JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 6623−6626 **6623**

In Vitro Hypoglycemic Effects of Different Insoluble Fiber-Rich Fractions Prepared from the Peel of *Citrus Sinensis* L. cv. Liucheng

CHI-FAI CHAU,* YA-LING HUANG, AND MAO-HSIANG LEE

Department of Food Science, National Chung Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan, Republic of China

Insoluble fiber-rich fractions (FRFs), including insoluble dietary fiber, alcohol-insoluble solid, and water-insoluble solid, were isolated from the peel of *Citrus sinensis* L. cv. Liucheng. We found that these three FRFs could effectively adsorb glucose, retard glucose diffusion, and inhibit the activity of α-amylase to different extents. These mechanisms might create a concerted benefit in decreasing the rate of glucose absorption and eventually lower the concentration of postprandial serum glucose. The potential hypoglycemic effects of these FRFs suggested that they could be incorporated as low-calorie bulk ingredients in high-fiber foods to reduce calorie level and control blood glucose level.

KEYWORDS: Insoluble dietary fiber; alcohol-insoluble solid; water-insoluble solid; fiber-rich fractions; in vitro hypoglycemic effect; *Citrus sinensis* L. cv. Liucheng; peel

INTRODUCTION

In addition to the well-known role of dietary fiber in reducing the risk of cardiovascular disease, colon cancer, and obesity (*1*, *2*), increased consumption of dietary fiber could also lower the glycemic response in diabetics (*3*, *4*). Some authors have demonstrated that some insoluble fiber-rich materials could retard starch digestion, adsorb glucose, reduce glucose absorption, and also control postprandial serum glucose level (*5*−*8*). Fibers of different composition, source, and preparation might vary in their effectiveness in controlling hyperglycemia (*9*).

The market for dietary fiber is highly competitive. New food fibers with healthy properties are necessary to satisfy the demands from consumers and market. Currently, there is a great variety of agricultural byproducts from which dietary fiber powders are obtained, such as fruits, cereals, and vegetables (*10*). Schieber et al. (*11*) has pointed out that agricultural byproducts could be exploited as a potential source of fibers and functional compounds for food applications.

Citrus sinensis L. cv. Liucheng (Liucheng sweet orange) indigenous to Taiwan is commonly used for juice production. After the juice extraction process, thousands of tons of orange peel are produced and discarded as feed. From our previous findings (*12*), the peel is rich in fiber-rich fractions (FRFs) including insoluble dietary fiber, alcohol-insoluble solid, and water-insoluble solid (476−515 g kg^{-1} of peel), which are mainly composed of pectic substances and cellulose. Insoluble dietary fiber is the dominant fiber fraction (∼ 83.5% of total dietary fiber). The physicochemical properties (e.g., swelling property, water- and oil-holding capacities, and cation-exchange capacity) of these three FRFs were found to be much higher than those of cellulose. These FRFs could therefore be a promising source of food fiber.

The aim of this study is to prepare various insoluble FRFs from the peel of Liucheng sweet orange via different methods and to evaluate their potential hypoglycemic effects by several in vitro tests. The influences of these FRFs on the availability and diffusion of glucose and the enzymatic degradation of starch were determined and compared with those of cellulose. The potential role of the peel insoluble FRFs in lowering postprandial serum glucose level will be discussed in this study.

MATERIALS AND METHODS

Preparation of Peel Sample. After the juice extraction process, the peel of *C. sinensis* L. cv. Liucheng (LSO) was collected from CHIA-MEEI Food Industrial Corp., Taiwan. The LSO peel sample was dried in an air oven at 40 °C for 48 h. The moisture content of the dried peel sample was 75.7 ± 0.42 g kg^{-1}. The dried sample was then finely ground to 0.5 mm in size and kept in a desiccator until used.

Separation of Insoluble Dietary Fiber (IDF). According to AOAC method 985.29 (*13*), the IDF in the peel sample was separated and quantified using the fiber assay kit (Megazyme K-TDFR, Wicklow, Ireland). DF contents were corrected for residual protein, ash, and blank.

