

Enzymatic modification to improve the water-absorbing and gelling properties of psyllium

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Abstract

Psyllium husks were treated with a commercial food-grade polysaccharidase mixture, under solid-state reaction conditions, to improve water-absorbing and gelling properties. The modified psyllium preparations were analyzed and compared to the original psyllium and the control, treated with no enzyme under the same reaction conditions, for their water-absorbing ability, gelling properties, fibre contents, and surface structures. The water-absorbing ability was determined by a gravimetric method, while the gelling property of the modified psyllium was measured using a texture analyzer. The results showed that the solid-state enzymatic modification was able to significantly reduce both water-absorbing and gelling abilities of psyllium. Compared to the control, reductions of 49% in water-absorbing ability, 71% in gel hardness, and 35% in gel adhesiveness were observed for the novel psyllium preparation that was treated with the enzyme mixture at a level of 36 units/g psyllium. The surface structure of the modified psyllium was examined using scanning electron microscopy (SEM). The SEM results showed that the enzymatic modification decreased the total surface area. This may contribute to the reduced water-absorbing ability of the modified psyllium. This study demonstrated the potential of preparing novel psyllium preparations, using a solid-state enzymatic method, for commercial food applications.

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1. Introduction

It is well accepted that a high intake of soluble fibre is associated with favourable effects on human health (Anderson et al., 2000; Anderson, Deakins, Floor, Smith, & Whitis, 1990; Romero, Romero, Galaviz, & Fernandez, 1998). Psyllium is an excellent source of natural soluble fibre, and contains approximately eight times more soluble fibre than oat bran on a per weight basis (Yu, Deway, Lai, Simmons, & Neilsen, 2001). Recently, psyllium has been widely recognized for its safe and cholesterol-lowering effects, effective laxative activity, and insulin sensitivity improvement capacity (Anderson et al., 2000; Song, Sawamura, Ikeda, Igawa, & Yamori, 2000). A few food products have been developed using psyllium as a bioactive component and marketed for cholesterol-reducing effects (Childs, 1999),

since there is a significant population, including more than 60 million adult Americans, who still need cholesterol-lowering dietary treatment to reduce both total serum and low-density lipoprotein (LDL) cholesterol (Aygustin & Dwyer, 1999; Jensen, Spiller, Gates, Miller, & Whittam, 1993). However, it is a real challenge to incorporate the required amount of psyllium in one serving of a food product for the cholesterol-lowering claim on the label, as required by the Food and Drug Administration in the United States (Childs, 1999; Yu et al., 2001). This is mainly due to the extremely strong gelling and water-absorbing abilities of psyllium.

Many physical and mechanical techniques have been investigated for their effects in reducing the gelling and water-absorbing abilities of psyllium and consequently to promote its utilizations in food products (Rudin, 1988; Wullschleger, Creek, Chen, Bowman, & Hawblitz, 1993). These previous investigations have indicated the possibility of improving the physicochemical properties of psyllium. However, none of them could sufficiently solve the strong gelling and extreme water-absorbing

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problems of psyllium. In 2001, Yu and others reported an enzymatic method for producing novel psyllium preparations with reduced water-absorbing ability and different gelling properties. It was possible to deliver the required soluble fibre in a single serving of cookies using the modified psyllium and assert the claim of cholesterol-lowering benefit on the label (Yu et al., 2001). However, the product had to be freeze-dried, after the conventional enzyme reaction, to remove water from the rubbery gels. This limited the potential application of this enzymatic procedure for commercial production of the modified psyllium.

In this study, the novel psyllium samples were prepared by treatment of the psyllium husks with a commercial food-grade polysaccharidase complex under a solid-state enzymatic reaction condition. The water-absorbing and gelling abilities of the modified psyllium preparations were examined and compared with that of the original commercial psyllium to evaluate the feasibility of producing the novel psyllium with improved physicochemical properties for food applications. Results from this study demonstrated the potential to improve the water-absorbing and gelling properties of psyllium using a solid-state enzyme reaction. The production of improved psyllium will promote its utilization in making functional foods for optimizing human health.

