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Effects of Xylanase Treatments on Gelling and Water-Uptaking Properties of Psyllium

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The effects of a commercial food-grade xylanase on the physicochemical properties of psyllium were evaluated. The enzymatic reactions were conducted in solid state at ambient temperature. The enzyme-treated psyllium preparations were analyzed and compared with the starting psyllium for their water-uptake properties, gelling capacities, soluble and insoluble fiber contents, and surface structures. The solid-state xylanase treatment significantly reduced both water-uptake and gelling capacities of psyllium ($p < 0.01$), with a slight decrease of soluble fiber content, whereas no effect on insoluble fiber content was observed. The xylanase treatment also resulted in a smoother surface structure of psyllium particles. In addition, no special equipment and operation were required to conduct the enzymatic reaction, which generated no waste. These data indicated a potential to improve the physicochemical properties of psyllium by use of the solid-state xylanase reactions to promote the utilization of psyllium fiber in functional foods for promoting human health.

KEYWORDS: Psyllium; *Plantago*; enzyme; xylanase; gelling capacity; water-uptake capacity; fiber; soluble fiber

INTRODUCTION

Dietary fiber has showed protective effects against a range of chronic diseases including coronary heart diseases (*1*, *2*). Coronary heart disease (CHD) is a major cause of morbidity and mortality for both men and women, with an estimated cost of $50−100 billion annually in the United States alone (*3*). Lowering total and low-density lipoprotein (LDL) cholesterol levels is considered to be an important strategy to reduce the risks of CHD, since there is a positive association with elevated LDL cholesterol level and CHD risks (*4*). According to National Cholesterol Education Program (NCEP) criteria, there are more than 60 million adult Americans that still need cholesterol-lowering dietary treatment to reduce total serum cholesterol and LDL cholesterol (*3*, *5*). Dietary fibers, mainly water-soluble fiber, are widely accepted for lowering total and LDL cholesterol levels.

Psyllium, a mucilaginous material prepared from the seed husk from the plants of *Plantago* genus, is an excellent source of both soluble and insoluble fibers. The soluble fiber content in psyllium is approximately 8 times greater than that of oat bran (*6*). In 1998, the U.S. Food and Drug Administration (FDA) approved that food products containing a certain amount of psyllium may have a claim of cholesterol-lowering effects on their labels. A number of studies have been conducted to investigate the health benefits of psyllium and its applications in food and other consumer products such as hair-setting lotions (*7*). In addition to its cholesterol-lowering activity, psyllium may also exhibit laxative effects, reduce the risk of colon cancer, treat gastric hypoacidity, and may be used for weight control (*8*−*11*).

However, the strong water uptaking/absorbing and gelling properties of psyllium make it a real challenge to incorporate psyllium in food/beverage formulas at the level required to have a health claim on label. In addition to its undesirable functionalities in food formulation and processing, an unpleasant slimy mouth feeling and an undesirable flavor characteristic are also related to these properties. To promote the application of psyllium in foods or other consumer products, it is necessary to improve its functional/biological/sensory properties.

There have been many efforts to improve the physicochemical/functional/sensory properties of psyllium by physical/mechanical means. These included coating and granulating psyllium to improve its water dispersibility (*6*, *12*), preparing extrudes of psyllium blended with other food ingredients (*13*), changing the pH or adding an edible acid, and mixing with sugar (*6*). These previous investigations have indicated the potential possibility of improving the physicochemical/sensory properties of psyllium to promote its applications in foods. However, none of them could sufficiently solve the strong gelling and extreme water-uptake problems of psyllium. No structural modification has been conducted to improve the functional properties of psyllium until Yu et al. (*6*) disclosed a method to produce novel psyllium preparations with improved sensory properties, reduced water-uptaking capacity, and different gelling properties. However, this enzymatic procedure for improving psyllium properties

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cannot be commercialized because a freeze-dry procedure had to be involved to remove water from the rubbery gels of the final reaction products (*6*).

In the present study, the effects of a commercial xylanase on the gelling and water-uptaking properties of psyllium were evaluated by a solid-state reaction procedure. This procedure requires no special equipment or operation and therefore could be easily scaled up for commercial production. In addition, no hazardous wastes are generated through the solid-state reaction process.

MATERIALS AND METHODS

Psyllium husks (95% purity, 40 mesh) were kindly provided by the Bio-Products Co. (Joliet, IL), while the xylanase (Shearzyme 500L) used in this research was a gift from Novo Nordisk Biochem North America, Inc. (Franklinton, NC). All other chemicals and solvents were the highest commercial grade and used without further purification.

