Production of Water-soluble Indigestible Polysaccharides Using Activated Carbon

Abstract: Water-soluble dietary fiber provides numerous health benefits. A novel procedure to efficiently manufacture water-soluble indigestible polysaccharides was developed by heating glucose at 180 °C in the presence of activated carbon. Aside from its ability to catalytically assist the polycondensation of saccharides, activated carbon provides the added benefits of being easily separable from the reactants and suppressing coloration of the product. Prior to purification, the indigestible fraction made up over 80% of the reaction mixture. After hydrolysis catalyzed by α-amylase and glucoamylase, and fractionation by ion-exchange chromatography, a total of 99.7% dietary fiber content was attained. This indigestible fraction, termed resistant glucan, was only minimally degraded by upper digestive tract enzymes, similar to the digestibility of polydextrose. Structural analysis by methylation and NMR indicated that the resistant glucan formed a highly branched structure containing α- and β-1,2-, 1,3-, 1,4-, and 1,6-linkages. On an industrial scale, the resistant glucan was obtained from glucose syrup (DE 86) by heating with activated carbon, enzymatic hydrolysis, refining, fractionating, and drying. Our facile method is an efficient means to obtain water-soluble dietary fiber.

Key words: dietary fiber, polysaccharides, polycondensation, activated carbon, glucose

INTRODUCTION

Most dietary fibers are resistant to hydrolysis catalyzed by human digestive enzymes.1,2) Dietary fibers include many types of polysaccharides such as plant derived cellulose, hemicelluloses, pectin and lignin, and chitin and chitosan originating from animal tissues. Chemically or enzymatically modified polysaccharides considered to be dietary fiber include polydextrose3) and indigestible dextrin.4)

The intake of water-soluble dietary fiber provides numerous health benefits including improving serum lipid values,5,6) increasing carbohydrate metabolism7) and intestinal flora,8) and modulating immune functions.9,10) Water-soluble dietary fibers are widely used in various processed foods and beverages as they are easy to incorporate and manipulate during processing11). However, water-soluble dietary fibers such as guar gum hydrolyzate, glucomannan, highly methoxylated pectin, and low molecular alginic acid make solutions very viscous, limiting their use in processed foods and beverages. Lower viscosity dietary fibers such as polydextrose12,13) and indigestible dextrin14,15) are easily incorporated during processing, and thus, are widely used in food products.

The synthesis of indigestible polysaccharides by various chemical or enzymatic methods has been reported.16-20) One method, dehydration polycondensation, did not require the use of a catalyst.21,22) However, all these methods require an purification process to remove residual catalyst and/or by-products originating from the starting materials to limit problems with taste and coloration. Therefore, there is a need to have a simple and efficient procedure to obtain water-soluble dietary fiber. In this study, an industrial process for producing indigestible polysaccharides for use as dietary fiber from glucose using activated carbon as a catalyst was developed.

MATERIALS AND METHODS

D-Glucopyranose was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Glucose syrup (DE86, moisture content of 29.1%) was obtained from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). D-Glucopyranose (Glc) was dried at 80 °C under vacuum before use. The partial hydrolysates of starch Pinedex no. 2 (DE8) and no. 100 (DE3), maltodextrin, and indigestible dextrin were from Matsutani Chemical Industry (Hyogo, Japan).

The steam-activated carbon (TaikoA; food additive grade, Futamura Chemical Co., Ltd. Aichi, Japan) was dried at 80 °C under vacuum before use. The 6 M hydrochloric acid (19.9%, w/w) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Ortho-phosphoric acid (85%, w/w) and citric acid (99%, w/w) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Amyloglucosidase (EC 3.2.1.3; A-9913), and heat-stable α-amylase (EC 3.2.1.1; A-3306) were purchased from Sigma-Aldrich Inc. (St. Louis, USA). The salivary amylase (EC 3.2.1.1) which is Type IX-A of human origin, the pancreatic amylase (EC 3.2.1.1) which is Type VI-B of porcine origin were both purchased from...
Sigma-Aldrich and rat intestinal mucosal enzymes were prepared from rat intestinal acetone powder (Sigma-Aldrich Inc.). Kleistase T5 (a thermostable amylase from *Bacillus subtilis*), Daizyme GPK mixture of glucoamylase (EC 3.2.1.3) from *Aspergillus niger* and pullulanase (EC 3.2.1.41) from *Bacillus brevis* were purchased from Amano Enzyme Inc. (Nagoya, Japan).