2. Materials and methods

2.1. Materials

Psyllium husks (95% purity, 40 mesh) were a gift kindly provided by the Bio-Products Co (Joliet, IL), while the Viscozyme L was a gift from Novo Nordisk Biochem North America, Inc (Franklinton, NC). Viscozyme L is a multienzyme complex, containing a wide range of carbohydrase activities, including cellulase, hemicellulase, xylanase, arabanase and β -glucanase activities. All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2. Preparation of the modified psyllium

A certain amount of Viscozyme L, in a liquid form, was mixed into psyllium husk powder to start the solid-state enzymatic reaction. Final concentrations of Viscozyme L were 0, 2.4, 4.8, 9.6, 19.2 and 36 units per gramme of psyllium. The reaction was conducted at ambient temperature and terminated by inactivating the enzyme. The enzyme was inactivated by microwaving the reaction mixture for 2 min using a commercial microwave oven (Sanyo Super Showerwave). The final product of the solid-state reaction was obtained after grinding the material and collecting the portion that passed through a

20 mesh sieve. Controls were performed using the above procedure without addition of the enzyme. The modified psyllium samples were prepared in triplicate, and stored at ambient temperature for further analyses.

2.3. Water-absorbing capacity

Water-absorbing capacity was determined gravimetrically, using a laboratory procedure previously described by Yu and others (2001). Briefly, all samples were dried at 65 °C for 72 h and equilibrated in a 10% relative humidity (RH) chamber at ambient temperature for 48 h. Then, samples were transferred to a 98% RH chamber and exposed to moisture for 10 min. The absolute amount of absorbed water was calculated from the weight change of the dry matter before and after being exposed to the high RH environment. The results were expressed as the mean \pm S.D. in mg water absorbed per gramme of psyllium per minute. All measurements were made in triplicate.

2.4. Gelling properties

Gelling properties of modified psyllium samples were measured and compared with that of the original psyllium according to a previously described procedure (Paraskevopoul & Kiosseoglou, 1997; Pons & Fisman, 1996; Yu et al, 2001). A TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY) was used with a 25 mm diameter probe. Gel samples were prepared by mixing 1.50 g of psyllium into 30 ml of water. After setting for 3 or 48 h, gel samples were subjected to a double compression test. Measurements were performed with a pretest speed of 2.0 mm/s, a test speed of 5.0 mm/s, a post-test speed of 5.0 mm/s, and a distance of 6 mm. The results were expressed as the mean \pm S.D. in gramme force for hardness and adhesiveness. All measurements were conducted in triplicate.

2.5. Surface structure analysis

Scanning electron microscopy (SEM) was performed to determine the changes of the surface structures of psyllium particles due to Viscozyme L treatments. SEM was carried out using a Philips SEM 505 instrument (Holland) at Colorado State University.

2.6. Fiber contents

Soluble and insoluble fibre contents in the modified psyllium were measured and compared to that of the original psyllium husks using a commercial kit purchased from Megazyme International Ireland Ltd (Wicklow, Ireland) according to the previously reported enzymatic procedure (Lee, Rodriguez, Storey, Farmakalidis, & Prosky, 1995).

2.7. Statistical analysis

All tests were conducted in triplicate, except for the fibre content determinations. Data were reported as means \pm S.D. Analysis of variance and least significant difference tests were conducted to identify differences among means. Statistical significance was assumed at $P < 0.05$.

3. Results and discussion

Elevated serum total and LDL cholesterol levels are known risk factors for the development of coronary heart disease (Ganji & Kies, 1996). Psyllium husk, which contains very high levels of soluble fibre, has been shown to inversely affect both total and LDL cholesterol in human and animal studies (Anderson et al., 2000; Ganji & Kies, 1996). The two properties that most limit the applications of psyllium in food products are its extremely high water-absorbing and strong gelling abilities (Yu et al., 2001). Psyllium takes up water rapidly and forms rubbery gels when water is added to solid ingredients containing psyllium during processing, while other ingredients may remain dry. Psyllium may also absorb a large amount of water during steam-cooking and change the mechanical and rheological properties of the formula. This may raise safety concerns for a food manufacturer. The strong water-absorbing and gelling ability makes it difficult to incorporate the required quantity of soluble fibre from psyllium in one serving of a food in order to have the health claim on the label, and also limits the type of food products that can be enriched with the soluble fibre. To promote utilization of psyllium as a dietary soluble fibre, it is critical to improve or reduce its water-absorbing and gelling properties.