Preparation of Xylanase-Treated Psyllium. A certain amount of xylanase was mixed into psyllium powder to start the solid-state enzymatic reaction, with the reaction mixture appearing as powdered solid. The tested final concentrations of xylanase were 0, 24, 48, 72, and 120 units/g of psyllium. The reaction was conducted at ambient temperature for up to 120 h and terminated by inactivating the enzyme. The enzyme was inactivated by microwaving the reaction mixture for 2 min in a commercial microwave oven (Sanyo Super Showerwave). The final product of the solid-state reaction was obtained after grinding the material through a 20-mesh sieve. Controls were performed using the above procedure without addition of the xylanase. The modified psyllium samples were prepared in triplicates and stored at ambient temperature for further analyses.

Water Uptake by Xylanase-Treated Psyllium. Water uptake was determined gravimetrically by a method previously described by Yu and others (*6*). Briefly, all samples were equilibrated in a 10% relative humidity (RH) chamber 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 after exposure to high RH environment. Water uptake was reported as the average amount of water taken up by each gram of the xylanase-treated psyllium per minute. All measurements were made in triplicate.

Gelling Properties. Gelling properties were measured for xylanase-treated psyllium samples and compared with the original psyllium by the method previously described (*6*, *14*, *15*). A TA-XT2 texture analyzer (Texture Technologies Corp., Scarsdale, NY) was used with a 25 mm diameter probe. Analytical samples were prepared by mixing 1.50 g of psyllium into 30 mL of water. After setting for 24 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 posttest speed of 5.0 mm/s, and a distance of 6 mm. All measurements were conducted in triplicate. The results were expressed as the mean ± SD in gram force for hardness and adhesiveness.

Fiber Contents. Soluble and insoluble fiber contents in modified and raw psyllium were measured with a commercial kit purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland) according to the previously reported procedure (*16*).

Scanning Electron Microscopy Measurement. SEM was conducted to determine the potential changes on the surface structures of psyllium particles due to the enzymatic treatments. SEM was performed on a Philips SEM 505 instrument (Holland) at Colorado State University.

Statistic Analysis. Data were reported as mean ± SD for triplicate measurements. An independent samples *t*-test (SPSS for Windows, version rel 10.0.5, 1999, SPSS Inc., Chicago, IL) was conducted to identify differences among means, while a Pearson correlation test was conducted to detect the correlations among means. A $p < 0.05$ was considered statistically significant.

RESULTS

Water-Uptake Properties of Xylanase-Treated Psyllium. Xylanase treatment significantly suppressed the water-uptake ability of the psyllium ($p < 0.01$) (**Figure 1**). Higher enzyme level resulted in a modified psyllium preparation that has a lower water uptake. A significant correlation was observed between the reduction in water uptake and the enzyme concentrations in the solid-state reaction mixtures. The correlation coefficient was −0.98 ($p < 0.05$).

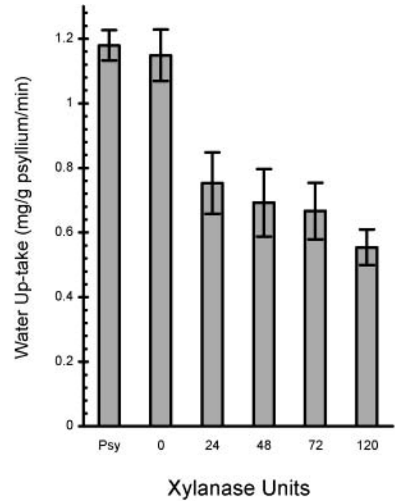

Figure 1. Effects of xylanase treatment on water uptake by psyllium. Bars marked 0, 24, 48, 72, and 120 represent the final xylanase levels of 0, 24, 48, 72, and 120 units/g of psyllium in the solid-state reaction mixtures, respectively, while Psy represents commercial psyllium, the starting material. Vertical bars represent the standard deviation of each data point.

Gelling Properties. Xylanase treatments significantly reduced the gelling capacity of psyllium (**Figure 2**). Less force was required to break the gels prepared from the xylanase-treated psyllium samples than that to break the gels prepared from the control psyllium (0 xylanase-treated) or starting material, the commercial psyllium (**Figure 2A**). Significant deductions were also detected in the adhesiveness of the gels prepared from xylanase-treated psyllium samples ($p < 0.01$) (**Figure 2B**). No significant difference in gel hardness or adhesiveness was detected in the gels prepared from 0 xylanase-treated control psyllium and the commercial psyllium, suggesting the reduced gelling capacities were caused by the xylanase treatments. Furthermore, greater reduction in gelling capacity was observed for psyllium samples treated with higher levels of xylanase (**Figure 2**). Correlations were exhibited between the xylanase concentrations in the final reaction mixtures and the hardness or adhesiveness of the gels prepared from xylanase-treated psyllium samples. The correlation coefficients were −0.90 and −0.80 for hardness and adhesiveness, respectively.