**Polymerization reaction.** Glucose (1 g) was ground with activated carbon (20 mg) using a mortar and pestle. The mixture was placed in a stainless-steel cup (18 cc) and heated at 180°C in a forced convection oven (Advantec Co., Ltd., PRK633DB, Japan) for 30 min. Glucose syrup (1 g, dried solid) was mixed well with activated carbon (30 mg). The mixture was heated as described above. The glucose syrup reaction mixture was incubated in the absence/presence of various catalysts (hydrochloric acid, ortho-phosphoric acid, citric acid, and activated carbon) at various times and heating temperatures.

**Preparation of the resistant glucan mixture (RGM).** Glucose syrup (100 kg, dry solid) was mixed well with activated carbon (3%, w/w), and heated at 180-230°C on a twin screw kneader (Kimura Chemical Plants Co., Ltd., Japan) under reduced pressure. The product was placed in a tank, diluted with water to make a 30% (w/w) solution and then filtered to completely remove the activated carbon. The obtained soluble saccharide fraction underwent decolorization using activated carbon and deionization with ion-exchange resin, evaporation and then it was spray-dried.

**Preparation of resistant glucan (RG).** After glucose syrup underwent polycondensation in the presence of activated carbon using the same method as for the production of RGM, the reaction mixture solution (30%, w/w) was adjusted to pH 4.5 with 1 M NaOH. Then, the product was treated with Kleistase T5 (0.01%-DS) and Daizyme GPK (0.2%-DS) at 50°C for 48 h. The reaction mixture was purified as described above for RGM. The lower molecular weight components (e.g., di- or mono-saccharides) were removed by simulated moving bed chromatography (Na⁺ form, i.d. 8 mm, 8 identical columns, 60 µm) and heated to 100°C for 30 min with the artificial gastric juice (16.7 mM HCl-KCl buffer, pH 2.0), then the solution was incubated at 37°C for 30 min with the salivary amylase (160 U/g of dry solid of sample) in 45 mM Bis-Tris buffer (pH 6.0) containing 0.9 mM CaCl₂ and then heated to 100°C for 10 min to stop the reaction.

**Measurement of indigestible content.** The digestibility of the sample was determined by measuring the indigestible content using enzymatic HPLC. The digestibility of the glucan was determined by measurement of the water-soluble dietary fiber content according to the AOAC Official Method 2001.03.23)

**HPLC analysis.** The chain length distribution of the RGs was analyzed by HPLC (MCI GEL CK02AS column, i.d. φ20 × 250 mm, Mitsubishi Chemical Co., Tokyo, Japan) set at 80°C equipped with a refractive index detector. A total of 50 µL of sample (50 mg/mL) was injected. The mobile phase was deionized water at a flow rate of 1.0 mL/min.

**In vitro digestion method.** The resistance to successive digestion was examined using a modification of the method described by Okada *et al.*25) The sample (4.55% (w/v), final concentration) was incubated at 37°C for 30 min with the salivary amylase (160 U/g of dry solid of sample) in 45 mM Bis-Tris buffer (pH 6.0) containing 0.9 mM CaCl₂ and then heated to 100°C for 10 min to stop the reaction.

**Polymerization reaction.** Glucose (1 g) was ground with activated carbon (20 mg) using a mortar and pestle. The mixture was placed in a stainless-steel cup (18 cc) and heated at 180°C in a forced convection oven (Advantec Co., Ltd., PRK633DB, Japan) for 30 min. Glucose syrup (1 g, dried solid) was mixed well with activated carbon (30 mg). The mixture was heated as described above. The glucose syrup reaction mixture was incubated in the absence/presence of various catalysts (hydrochloric acid, ortho-phosphoric acid, citric acid, and activated carbon) at various times and heating temperatures.