In this study, Viscozyme L, a commercial food-grade enzyme mixture, was evaluated for its ability to reduce the water-absorbing and gelling abilities of psyllium under a solid-state reaction condition. The Viscozyme L treatments resulted in a reduction of the water-absorbing rate of psyllium under the testing conditions (Fig. 1). Compared to the control, significant decreases of water-absorbing rate were observed in the modified psyllium samples treated with Viscozyme L at levels of 19.2 and 36 units per gramme of psyllium. The correlation coefficient was -0.95 between the Viscozyme L concentration and the final water-absorbing rate of the modified psyllium samples. A 49% reduction in water-absorbing rate was detected in the modified psyllium treated with Viscozyme L at a concentration of 36 units/g psyllium. This reduction is similar to that of the modified psyllium treated with Viscozyme L at a level of 60 units/g psyllium in a suspension, under a conventional enzymatic reaction condition (Yu et al., 2001), suggesting

that less enzyme may be required if the reaction is performed under the solid-state condition(s).

The Viscozyme L treatments also decreased the gelling abilities of psyllium. Reductions in gel hardness and adhesiveness were observed in the modified psyllium samples (Figs. 2 and 3). Compared to the control, the Viscozyme L treatment, at a level of 36 units/g psyllium, resulted in a 71% reduction in gel hardness (Fig. 2), and a 35% reduction in gel adhesiveness (Fig. 3). The psyllium modified with a higher level of the Viscozyme L correlated with lower peak forces and shorter times to return to the baseline when the external force was removed, which represented less gelling ability (Fig. 4). A correlation coefficient of -0.96 between the Viscozyme L concentration and gel adhesiveness was observed, while no correlation was detected between enzyme level and gel hardness. In 2001, Yu and others reported a reduction of 69% in gel hardness for psyllium modified with Viscozyme L at a final enzyme concentration of 60 units/g of psyllium under conventional enzymatic reaction conditions, as compared to the control (Yu et al., 2001). These again indicate that less Viscozyme L may be needed if the enzymatic reaction is conducted in a solid-state. Furthermore, the final modified psyllium products through a solid-state reaction are common solid powders, and no additional steps are needed to remove moisture after inactivation of the enzyme. This makes the solid-state enzymatic procedure preferred for commercial production of novel psyllium fibres because a freeze-dry procedure must be used to

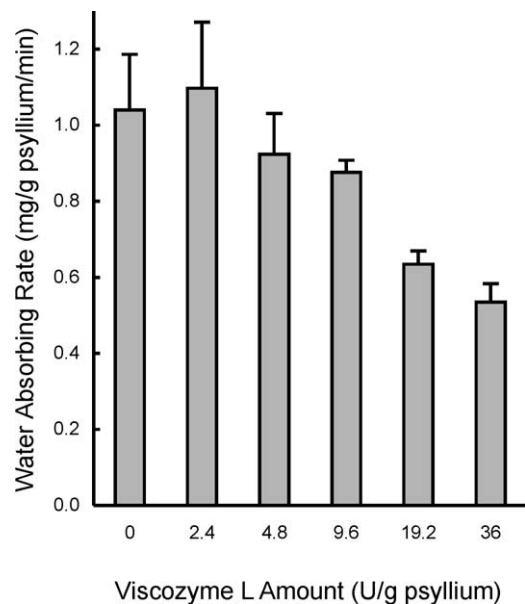


Fig. 1. Effects of Viscozyme L on the water-absorbing property of psyllium. 0, 2.4, 4.8, 9.6, 19.2 and 36 represent the final Viscozyme L levels of 0, 2.4, 4.8, 9.6, 19.2 and 36 units/g of psyllium in the solid-state reaction mixtures, respectively. All tests were conducted in triplicate and means are used. Vertical bars represent the standard deviation of each data point ($n = 3$).

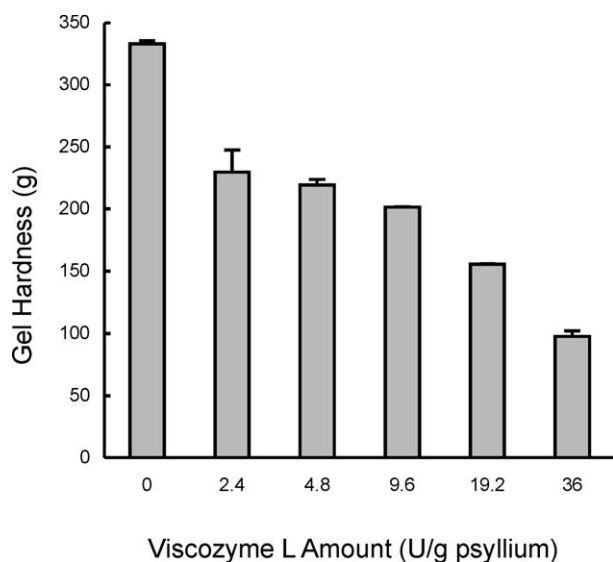


Fig. 2. Hardness of the gels prepared from the modified psyllium. 0, 2.4, 4.8, 9.6, 19.2 and 36 represent the final Viscozyme L concentrations of 0, 2.4, 4.8, 9.6, 19.2 and 36 units/g of psyllium in the solid-state reaction mixtures, respectively. The setting time was 48 h for all samples. All tests were conducted in triplicate and means are used. Vertical bars represent the standard deviation of each data point ($n=3$).

