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PREPARATION AND CHARACTERISTICS OF ENZYME-RESISTANT PYRODEXTRINS FROM CORN STARCH

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Key words: pyroconversion, soluble dietary fiber, dextrins, enzyme-resistance, corn starch

Corn starch was modified by pyroconversion at 130°C in the presence of acid catalyst in a sealed container to produce non-digestible pyrodextrins. The effect of reaction time on the dextrinization process was investigated. Physicochemical properties of the pyrodextrins were analyzed, and the effect of the structure of pyrodextrins on the digestibility was evaluated. The pyrodextrin, prepared by heating corn starch with hydrochloric acid (0.1%, dry starch basis) at 130°C for 180 min, was well-soluble (97% at 25°C) and contained only one fraction of average molecular weight of 2.2 x 10^4 g/mol. The enzyme resistance of pyrodextrin, measured according to AOAC method 991.43 for Total Dietary Fiber, reached 42%.

INTRODUCTION

Recently pyroconversion of starch has been proposed as the method of preparation non-digested dextrins. Wang *et al* [2001] have reported the preparation, and characteristics of enzyme-resistant dextrins from high-amylose corn mutant starches. Preparation and characteristics of indigestible pyrodextrins from waxy sorgum starch [Kwon *et al.,* 2005], lima bean starch [Orozco-Martinez & Betancur-Ancona, 2004], lentil, dark sorghum, cocoyam, sagu [Laurentin *et al.,* 2003], cowpea [Campechano-Carrera *et al.,* 2007] starches have been reported.

Pyroconversion involves different chemical reactions, including hydrolysis, transglucosidation, and repolymerization [Wurzburg, 1986]. After hydrolysis, the new reducing end becomes a glucosyl cation, which can undergo either intermolecular dehydration, producing 1,6-anhydro-β-D-glucopiranose or intermolecular bond formation (transglucosidation) leading to the formation of random 1→2, 1→3, 1→4, and 1→6 glucosidic linkages [Tomasik *et al.,* 1989]. Since glucosidic linkages other than α-D-(1→4), and α-D-(1→6) are formed during dextrinization, highly converted dextrin should also contain indigestible starch that is similar to resistant starch [Wang *et al.,* 2001], but does not fall within three categories classified by Englyst *et al.* [1992]. Products of starch thermal depolymerization – dextrins – belong to the type 4 resistant starch (RS 4) [Leszczyński, 2004].

Pyrodextrinization has been also recognized as a way of producing new type soluble dietary fibers called resistant maltodextrins (in US), resistant dextrins (in EU) or indigestible dextrins (in Japan). Resistant maltodextrins are short chain polymers of glucose that are hardly resistant to digestion in the human digestive system. Nowadays most of the resistant maltodextrins in food products are manufactured from starch by treatments with heat and/or acid, and/or enzymes [Panel on the Definition of Dietary Fiber, 2001]. Ohkuma *et al.* [1997] reported that at high temperature the α-1,4 and α-1,6 glycosidic bonds in the starch have been hydrolyzed. The newly developed hemiacetal/aldehyde groups generated from the bond breakage reacted with -OH groups of the glucose units at random positions to form α-1,2, α-1,3, and other bonds. Starch molecules with α-1,2 and α-1,3 bond linkages or highly clustered bonds could not be digested by enzymes present in the digestive track, exhibited functional properties similar to those of dietary fibers.

Resistant maltodextrins are commercially available. Fibersol-2 is well-known soluble, non-digestible, starch-derived resistant maltodextrin [Ohkuma *et al*., 1997]. Fibersol-2 is produced from cornstarch by pyrolysis and subsequent enzymatic treatment to convert a portion of the normal α-1.4 glucosidic linkages to random 1,2-, 1,3-, and 1,4- α or β linkages [Ohkuma & Wakabayashi, 2001].

The example of commercially available resistant dextrin is Nutriose. It is a soluble dextrin, produced from wheat or maize starch using a highly controlled process of dextrinization followed by chromatographic fractionation step [Fouache *et al*., 2003]. The resistant dextrins have found wide application in food and pharmaceutical industries, as components of fiber-enriched drinks [Serpelloni, 2003], components of a fiber-enriched composition for enteral nutrition [Saniez, 2004], granulation binders [Serpelloni, 2006], in the preparation of low-calorie food [Brendel *et al*., 2002], and sugar-free

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confectionery [Serpelloni, 2002]. Both indigestible dextrins Fibersol-2 and Nutriose [Pasman *et al*., 2006; van den Heuvel *et al*., 2004, 2005; Vermorel *et al*., 2004] had very important physiological properties.