Separation of Alcohol-Insoluble Solid (AIS) and Water-Insoluble Solid (WIS). According to the method of Chau and Huang (*12*), AIS and WIS were separated from the LSO peel sample. AIS was prepared by homogenizing the peel sample in 85% (v/v) boiling alcohol (peel-to-alcohol ratio of 1:30, w/v) using an Osterizer (Sunbeam-Oster, Chicago, IL) at the "Hi" speed for 1 min, followed by further boiling for 40 min. WIS was prepared by homogenizing the peel sample in cold distilled water (peel-to-water ratio of 1:10, w/v) using an Osterizer (Sunbeam-Oster) at the "Hi" speed for 1 min. After filtration, AIS and WIS were washed with 70% ethanol and dried by solvent exchange

* To whom correspondence should be addressed. Tel: 886-4-22840385-63. Fax: 886-4-22876211. E-mail: chaucf@dragon.nchu.edu.tw.

10.1021/jf034449y CCC: $25.00
Published on Web 09/26/2003

Table 1. Glucose-Adsorption Capacity[a] of Various Fibers at Different Glucose Concentrations

fiber sample	glucose adsorbed (mmol g^{-1}) 200 mmol L^{-1}	100 mmol L^{-1}	50 mmol L^{-1}	10 mmol L^{-1}
cellulose[b]	18.5w	8.75w	3.68x	tr[c]
IDF	23.3x	11.3x	5.14y	tr
AIS	20.0y	9.36y	3.97x	tr
WIS	19.2z	9.95wy	4.09z	tr

[a] Means of triplicates. Values in the same column with different letters are significantly different (Duncan, $P < 0.05$). [b] Alphacel-Nonnutritive fiber, ICN Nutritional Biochemicals, Cleveland, OH. [c] Trace amount (<0.01).

and air at 30 °C. These FRFs were determined on a weight basis without correction for protein and ash.

Determination of Glucose-Adsorption Capacity. According to the method of Ou et al. (*6*), 1 g of fiber sample was mixed with 100 mL of glucose solution (10−200 mmol L^{-1}) at 37 °C for 6 h. After centrifugation at 3500*g* for 15 min, the final glucose content in the mixture solution was determined to estimate the glucose-adsorption capacity (millimoles per gram) of the fibers.

Measurement of Glucose Dialysis Retardation Index (GDRI). On the basis of the methods of Ou et al. (*6*) and López et al. (*14*) with slight modifications, a fiber sample (0.5 g) was mixed with 25 mL of glucose solution (50 mmol L^{-1}), and the mixture solution was dialyzed against 80 mL of distilled water at 37 °C using a dialysis membrane with a cutoff molecular weight of 12 000. After 20, 30, 60, 120, and 180 min, the glucose content in the dialysate was measured using the glucose assay kit (Megazyme K-GLUC, Wicklow, Ireland) to determine the GDRI as a function of time. A control test was carried out without the addition of fiber. The GDRI was calculated with the following equation: glucose dialysis retardation index = 100 − [((glucose content with the addition of fiber)/(glucose content of control)) × 100].

Determination of Amylolysis Kinetics. A starch solution (4%, w/v) was obtained by stirring 10 g of potato starch in 200 mL of 0.05 M phosphate buffer (pH 6.5) at 65 °C for 30 min, followed by making up to a final volume of 250 mL. Into 10 mL of starch solution were added 0.2 g of fiber sample and 0.04 g of α-amylase (Cat. No. 100447, ICN Biomedicals), and this mixture solution was dialyzed against 200 mL of distilled water at 37 °C using a dialysis membrane with a cutoff molecular weight of 12 000. Within the digestion process (2 h), the content of maltose, being a kinetic component of amylolysis, in the dialysate (1 mL) was determined after 20, 30, 60, and 120 min by adding 1 mL of 3,5-dinitrosalicyclic acid reagent (*15*). The effects of fibers on the starch digestion were determined as a function of time. A control test was carried out without the addition of fiber.

In addition, the amylase inhibitory activity (%) of the fibers was also determined by stirring 1 g of fiber sample and 4 mg of α-amylase (Cat. No. 100447, ICN Biomedicals) in 40 mL of starch solution (4%, w/v) at 37 °C for 60 min. The starch degradation was terminated by the addition of 0.1 M NaOH (80 mL). After centrifugation (3500*g*) for 15 min, the supernatant (1 mL) was analyzed for maltose content by using 1 mL of 3,5-dinitrosalicyclic acid reagent (*15*) to obtain the maltose production rate (micromoles per hour). A control test was done without the addition of fiber. The amylase inhibitory activity (%) was defined as the percent decrease in the maltose production rate over the control.

