg)	Inhibition ^b %
	Mucosal side	Serosal side		
0	0.39±0.002	0.257±0.066	0.647	-
200	0.27±0.032	0.199±0.071	0.469	27.51 ^a

Results are expressed as means ± SE of experiment, with total of 4 gut sections.

^bThe inhibition percentage was calculated as the ratio of total glucose in the presence and absence of galactomannan.

lowering postprandial glycaemia not only involve modifications of gut physiology, but also include direct inhibition of the first stage in the biochemical degradation of starch^[25].

Influence of Galactomannan on Starch Digestion:

The influence of galactomannan on the digestion of potato starch using inverted sac technique was studied. One concentration of galactomannan (200 mg) and inverted gut from rat were used. Control assay was carried out without a galactomannan. The obtained results are summarized in Table (4). Data showed that galactomannan at concentration of 200 mg inhibited the starch digestion in the mucosal side and also inhibited glucose absorption into the serosal side. The glucose liberated in the serosal side was reduced from 0.257 to 0.199 mg/dl. The concentration of glucose remained in the mucosal side after starch digestion and glucose absorption was also reduced as a result of inhibitory effect of galactomannan. At the same time, starch inhibition was reached to 27.51% with this galactomannan concentration when the results are expressed as total inhibition of glucose found in the serosal and mucosal sides. These results indicated that the galactomannan has a direct effect on digestive enzymes of starch, confirmed the results obtained previously indicated that galactomannan has a direct inhibitory effect on amylase (Table 1). Thus, the results obtained previously support these data. The results obtained are in a good agreement with those^[18] who found that the gel fraction derived from fenugreek decreased both digestion and absorption of starch.

In addition to the mechanisms mentioned previously concerning the inhibitory effects of galactomannan, it has a high viscosity, there by reducing enzyme-substrate contact. *In vitro* experiments and perfusion studies have suggested that the fiber gelling agents (galactomannan) may directly inhibit certain digestive and transport functions in the rat intestine^[12,6,9,29]. The blood glucose lowering effect of guar and similar sNSP observed in acute studies seems to depend mainly on the capacity of the polymer to increase the viscosity of gastric and upper intestinal contents^[10,7]. The increase in viscosity can affect gastric function (e.g. emptying and sieving) and inhibit propulsive and mixing effects generated by peristalsis^[4, 23]. Under these conditions, therefore, where interactions between substrates and

digestive enzymes are less frequent, not only is there likely to be a decrease in the rate of digestion of starch by α -amylase, but the products of amylolysis (e.g. maltose, -limit dextrins) will almost certainly be presented to the mucosa at a slower rate^[3].

Concluding Remarks: Finally, it could be concluded that galactomannan can be used in the medical and pharmaceutical fields as non competitive inhibitor of digestive enzymes associated with carbohydrate, protein and lipid digestion.

REFERENCES

1. Bernfeld, P., 1955. Amylases, and . In Methods in Enzymology. Eds., Colowick, S.P. and N.O. Kaplan, Academic press, New York., 1: 149-58.
2. Blake, D.E., C.J. Hamblett, P.J. Frost, P.A. Judd and P.R. Ellis, 1997. Wheat bread supplemented with depolymerised guar gum reduces plasma cholesterol concentration in hypercholesterolaemic human subjects. American J. Clinical Nutrition, 65: 107-113.
3. Brennan, C.S., D.E. Blake, P.R. Ellis and J.D. Schofield, 1996. Effect of galactomannan on wheat bread microstructure and on the *in vitro* and *in vivo* digestibility of starch in bread, J. Cereal Science, 24: 151- 160.
4. Bueno, L., F. Praddaude, J. Fioramonte and Y. Ruckesbusch, 1981. Effect of dietary fibre on gastrointestinal motility and jejunal transit time in dogs, Gastroenterology, 80: 701- 707.
5. Chandrasekher, G., D.S. Raju and T.N. Pattabiraman, 1981. Nature plant enzyme inhibitors: α -amylase inhibitors in millets. J. Science of Food and Agriculture, 32: 9-16.
6. Cherbut, C., E. Albina, M. Champ, J.L. Doublier and G. Lecannu, 1990. Action of guar gum on the viscosity of digestive contents and on gastrointestinal motor function in pigs. Digestion, 4: 205-213.
7. Edwards, C.A., I.T. Johnson and N.W. Read, 1998. Do viscous polysaccharides slow absorption by inhibiting diffusion or convection, European J. Clinical Nutrition, 42: 307-312.