The objective of the present study was to prepare enzyme-resistant, and water-soluble dextrins by heating normal corn starch in the presence of acid catalyst in a sealed container, to investigate effect of reaction time on the dextrinization process and physicochemical properties of dextrins.

MATERIALS AND METHODS

Materials. Corn starch was obtained from American Maize-Products Company (Hammond, IN). Hydrochloric acid (HCl, Cat. No. A142-212 was purchased from Fisher Scientific (Fairlawn, NJ). α-Amylase from *Bacillus licheniformis* (Cat. No. A3403), protease from *Bacillus licheniformis* (Cat. No. P3910), amyloglucosidase from *Aspergillus niger* (Cat. No. A9913), Tris(hydroxyl-methyl)aminomethane (Tris, Cat. No. T1503), 2-Morpholinoethanesulfonic acid (MES, Cat. No. M3671), Celite (Cat. No. C8656) were purchased from Sigma-Aldrich Corp. (St. Louis, MO) and used as received.

Starch pyrodextrinization. Corn starch (80 g, moisture content <5%) was sprayed with hydrochloric acid solution (5% ww., 1.28 mL) to obtain the final HCl concentration of 0.1% dry starch basis (dsb). The mixture was blended in a blender to disperse HCl evenly, and then transferred into anti-pressure Pyrex bottles (250 mL) and capped. The sample was equilibrated overnight at room temperature. The sample in the bottle was then heated at 130°C in a furnace. The various time of heating has been tested: 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160 and 180 min. After heating samples were cooled in a desiccator and analyzed.

For the comparison the pyrodextrins by heating corn starch without hydrochloric acid were prepared.

Determination of moisture content. Samples (0.1000 g each) were weighed in the weighing bottles previously dried and weighed to the nearest 0.0001 g. The bottles with samples were put into oven and dried at 105°C also to the nearest 0.0001 g. Before weighing, the bottles with samples were equilibrated in a desiccator for a minimum 40 min. Measurements were run in triplicates.

Water solubility. Sample suspensions were stirred at a room temperature for 30 min. After stirring the suspensions were centrifuged at 7000 rpm for 20 min, and the amount of dried matter in the supernatant was weighed after drying in an 60°C oven overnight and subsequently in a 110°C oven for 1 hour. Solubility (%) was the weight of dried matter in supernatant x 100/sample weight as dry matter. Measurements were run in triplicates.

Reducing sugar content. Reducing sugar content was measured according to Somogyi-Nelson method using 10–100 (g of glucose/mL as the standard [Somogyi, 1952]. Anhydrous sodium carbonate (25 g), sodium potassium tartrate (25 g), sodium bicarbonate (20 g), anhydrous sodium sulfate (200 g) were dissolved in 800 mL of distilled water, then diluted to 1000 mL (Reagent A). Cupric sulfate pentahydrate (30 g) was dissolved in 200 mL distilled water, and then 4 drops of concentrated sulfuric acid was added (Reagent B). Ammonium molybdate (25 g) was dissolved in 450 mL of distilled water containing 21 mL of concentrated sulfuric acid. Disodium hydrogen arsenate heptahydrate was dissolved in 25 mL of distilled water. The disodium arsenate solution was added to ammonium molybdate solution slowly with stirring, then diluted to 500 mL. The solution was kept at 37-40°C overnight, then stored in a brown bottle (Reagent C). Prior to the experiment Reagent D was prepared by mixing 25 mL of Reagent A and 1 mL of Reagent B. Next, 1 mL of Reagent D was added to 1 mL of sample (standard), heated in a boiling water bath for 20 min, and then cooled for 5 min. in running tap water. Then, 1 mL of reagent C was added to the test tube and shaken until no bubbles were evolved. After standing for 20 min, the solution was diluted to 25 mL, and then absorbance was determined spectrophotometrically at 520 nm. The measurements were run in triplicates.