Statistical Analysis. All determinations, which were carried out in triplicate, were analyzed by one-way analysis of variance using the Statistical Analysis System (SAS). An α level of 0.05 was set to determine statistical significance.

RESULTS AND DISCUSSION

In this study, the LSO peel (262 ± 21.6 g kg^{-1} of fresh fruit) was rich in insoluble FRFs, including IDF, AIS, and WIS (476, 515, and 502 g kg^{-1}, respectively). **Table 1** reveals that all of the fiber samples at different glucose concentrations (50−200 mmol L^{-1}) could bind glucose (3.68−5.14 to 18.5−23.3 mmol g^{-1}) effectively, and the amounts of glucose bound to these fibers were concentration-dependent. In general, the glucose adsorption capacity of these FRFs was higher than those of cellulose. The abilities of insoluble fibers (e.g., insoluble fibers from artichoke and wheat bran, and resistant starch) to adsorb glucose have also been reported by Ou et al. (*6*) and López et al. (*14*). When the glucose concentration decreased to 10 mmol L^{-1}, all of the fibers could still adsorb a trace amount of glucose (Table 1). It was speculated that the three FRFs could keep the glucose in the intestinal lumen at a low concentration.

Table 2 presents the effects of various fibers on glucose diffusion and glucose dialysis retardation index (GDRI). The glucose contents in the dialysate among the three FRFs and cellulose were elevated from 98.3 to 121 μmol (at 20 min) to 292−355 μmol (at 180 min) as the time increased from 20 to 180 min. While comparing with the control, it was revealed that all of the FRFs could significantly ($P < 0.05$) decrease the contents of diffused glucose. On the basis of the retardation in glucose diffusion, GDRI for different fibers could be obtained (**Table 2**). GDRI is a useful in vitro index to predict the effect of fiber on the delay in glucose absorption in the gastrointestinal tract (*14*). At 20 min, the GDRI of the three FRFs (17.2−26.6%) was significantly ($P < 0.05$) higher than that of cellulose (9.70%). It was observed that GDRI maximal values were reached after 30 min for cellulose and WIS, but IDF and AIS peaked by 60 and 20 min, respectively. As no fiber prehydration step was included in these assays, the perceived variability in the hydration properties of the various materials might have contributed to the differences recorded. When the time increased to 60, 120, and 180 min, the FRFs (8.40−22.0, 8.02−21.9, and 6.98−18.4%, respectively) also had greater GDRI than cellulose (0.84−4.80%). Among all of the fiber samples, the GDRI values generally diminished as the time increased. Findings from Jenkins et al. (*16*) have shown that the delay in glucose absorption in the gastrointestinal tract is determined mainly by the viscosity contributed by fibers. However, the retardation effects due to the three FRFs (**Table 2**), which only contributed little to the viscosity of solution, might be probably attributed to their adsorption capacities (**Table 1**). In addition to the glucose adsorption, the retardation in glucose diffusion might also be attributed to the physical obstacle presented by insoluble fiber particles toward glucose molecules and the entrapment of glucose within the network formed by fibers (*4*, *14*). López et al. (*14*) has demonstrated that the insoluble fibers might have greater GDRI than the soluble fibers. From these results, it was conceivable that these insoluble FRFs could effectively adsorb glucose, delay the glucose diffusion, and then postpone the glucose absorption in the gastrointestinal tract.

Table 3 shows the effects of various fibers on amylolysis kinetics. The maltose contents in the dialysate among the three FRFs and cellulose were elevated as the time increased from 20 min (86.4−121 μmol) to 120 min (418−518 μmol). In comparison with the control, the diffused maltose in the dialysate among the three FRFs at 20, 60, and 120 min were 59.3−76.1, 82.0−82.8, and 69.7−86.4% of control, respectively, and those of cellulose were 82.7, 96.7, and 81.5% of control, respectively. Therefore, the FRFs appeared to be more effective in retarding the amylolysis relative to cellulose along the enzymatic digestion process. In the human body, although glucose is absorbed into the small intestine through an active process, the results of our experiments (**Tables 2** and **3**) at least demonstrated that the glucose diffusion and starch degradation could be delayed by the presence of the insoluble FRFs.