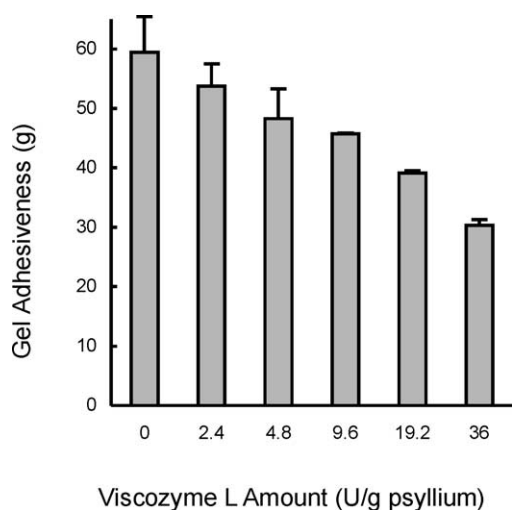


Fig. 3. Adhesiveness of the gels prepared from the modified psyllium. 0, 2.4, 4.8, 9.6, 19.2 and 36 represent the final Viscozyme L concentrations of 0, 2.4, 4.8, 9.6, 19.2 and 36 units/g of psyllium in the solid-state reaction mixtures, respectively. The setting time was 48 h for all samples. All tests were conducted in triplicate and means are used. Vertical bars represent the standard deviation of each data point ($n=3$).

remove water from the rubbery gel products if the enzymatic modification of psyllium is performed under conventional aqueous-phase reaction conditions (Yu et al., 2001). In addition, the solid-state enzymatic reaction generates no hazardous wastes.

It is well accepted that the gelling process begins with the formation of junction zones (Whistler & BeMiller, 1997). The junctions grow and join the polysaccharide

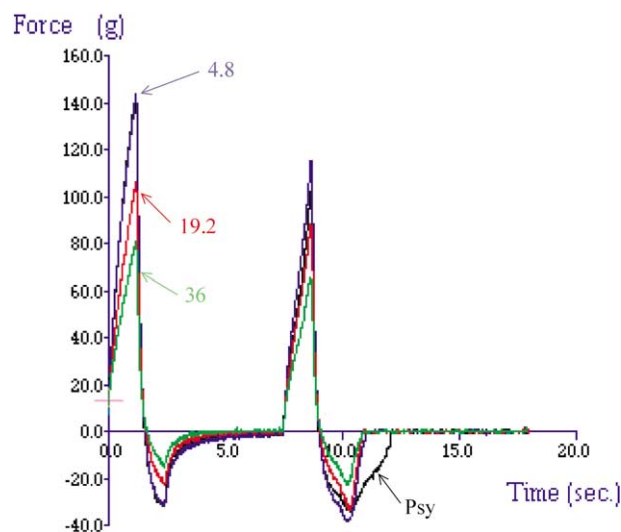


Fig. 4. Effects of Viscozyme L on the gelling properties of psyllium. 4.8, 19.2 and 36 represent the final Viscozyme L concentrations of 4.8, 19.2 and 36 units/g of psyllium in the solid-state reaction mixtures, respectively, while Psy stands for original psyllium. The setting time was 3 h for all samples.

molecules to form the gel network. It is also well known that the functionalities or physicochemical properties of a polysaccharide are determined by their chemical and molecular structures. The Viscozyme L treatment may alter the chemical or/and molecular structures of psyllium and reduce its ability to form the junction zones. This eventually results in a reduction in its gelling abilities. In addition, different chemical mechanism(s) may be involved in the solid-state and conventional aqueous-phase enzymatic reactions. Psyllium is a highly branched acidic arabinoxylan (Chan & Wypyszyk, 1988). Viscozyme L contains several carbohydrase activities and may cleave the xylan backbone as well as branch linkages in a psyllium polysaccharide molecule. In a conventional enzyme reaction, adequate amounts of free water are available as reagents, and therefore an enzymatic hydrolysis is the predominant reaction. Under the solid-state reaction condition, where a limited number of free water molecules are available, transglycosylations might occur. The possible occurrence of more than one reaction in the solid-state enzymatic reaction provides a possible explanation for the observation that a 71% reduction in gel hardness was obtained under the solid-state reaction condition, while a 69% reduction in gel hardness was obtained in a conventional enzyme reaction with a similar level of Viscozyme L (Yu et al., 2001).