Fiber Contents. Soluble and insoluble fiber contents in xylanase-treated psyllium were measured and compared to that of the commercial psyllium, because fibers might account for the beneficial effects of psyllium. Doubled level of xylanase did not necessarily result in a doubled reduction of soluble fiber contents, while no change of the insoluble fiber contents was observed with xylanase treatments (**Table 1**). The reduction of soluble content was correlated to the level of the xylanase in the reaction mixtures with a correlation coefficient of −0.95.

SEM Measurement. SEM determination showed that xylanase treatment resulted in a smoother structure on the surface of the psyllium particles (**Figure 3B**), as compared to that of the control treated with 0 xylanase (**Figure 3A**).

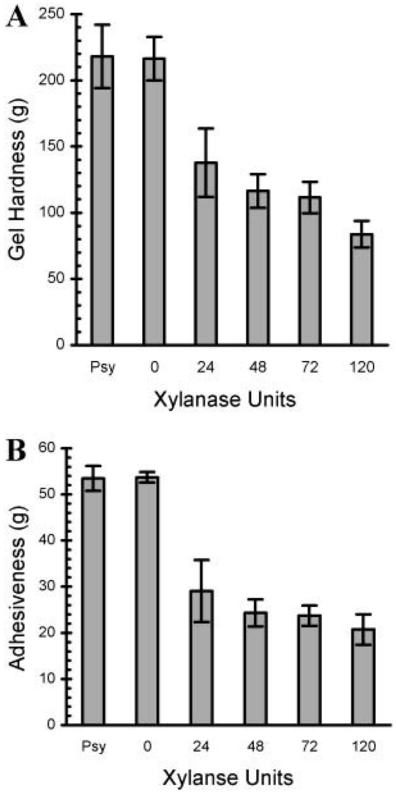

Figure 2. Effects of xylanase treatment on the gelling capacity of psyllium. Bars marked 0, 24, 48, 72, and 120 represent the final xylanase levels of 0, 24, 48, 72, and 120 units/g of psyllium in the solid-state reaction mixtures, respectively, while Psy represents commercial psyllium, the starting material. Vertical bars represent the standard deviation of each data point.

Table 1. Effects of Xylanase Treatment on Fiber Content

xylanase concentration (units/g of psyllium)	soluble fiber (g/100 g of psyllium)	insoluble fiber (g/100 g of psyllium)
0	78.5	13.0
24	79.2	12.7
48	77.4	13.1
72	74.3	12.3
120	73.1	12.5
Psy[a]	79.6	12.4

[a] Psy stands for commercial psyllium, the starting material of the solid-state xylanase reactions.

DISCUSSION

It has been widely accepted that eating is not as straightforward as it used to be. There is an increasing consumer desire to ensure overall good health and quality life, rather than balanced nutrition, through functional foods that may reduce the risk of certain diseases or promote general human health (*17*, *18*). The key to producing a functional food is to develop a novel health beneficial factor, a nutraceutical, that has desirable physicochemical and sensory properties. A practical way for a nutraceutical development is to improve the physicochemical or sensory characteristics of a known bioactive component by chemical, physical, or enzymatic means to promote its utilization in food products. Enzymatic treatments/modifications have been successfully used in food industries to prepare novel food ingredients, refine processing procedures, or improve food qualities (*6*, *19*, *20*). To promote the applications of psyllium as a bioactive food additive for lowering cholesterol, controlling body weight, or as a laxative agent, it is important to improve the physicochemical properties of psyllium without reducing its health-beneficial effects. This study was conducted to evaluate the effects of xylanase, a commercially available food-grade enzyme preparation, on the water-uptake and gelling properties of psyllium under a special solid-state reaction condition.