Table 2. Effects of Various Fibers on Glucose Diffusion[a] and Glucose Dialysis Retardation Index[b]

	glucose in dialysate (μmol)				
fiber sample	20 min	30 min	60 min	120 min	180 min
control	134 (0)w	156 (0)w	250 (0)w	324 (0)w	358 (0)w
cellulose[c]	121 (9.70)x	132 (15.4)x	238 (4.80)wx	313 (3.40)wx	355 (0.84)wx
IDF	111 (17.2)y	138 (11.5)x	195 (22.0)y	253 (21.9)z	292 (18.4)y
AIS	98.3 (26.6)z	139 (10.9)x	229 (8.40)wx	298 (8.02)xy	332 (7.26)x
WIS	104 (22.4)yz	107 (31.4)y	212 (15.2)xy	278 (14.2)y	333 (6.98)x

[a] Means of triplicates. Values in the same column with different letters are significantly different (Duncan, $P < 0.05$). [b] Data in parentheses show the glucose dialysis retardation indexes of various fibers. Glucose dialysis retardation index = 100 − [((glucose content with the addition of fiber)/(glucose content of control)) × 100]. [c] Alphacel-Nonnutritive fiber, ICN Nutritional Biochemicals, Cleveland, OH.

Table 3. Effects of Various Fibers on Amylolysis Kinetics

	maltose in dialysate (μmol)[a]			
fiber sample	20 min	30 min	60 min	120 min
control	146v	211v	377v	600v
cellulose[b]	121w	211v	364v	489wx
IDF	105wx	170w	312w	478x
AIS	86.4x	167w	309w	418y
WIS	111wx	177w	309w	518w

[a] Means of triplicates. Values in the same column with different letters are significantly different (Duncan, $P < 0.05$). [b] Alphacel-Nonnutritive fiber, ICN Nutritional Biochemicals, Cleveland, OH.

Table 4. Inhibitory Activity of Various Fibers Against α-Amylase

	control	cellulose[a]	IDF	AIS	WIS
maltose production rate (μmol h^{-1})[b]	726x	672xy	561z	678xy	642y
amylase inhibitory activity (%)[b,c]	-	7.44x	22.8y	6.61x	11.6z

[a] Alphacel-Nonnutritive fiber, ICN Nutritional Biochemicals, Cleveland, OH. [b] Means of triplicates. Values in the same row with different letters are significantly different (Duncan, $P < 0.05$). [c] The amylase inhibitory activity (%) was defined as the percent decrease in the maltose production rate over the control (without fiber addition).

As shown in **Table 4**, the amylase inhibitory activities (%) of the three FRFs relative to cellulose were given. The rates of maltose production for IDF and WIS (560 and 642 μmol h^{-1}, respectively) were significantly ($P < 0.05$) lower than that of control. The results revealed that IDF and WIS could exhibit a stronger effect in reducing the activity of α-amylase (by 11.6 and 22.8%, respectively) as compared to those of AIS and cellulose (by 6.61 and 7.44%, respectively). The apparent reduction in the enzymatic activity revealed that the activity of α-amylase was directly affected by the insoluble fibers to different extents. The variations in the inhibitory activity among the fiber samples showed that the inhibition depended on the kind of fiber. The retardation of α-amylase activity (**Table 4**) and amylolysis kinetics (**Table 3**) by the insoluble FRFs might be attributed to several possible factors, such as fiber concentration, the presence of inhibitors on fibers, capsulation of starch and enzyme by fibers, reduced accessibility of the enzyme to starch, and the direct adsorption of enzyme on fibers leading to the decrease in amylase activity (*6*−*8*, *17*). In comparison to AIS, the significantly ($P < 0.05$) higher inhibitory activity of IDF and WIS against α-amylase implied that the amylase-inhibiting substances present in these LSO peel FRFs might be alcohol-soluble or water-insoluble. From the results of this in vitro study, the abilities of the three FRFs to adsorb glucose and reduce the activity of α-amylase suggested that they might create a concerted function in postponing the release of glucose from starch, delaying the rate of glucose absorption, and then controlling the concentration of postprandial serum glucose. Further studies are needed to investigate whether these FRFs act as inhibitor of α-amylase or a barrier between the amylase and starch.

This study revealed that the LSO peel insoluble FRFs (IDF, AIS, and WIS) could effectively adsorb glucose, retard the diffusion of glucose, and inhibit the activity of α-amylase to different extent. All of these mechanisms might work together to decrease the rate of glucose absorption and control the concentration of postprandial serum glucose. The potential hypoglycemic effects of these FRFs suggested that they could be incorporated as low-calorie bulk ingredients in high-fiber foods to lower postprandial serum glucose level and reduce calorie levels. Further investigations on the in vivo hypoglycemic effect and other physiological functions of these FRFs using animal-feeding experiments are underway.