**Color measurement.** The color of all samples was measured using the ICUMSA (International Commission for Uniform Methods of Sugar Analysis) method GS 1-7 1994.24) Samples were filtered through a 0.45 µm membrane to remove turbidity and the pH of the sample was adjusted to 7.0. The absorbance of the neutralized solution was measured at 420 nm (U-2990 spectrophotometer, Hitachi Co., Japan). The color of the sample was determined using the following formula:

\[
\text{Color (IU)} = \text{optical density} \times 1,000 / \text{concentration of solids} \times \text{cell length}
\]

**Molecular weight distribution of RG.** The molecular weight distribution of RGs was determined by HPLC (Showa OHpak SB803HQ column, i.d. φ8 × 300 mm, Showa Denko Co., Ltd., Kawasaki, Japan and Shodex OHpak SB802HQ column, i.d. φ8 × 300 mm). The mobile phase...
was distilled water containing 50 mM NaNO₃, the flow rate was 0.4 mL/min, and the column temperature was 70°C. The molecular masses of the RGs were calculated from a calibration curve constructed using a series of pullulan standards (Standard P-82, Showa Denko K.K., Tokyo, Japan).

Bacterial reverse mutation assay on RGM and RG. The bacterial reverse mutation assay was performed in accordance with OECD guideline 471 and standard procedures previously described by Maron and Ames. Escherichia coli WP2uvrA, and Salmonella Typhimurium TA98, TA100, TA1535, and TA1537 were used as indicative bacteria. In the presence of metabolic activation, S9 Mix, fractionated SD male rat liver microsomes prepared after intraperitoneal administration of phenobarbital, and 5,6-benzoflavone were used. The sample was added at 313 µg/plate as a solution in purified water. After incubation (37°C, 48 h) of the plate containing 0.1 mL of the bacterial suspension, 0.1 mL of the sample solution, 0.5 mL of the Phosphate buffer, and 2.0 mL of soft agar solution (2.7 mL in total), the reverse colonies per plate were counted. In the absence of metabolic activation, a Phosphate buffer was used instead of S9 Mix.

Acute toxicity of RGM and RG in rat. The single-dose oral toxicity study was performed in accordance with toxicity study guidelines. Test species: Sprague-Dawley male and female rats (5 weeks old, Charles River Japan) were divided into three groups with five rats per group. Either 5,000 or 10,000 mg/kg body weight of the sample was administered as a single oral dose following the good laboratory practices guidelines. The rats were divided into the three groups after pre-feeding with stock feed for 1 week. After fasting for 16 h, the sample solution was administered at 20 mL/kg body weight with a stomach sonde. After administration, the rats were observed for 4 h to check for any unusual symptoms, and then observed once a day in the following 14 days. After this observation period, all rats were dissected and the major thoracoabdominal organs were examined.

NMR analysis. NMR spectral data were recorded for 5% (w/v) solutions in D₂O at 25°C using a 400 MHz NMR spectrometer (Bruker Ultrashield™ 400 PLUS, Germany). The chemical shifts are expressed in ppm relative to sodium 3-(trimethylsilyl)-1-propanesulfonate sodium salt as an internal standard.

**Methylation analysis.** A sample (5 mg) was permethylated according to the method of Ciucanu and Kerek, and the products were isolated by partitioning between CHCl₃ and water. The methylated sample was then hydrolyzed in acid, reduced with NaBH₄, and acetylated by standard methods. The resulting partially methylated alditol acetates were analyzed by gas chromatography-mass spectrometry (GC: CP-3800 Varian, MS/MS: 1200L, Varian, DB-5 silica gel capillary column i.d. φ0.25 mm × 60 m, GL Sciences). Column temperature was programmed from 130°C (after 3 min) to 250°C at 5°C/min and held for 10 min. The carrier gas was He with an injector split of 20:1.

Other analytical methods. The concentration of sample as a dried solid was calculated from the following formula:

Sample concentration (dried solid) = (weight after drying under reduced pressure (105°C, 4 h))/(weight before drying) × 100%.

The total sugar content was measured by the phenolsulfuric acid method. The reducing sugar was measured by the Somogyi-Nelson method as follows. Reducing power = (amount of reducing sugar as glucose)/(amount of total sugar as glucose) × 100%.

The dextrose equivalent (DE) was measured by the Lane-Eynon general volumetric method. The viscosity of the sugar syrup was measured with a B-type viscometer (TV-10M; Tokyo Keiki, Inc., Tokyo, Japan) at 20°C.

**RESULTS AND DISCUSSION**

Comparison of the efficiency of various catalysts.

Table 1 shows the differences in the indigestibility and color of polycondensation polymers obtained in the presence of various catalysts and glucose or glucose syrup as a starting material. In the case of glucose, the content of the indigestible fraction obtained in the absence of activated carbon was 55.4% when heated for 30 min at 180°C (sample A). The content of the indigestible fraction in the presence of activated carbon was 85.8% (sample B). We concluded that activated carbon acted as a catalyst for the polycondensation reaction of saccharides.