To better understand the reduction in water-absorbing capacity of the modified psyllium, SEM was conducted to measure and compare surface structures of modified psyllium with those of original psyllium husks and the control psyllium. SEM results showed that the psyllium treated with a higher level of Viscozyme L had a smoother surface than that treated with a lower level

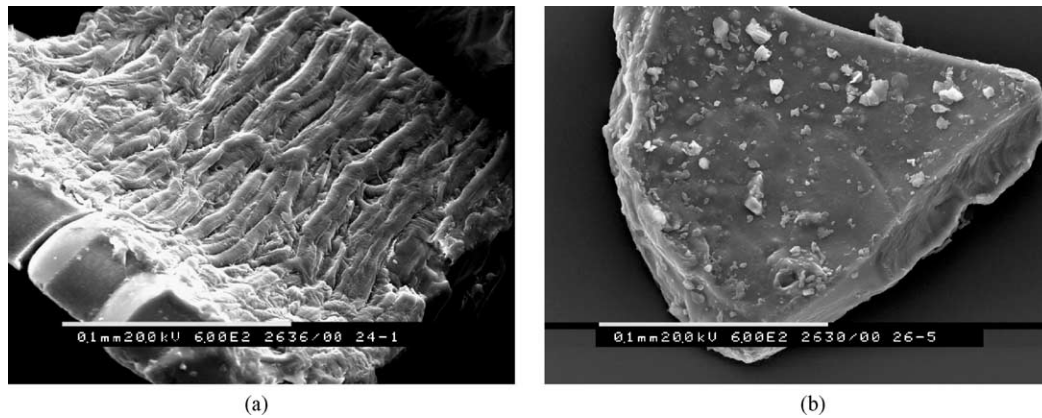


Fig. 5. Comparison of the surface structures of modified psyllium samples. A and B represent the SEM results of the psyllium samples treated with the Viscozyme L at levels of 2.4 and 30 units/g of psyllium, respectively, under the same experimental conditions.

of Viscozyme L (Fig. 5). In other words, the Viscozyme L treatment resulted in a reduced surface area. The reduction of particle surface area provides a reasonable explanation for the suppressed water-absorbing rates of the modified psyllium preparations.

Fibre contents of the modified psyllium preparations were determined, since soluble fibre content is an important indicator of their potential cholesterol-lowering activities (Yu et al., 2001). Compared to the control, there appeared to be a loss of soluble fibre but no change in insoluble fibre for the psyllium samples treated with Viscozyme L (Table 1). Earlier in 2001, Yu and others reported a reduction of 15 and 26% in the soluble fibre contents for the psyllium preparations treated with Viscozyme L at levels of 12 and 60 units/g psyllium. These results indicate that the Viscozyme L treatment damages less soluble fibre under the solid-state reaction condition and is a preferred procedure for preparing novel psyllium for applications in lowering the total or LDL cholesterol. A hamster feeding study is underway to compare the cholesterol-lowering activity of the modified psyllium from this study with that of the original psyllium.

Table 1
Effects of the Viscozyme L treatment on fibre contents

Viscozyme L concentration (U/g psyllium)	Soluble fibre (g/100 g psyllium)	Insoluble fibre (g/100 g psyllium)
Cont ^a	78.5	13.0
2.4	72.9	12.4
4.8	77.3	12.5
9.6	75.9	13.2
19.2	73.1	11.9
36	68.3	12.3
Psy ^b	79.6	12.4

^a Cont is the control containing no Viscozyme L in the solid-state reaction mixture.

^b Psy stands for the commercial psyllium husk, the starting material of the solid-state Viscozyme L reactions.

In conclusion, Viscozyme L treatment, following a special solid-state reaction procedure, has the potential to prepare novel psyllium fibres with improved water-absorbing ability and gelling properties. These novel psyllium fibres could be used to prepare functional foods that have cholesterol-lowering effect.

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