Determination of enzyme-resistant fraction content in pyrodextrins. Enzymatic-gravimetric AOAC official 991.43 method [2003] was used to analyse the enzyme-resistant fraction content of the pyrodextrins. A sample (1.00 g, dsb) was dispersed in Mes-Tris buffer solution, and digested consecutively by heat-stable α-amylase solution (500 U), protease (30 U), and amyloglucosidase (600 U). After digestion 4 volumes of ethanol (95%, preheated to 60°C) was added. The precipitate was filtered on a tarred sintered-glass crucible with a bed of dried Celite as filter aid under vacuum. The solid residue was washed with ethanol, and acetone. The crucible with the residue was dried overnight in an oven at 110°C, cooled to room temperature in a desiccator, and weighed. Resistant fraction content was calculated as the dried residue divided with the initial weight of dry sample.

Molecular weight distribution of samples determined by High Performance Size Exclusion Chromatography (HPSEC). The molecular weight distribution of pyrodextrins was determined by means of high-performance size-exclusion chromatography. An HPSEC system consisted of an HP 1050 isocratic pump (Hewlett Packard, Valley Forge, PA) equipped with an injection valve (100 μL sample loop, Model 7125, Rheodyne), a multi-angle laser-light-scattering detector (Dawn DSP-F, Wyatt Tech. Corp., Santa Barbara, CA) with a He-Ne laser source (λ=632.8 nm), and K-5 flow cell, and an HP 1047A RI detector (Hewlett Packard, Valley Forge, PA). To separate fractions Shodex OH pack KB-806, and KB-804 analytical columns with a KB-G guard column (Showa Denko K.K, Tokyo, Japan) were used. The temperature of the injector and columns was maintained at 50°C using a CH-460 column heater and a TC-50 controller (Eppendorf, Madison, WI). Temperature of RI detector was set at 35°C. The mobile phase was distilled-deionized water (18.2 MΩcm) passed through in-line membrane filters (0.2 and 0.1 μm, Millipore, Bedford, MA) at a flow rate of 0.5 mL/min. Data obtained from MALLS and RI detectors were analyzed using Astra software (Version 4.7.07, Wyatt Technology, Santa Barbara, CA).

RESULTS AND DISCUSSION

The screening studies [Kapusniak & Jane, 2006] revealed that the optimum conditions for the preparation of enzyme-resistant pyrodextrin from normal corn starch were as follows: the final concentration of hydrochloric acid of 0.1% (dry starch basis, dsb), temperature of 130°C, time of heating of 180 min.

During heating at 130°C the moisture content decreased rapidly for the first 10 min and then stabilized, reflecting reaction of hydrolysis (Figure 1). After 30 min of heating, the moisture content began to rise, possibly as a result of polymerization. At higher temperature and low moisture content, the rearrangement involving breaking the glucosidic linkage at one point in the molecule and reattaching the severed part of the chain to another point in the same or a different molecule by formation of another glucosidic bond attached at the C-2, C-3, C-4 or C-6 position of the anhydroglucose unit at the attachment point was postulated. At high temperature in the presence of acid and anhydrous conditions, the small moieties repolymerize to form larger, highly branched molecules [Wurzburg, 1986; Tomasik *et al.,* 1989; BeMiller, 1993].

Water solubility is an important factor for the use of pyrodextrins as food ingredients. In general, pyroconversion considerably increases the solubility. A low initial moisture content allows full solubility to be reached much faster than in the case of moister starches. Previously authors have revealed that the solubility showed an opposite trend to indigestible fraction content of pyrodextrins [Kwon *et al*., 2005]. That might be caused by polymerization or formation of non-starchy material such as char during pyroconversion. On the other hand high solubility may be very important factor influencing accessibility and/or affinity of the bacterial enzymes to pyrodextrins. In the present study the water solubility at 25°C of pyrodextrins, prepared by heating normal corn starch with HCl (0.1%, dsb) at 130°C, increased rapidly for the first 50 min of conversion before it reached a plateau (Figure 2).

High solubility of the pyrodextrins was related to the reduction in the molecular weight, and high-performance size-exclusion chromatography (HPSEC) confirmed the changes in the molecular weight (Figure 3). The HPSEC chromatogram of native corn starch showed two fractions amylopectin with weight average molecular weight (M_w) of 5.3 x 10^8 g/mol and amylose M_w 3.3 x 10^6 g/mol (Table 1). After 20 min heating at 130°C M_w decreased, and two fractions at M_w of 2.2 x 10^7 and 7.9 x 10^5 g/mol were produced. After 60-min heating the average molecular weight decreased to 2.2 x 10^4 g/mol. The heating up to 180 min did not cause further reduction in the molecular weight.