8. Ellis, P.R., 1999. The effect of fibre on diabetes, In *The Right Fibre for the Right Disease*, Ed., Hill, M. The Royal Society of Medicine Press, London, pp. 33-42.
9. Ellis, P.R., F.G. Roberts, A.G. Low and L.M. Morgan, 1995. The effect of highmolecular weight guar gum on net apparent glucose absorption and net apparent insulin and gastric inhibitory polypeptide production in the growing pig: relationship to rheological changes in jejunal digesta, *British Journal of Nutrition*, 74: 539- 556.
10. Ellis, P.R., P. Rayment and Q. Wang, 1996. A physico-chemical perspective of plant polysaccharides in relation to glucose absorption, insulin secretion and the entero-insular axis, *Proceedings of the Nutrition Society*, 55: 881- 898.
11. Ellis, P.R., Q. Wang, P. Rayment, Y. Ren, S.B. Ross-Murphy, 2001. Guar gum: agricultural and botanical aspects, physico-chemical and nutritional properties and its use in the development of functional foods, In *Handbook of Dietary Fibre*, Eds., Cho, S.S. and M. Dreher, Marcel Dekker,
12. Elsenhans, B., D. Zenker, W.F. Caspary and R. Blume, 1984. Guar gum effect on rat intestinal absorption. *Gastroenterology*, 86: 635-645.
13. Gatenby, S.J., P.R. Ellis, L.M. Morgan and P.A. Judd, 1996. Effect of depolymerised guar gum on acute metabolic variables in patients with noninsulin dependent diabetes, *Diabetic Medicine*, 13: 358-364.
14. Hamerstrand, G.E., L.T. Black and J.D. Glover, 1981. Trypsin inhibitors in soy products: Modification of the standard analytical procedure. *Cereal Chemistry*, 58(1): 42-5.
15. Hansen, W.E., 1987. Effects of dietary fibre on pancreatic lipase activity *in vitro*. *Pancreas*, 2: 195-198.
16. Hussein, M.M.E., W.A. Helmy and H.M. Salem, 1998. Biological activities of some galactomannans and their sulfated derivatives. *Phytochemistry*, 48(3): 479-484.
17. Lairon, D.H., J.L. Lafont, G. Vigne, J. Nalbone, J. Leonardi and J.C. Hauton, 1985. Effects of dietary fibres and cholestyramine on the activity of pancreatic lipase *in vitro*. *American J. Clinical Nutrition*, 42: 629-638.
18. Madar, Z. and I. Shomer, 1990. Polysaccharide composition of a gel fraction derived from fenugreek and its effect on starch digestion and bile acid absorption in rats. *J. Agriculture and Food Chemistry*, 38: 1535-9.
19. Matsuo, A.L., I.I.L. Tersariol, S.I. Kobata, L.R. Travassos, A.K. Carmona and R. Puccia, 2006. Modulation of the exocellular serine-thiol proteinase activity of *Paracoccidioides brasiliensis* by neutral polysaccharides. *Microbes Infection*, 8: 84-89.
20. Mia, A.S., H.D. Koger and M.M. Tierney, 1978. Rapid turbidimetric determination of serum pancreatic lipase in the dog. *American Journal of Veterinary Research*, 39(2): 317-8.
21. Morgan, L.M., J.A. Tredger, J. Wright and V. Marks, 1990. The effect of soluble and insoluble-fibre supplementation on post-prandial glucose tolerance, insulin and gastric inhibitory polypeptide secretion in healthy subjects, *British J. Nutrition*, 64: 103-110.
22. Pasquier, B., M. Armand, C.Castelain, F.Guillon, P. H. Borel, H. Lafont and D. Lairon, 1996. Emulsification and lipolysis of triacylglycerols are altered by viscous soluble dietary fibres in acidic gastric medium *in vitro*, *Biochemical J.*, 314: 269-275.
23. Read, N.W. and M.A. Eastwood, 1992. Gastro-intestinal physiology and function, In *Dietary Fibre-A Component of Food*, Eds., Schweizer, T.F. and C.A. Edwards, Springer-Verlag, London, 1992, pp. 103-117.
24. Sittikijyothin, W., D. Torres and M.P. Goncalves, 2005. Modelling the rheological behaviour of galactomannan aqueous solutions. *Carbohydrate Polymers.*, 59: 339-350.
25. Slaughter, S.L., P.R. Ellis, E.C. Jackson, and P.J. Butterworth, 2002. The effect of guar galactomannan and water availability during hydrothermal processing on the hydrolysis of starch catalysed by pancreatic α -amylase. *Biochimica et Biophysica Acta*, 1571: 55- 63.
26. Sudhakar, V., R.S. Singhal and P.R. Kulkarni, 1996. Starch-galactomannan interactions: functionality and rheological aspects. *Food Chemistry*, 55(3): 259-264.
27. Tavares, C. and J.A. Lopes da Silva, 2003. Rheology of galactomannan-whey protein mixed systems. *International Dairy Journal*, 13: 699-706.
28. Whistler, R.L. and J. Saarnio, 1957. Galactomannans from soy hulls. *J. American Chemical Society*, 79: 6055-6057.
29. Wood, P.J., 2001. Cereal β -glucans: structure, properties and health claims, In *Advanced Dietary Fibre Technology*, Eds., McCleary, B.V. and L. Prosky, Blackwell, Oxford, UK, pp. 315-327.