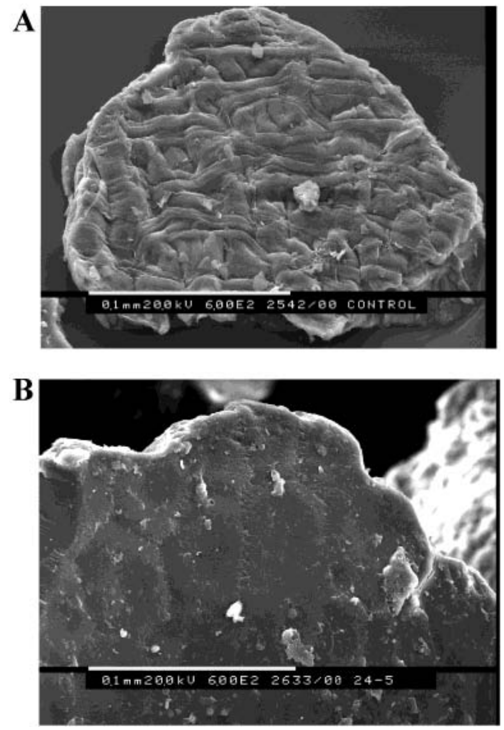

Figure 3. Surface structures of the xylanase-treated psyllium samples measured by SEM. Panels **A** and **B** are the SEM results for psyllium samples treated by 0 and 120 units of xylanase under the experimental conditions, respectively.

It is well-known that the formation of a junction zone is the initial step of the gelling process (*21*). The junction zones grow and join the polymers, such as polysaccharides or proteins, together to form a gel network by intermolecular hydrogen bonding, hydrophobic interactions, ionic associations, entanglement, or covalent bonds. Enzymatic treatments may alter the chemical or molecular structure of a polymer and consequently alter the capacity of the polymer to form a junction zone and a gel. It also has to be noticed that different chemical mechanism(s) may be involved in the conventional aqueous-phase and solid-state enzymatic reactions. Psyllium is a highly branched acidic arabinoxylan (*6*). The xylan backbone has both 1→4 and 1→3 linkages (*7*). In a conventional enzyme reaction, an adequate amount of free water molecules are available as reagents, and therefore an enzymatic hydrolysis catalyzed by the xylanase may be the predominant reaction. This is supported by the observation that a great reduction in soluble fiber content was detected in the modified psyllium prepared by the conventional aqueous-phase enzymatic procedure (*6*). In contrast, a limited amount of free water molecules are available in the solid-state enzymatic reaction, and other types of reactions, such as isomerization, might occur. These reactions may change the molecular structures of psyllium and inhibit the formation of junction zones, and consequently reduce the gelling capacity of the modified psyllium, with less reduction in soluble fiber content. Further research is needed to investigate the mechanism(s) involved in solid-state enzymatic procedure.

The reduction of water-uptake ability cannot be explained well by the breaking down of the xylan backbone, although that may be a reasonable mechanism of reducing the gelling capacity of the modified psyllium. To better understand the mechanism(s) involved in the reducing water-uptake ability, SEM was conducted to measure and compare the surface structures of the psyllium samples treated with 0 and 120 units of xylanase/g of psyllium. The xylanase treatments resulted in a smoother surface on the psyllium particles (Figure 3). In other words, xylanase treatment reduced the total surface areas of the psyllium particle. The reduction of the surface area may be associated with the decreased water-uptaking properties of the polysaccharide particles.

It is also realized that the chemical or enzymatic reactions may result in a change of the biological activities of the nutraceutical(s) due to the possible alterations of their chemical and molecular structures under the reaction conditions. The effects of the xylanase on both soluble and insoluble fiber contents also were determined because they are important parameters associated with the potential health-beneficial effects of psyllium. The xylanase-treated psyllium preparations are believed to retain their beneficial effects since less change in fiber content was observed (*6*).

In this study, xylanase treatment at a ratio of xylanase to psyllium of 70 units/g of psyllium reduced water-uptaking rate by 42%, gel hardness by 48%, and gel adhesiveness by 55% with a 5% reduction in soluble fiber content (**Table 1**). In 2001, Yu and co-workers (*6*) reported that the xylanase at a level of 100 units/g of psyllium resulted in a reduction of 26% in water-uptaking rate, 23% in gel hardness, and 23% in gel adhesiveness with a 6.6% decrease in soluble fiber content. This suggests that the solid-state enzymatic reaction may result in a greater reduction in both gelling capacity and water-uptaking capacity with same level of xylanase. In addition, the final psyllium products of the solid-state xylanase reactions are solid powders and no additional step is required to remove water, while a freeze-dry procedure has to be involved to remove moisture from the wet-enzymatic treated psyllium products that appear as rubbery gels (*6*). The solid-state enzymatic reaction may be easily scaled up for commercial production of novel psyllium preparations with desirable properties.

In conclusion, the present study indicated that the xylanase treatment might improve the water-uptake and gelling properties of psyllium under a solid-state reaction condition, with a slight reduction of soluble fiber content. The solid-state procedure has great potential to be scaled up for commercial production. The xylanase-treated psyllium preparations are considered to retain their beneficial effects because significant levels of both soluble and insoluble fibers are still available (*6*). A hamster feeding study has been conducted to demonstrate the cholesterol-lowering activity of the xylanase-treated psyllium preparations, and the results will be reported separately.

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