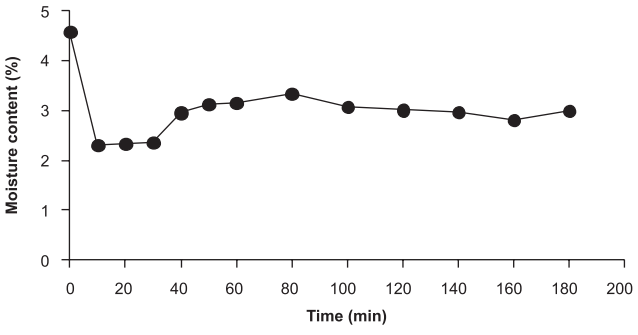

FIGURE 1. Changes of moisture content of the normal corn starch acidified with HCl (0.1%, dsb) during heating at 130°C.

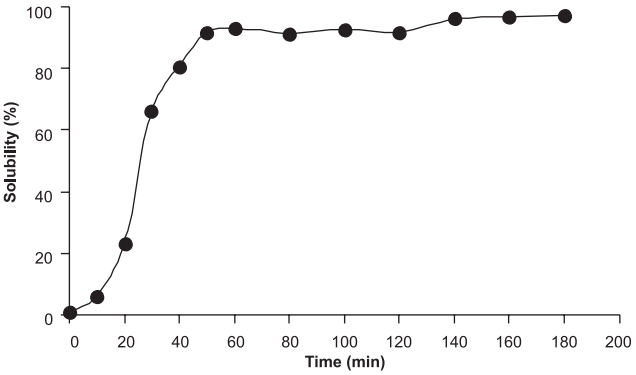

FIGURE 2. Water solubility (25°C) of pyrodextrins prepared with HCl (0.1%, dsb) as a catalyst at 130°C for different time course.

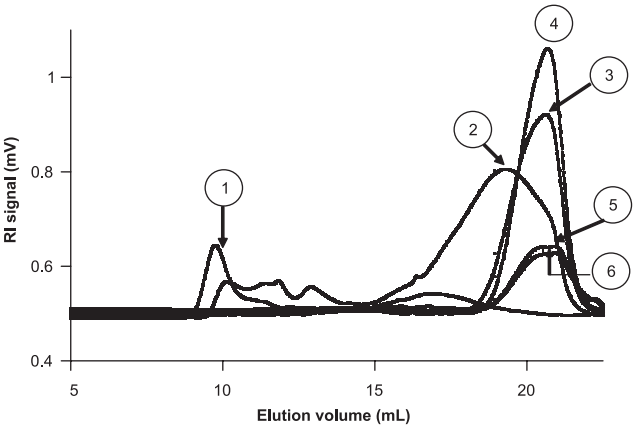

FIGURE 3. HPSEC profiles of normal corn starch (1), and pyrodextrins prepared at 130°C for different time course: 20 min (2), 40 min (3), 60 min (4), 140 min (5), 180 min (6).

TABLE 1. Weight-average molecular weight (Mw) of corn starch heated with hydrochloric acid (0.1%, dsb) at 130°C for different time course.

Pyrodextrinization condition		Fraction 1 M_w (g/mol)	Fraction 2 M_w (g/mol)
Temperature (°C)	Time (min)		
Normal corn starch		5.3 x 10^8	3.3 x 10^6
130	20	2.2 x 10^7	7.9 x 10^5
130	40	nd	1.1 x 10^5
130	60	nd	2.2 x 10^4
130	140	nd	3.0 x 10^4
130	180	nd	8.2 x 10^4

During heating of normal corn starch with HCl (0.1%, dsb) at 130°C the reducing sugar content increased rapidly for the first 80 min, and then stabilized (Figure 4). The significant increase in reducing sugar content indicated that a measurable hydrolysis of glycosidic linkages had occurred. After 80 min of heating the reducing sugar content did not change or slightly decreased indicating involvement of new formed reducing ends in the formation of random α, and β 1→2, 1→3, 1→6 glycosidic linkages.

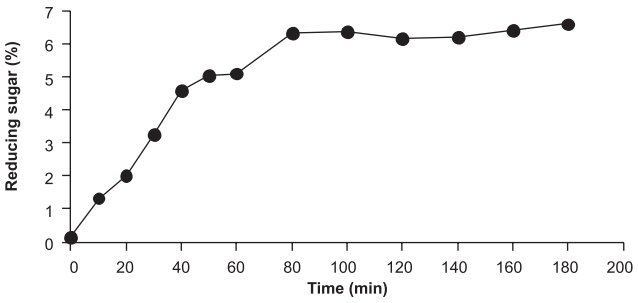

FIGURE 4. Reducing sugar content in pyrodextrins prepared at 130°C for different time course.