LITERATURE CITED

(1) Slavin, J. L. Dietary fibre and colon cancer. In *Handbook of Dietary Fiber*; Cho, S. S., Dreher, M. L., Eds.; Marcel Dekker: New York, 2001; pp 31−45.

(2) Rosamond, W. D. Dietary fiber and prevention of cardiovascular disease. *J. Am. Coll. Cardiol*. **2002**, *39*, 57−59.

(3) Berger, M.; Venhaus, A. Dietary fibre in the prevention and treatment of diabetes mellitus. In *Dietary Fiber−A Component of Food Nutritional Function in Health and Disease*; Schweizer, T. F., Edwards, C. A., Eds.; Springer-Verlag: London, 1992; pp 181−196.

(4) Nishimune, T.; Yakushiji, T.; Sumimoto, T.; Taguchi, S.; Konishi, Y.; Nakahara, S.; Ichikawa, T.; Kunita, N. Glycemic response and fiber content of some foods. *Am. J. Clin. Nutr*. **1991**, *54*, 414−419.

(5) Flourie, B. The influence of dietary fiber on carbohydrate digestion and absorption. In *Dietary Fiber−A Component of Food Nutritional Function in Health and Disease*; Schweizer, T. F., Edwards, C. A., Eds.; Springer-Verlag: London, 1992; pp 181−196.

(6) Ou, S.; Kwok, K. C.; Li, Y.; Fu, L. In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. *J. Agric. Food Chem.* **2001**, *49*, 1026−1029.

(7) Morón, D.; Melito, C.; Tovar, J. Effect of indigestible residue from foodstuffs on trypsin and pancreatic amylase activity in vitro. *J. Sci. Food Agric.* **1989**, *47*, 171−179.

(8) Gourgue, C. M. P.; Champ, M. M. J.; Lozano, Y.; Delort-Laval, J. Dietary fiber from mango byproducts: characterization and hypoglycemic effects determined by in vitro methods. *J. Agric. Food Chem.* **1992**, *40*, 1864−1868.

(9) Bornet, F. R. J.; Billaux, M. S.; Messing, B. Glycemic index concept and metabolic diseases. *Int. J. Biol. Marcomol.* **1997**, *21*, 207−219.

(10) Larrauri, J. A. New approaches in the preparation of high dietary fiber powders from fruit byproducts. *Trends Food Sci. Technol.* **1999**, *10*, 3−8.

(11) Schieber, A.; Stintzing, F. C.; Carle, R. Byproducts of plant food processing as a source of functional compounds − recent developments. *Trends Food Sci. Technol.* **2001**, *12*, 401−413.
(12) Chau, C. F.; Huang, Y. L. Comparison of the Chemical Composition and Physicochemical Properties of Different Fibers Prepared from the Peel of *Citrus sinensis* L. Cv. Liucheng. *J. Agric. Food Chem.* **2003**, *51*, 2615−2618.
(13) AOAC. *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Washington, DC, 1995.
(14) López, G.; Ros, G.; Rincón, F.; Periago, M. J.; Martínez, M. C.; Ortuño, J. Relationship between physical and hydration properties of soluble and insoluble fiber of artichoke. *J. Agric. Food Chem.* **1996**, *44*, 2773−2778.
(15) Chau, C. F. Nutritional values of three leguminous seeds and functional properties of their protein and fiber fractions. Ph.D. Dissertation, The Chinese University of Hong Kong, Hong Kong, 1998; pp 80−85.
(16) Jenkins, D. J. A.; Jenkins, M. J. A.; Wolver, T. M. S.; Taylor, R. H.; Ghafari, H. Slow release carbohydrate: mechanism of action of viscous fiber. *J. Clin. Nutr. Gastroenterol.* **1986**, *1*, 237−241.
(17) Isaksson, G.; Lundquist, I.; Ihse, I. Effect of dietary fiber on pancreatic enzyme activity in vitro. The importance of viscosity, pH, ionic strength, adsorption and time of incubation. *Gastroenterol.* **1982**, *82*, 918−924.

Received for review April 30, 2003. Revised manuscript received August 13, 2003. Accepted August 28, 2003. This study was carried out with financial support from the National Science Council of the Republic of China (NSC: Grant No. 92-2313-B-005-068).

JF034449Y