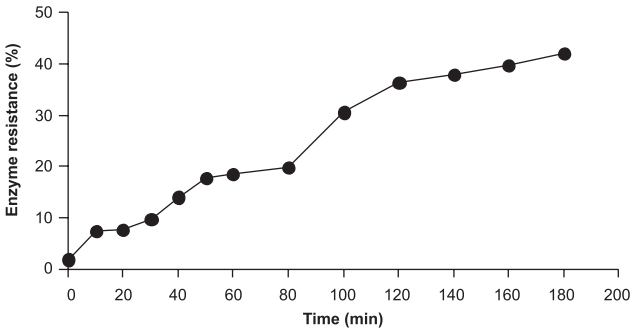

FIGURE 5. Enzyme resistance of pyrodextrins prepared by heating normal corn starch with HCl (0.1%, dsb) as catalyst at 130°C for different time course.

The pyroconversion in the presence of hydrochloric acid resulted in significant increase in enzyme-resistant fraction content. The enzyme resistance increased with increasing temperature and time of heating to about 42% for the sample prepared at 130°C for 180 min (Figure 5). The resistance of pyrodextrins to enzymatic activity results from changes in their structure, compared to starch. Upon heating of starch, depolymerization, transglucosidation and repolymerization proceed in the interior of its particles [Leszczyński, 2004]. With elongation of the dextrinization process, an increase in the number of 1→2 and 1→3 linkages between glucoside residues of resultant dextrins was observed [Ohkuma *et al.*, 1990]. The methylation of dextrins, followed by hydrolysis, and thereafter by gas chromatography [Ohkuma *et al.,* 1994; Ohkuma & Matsuda, 2002] or mass spectrometry [Roturier & Looten, 2006] proved that during pyroconversion the number of 1→2, 1→3, 1→6 linkages increased while 1→4 linkages decreased. By heating, the terminal glucose unit present in reducing ends underwent intermolecular dehydration to form 1.6-dehydroglucose, which subsequently reacted with hydroxyl groups on other chains resulting in new linkages. Also, new bonds could form among cleaved molecules during heating. Consequently, carbohydrate-hydrolyzing enzymes were limited to access their substrate. It was found that the content of indigestible components of dextrin was in inverse proportion to the amount of 1→4 glycosidic linkages among other glycosidic linkages thereof [Ohkuma *et al.,* 1994].

CONCLUSIONS

Heating of corn starch with hydrochloric acid (0.1%, dsb) as catalyst at 130°C for 180 min led to a significant increase in enzyme-resistant fraction content. The pyrodextrin prepared under those conditions was well-soluble (97% at 25°C) and possessed 42% enzyme resistance. One may conclude that pyrodextrinization can be considered as a way of producing soluble dietary fiber.

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PRZYGOTOWANIE I CHARAKTERYSTYKA OPORNYCH NA TRAWIENIE ENZYMATYCZNE PIRODEKSTRYN ZE SKROBI KUKURYDZIANEJ

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Skrobię kukurydzianą poddano procesowi ogrzewania w temperaturze 130°C w obecności katalizatora kwasowego w zamkniętych butelkach szklanych w celu otrzymania opornych na trawienie enzymatyczne pirodekstryn. Przebadano wpływ czasu ogrzewania na przebieg procesu dekstrynizacji. Otrzymane pirodekstryny poddano charakterystyce fizykochemicznej oraz określono wpływ ich struktury na trawienie enzymatyczne. Badania pokazały, że dekstryna otrzymana poprzez ogrzewanie skrobi kukurydzianej z kwasem chlorowodorowym (0,1% suchej masy skrobi) w temperaturze 130°C przez 180 min była bardzo dobrze rozpuszczalna w wodzie (97% w temp. 25°C) i zawierała tylko jedną frakcję o średniej wagowo masie cząsteczkowej wynoszącej 2,2 x 10^4 g/mol. Oporność na trawienie enzymatyczne, wyznaczona z wykorzystaniem metody AOAC 991,43 – oznaczania całkowitej zawartości włókien dietetycznych – wyniosła dla tej dekstryny 42%.