In the case of glucose syrup, the polycondensation polymers were prepared by controlling the additive rate of the catalyst to the starting material and reaction temperature

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### Table 1. Polymerization of glucose and glucose syrup in the presence of different catalysts at normal pressure.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Raw material</th>
<th>Catalyst</th>
<th>Reaction temp. (°C)</th>
<th>Reaction time (min)</th>
<th>Indigestibility content (%)</th>
<th>Color (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Glucose</td>
<td>—</td>
<td>180</td>
<td>30</td>
<td>55.4</td>
<td>31</td>
</tr>
<tr>
<td>B</td>
<td>Glucose</td>
<td>2.0% (w/w) Activated carbon</td>
<td>180</td>
<td>30</td>
<td>85.8</td>
<td>116</td>
</tr>
<tr>
<td>C</td>
<td>Glucose syrup</td>
<td>—</td>
<td>180</td>
<td>30</td>
<td>44.1</td>
<td>53</td>
</tr>
<tr>
<td>D</td>
<td>Glucose syrup</td>
<td>—</td>
<td>220</td>
<td>30</td>
<td>81.8</td>
<td>1693</td>
</tr>
<tr>
<td>E</td>
<td>Glucose syrup</td>
<td>3.0% (w/w) Activated carbon</td>
<td>180</td>
<td>30</td>
<td>81.2</td>
<td>37</td>
</tr>
<tr>
<td>F</td>
<td>Glucose syrup</td>
<td>0.0025% (w/w) Hydrochloric acid</td>
<td>180</td>
<td>30</td>
<td>80.5</td>
<td>108</td>
</tr>
<tr>
<td>G</td>
<td>Glucose syrup</td>
<td>0.2% (w/w) Phosphoric acid</td>
<td>180</td>
<td>30</td>
<td>81.4</td>
<td>307</td>
</tr>
<tr>
<td>H</td>
<td>Glucose syrup</td>
<td>3.0% (w/w) Citric acid</td>
<td>180</td>
<td>30</td>
<td>80.5</td>
<td>166</td>
</tr>
</tbody>
</table>
such that the indigestible components were in the range of 80 to 82%, except for sample C. To obtain a polycondensation polymer which contained 80 to 82% of indigestible components without use of a catalyst, a temperature of 220°C was required (sample D).

Polymers which contained 80 to 82% of indigestible components were obtained in the presence of 3.0% (w/w) activated carbon (sample E), 0.0025% (w/w) hydrochloric acid (sample F), 0.2% (w/w) ortho-phosphoric acid (sample G), and 3.0% (w/w) citric acid (sample H). When acids were employed as catalysts, samples with over 80% indigestible components were attained but the reaction mixtures were extremely colored (samples F, G, and H). Conversely, when activated carbon was used as a catalyst, the same amount of indigestible components was obtained but the coloration was low (samples B and E). Activated carbon was a good catalyst for the polycondensation, because the temperature of the polycondensation reaction could be lowered and the coloration could be suppressed.

Activated carbon has been reported to catalyze the hydrolysis of cellulose. In contrast, we developed a manufacturing procedure to produce indigestible polysaccharides from glucose syrup using activated carbon as a catalyst for polycondensation reaction.

The production of the indigestible fraction over time.

Figure 1 shows the production of the indigestible fraction over time using glucose syrup at 180°C under ordinary pressure, with or without activated carbon. The amount of indigestible fraction obtained in the presence of activated carbon increased sharply within the first 30 min, and then slowly increased. The content of the indigestible fraction with activated carbon was 82.6% at 30 min, and 87.9% at 120 min. Although the content of the indigestible fraction increased without activated carbon, the reaction was less efficient as evidenced by the lower yield of indigestible content.

The effect of heating temperature on the indigestible fraction content.

Glucose syrup with or without activated carbon was heated at various temperatures for 30 min (Fig. 2). From 100 to 120°C, very little increase in the content of the indigestible fraction was observed. The content of the indigestible fraction with and without activated carbon was 41.4 and 4.9% at 150°C. From 120 to 180°C, the content of the indigestible fraction in the presence of activated carbon significantly increased. At temperatures greater than 180°C, the production of the indigestible fraction was constant. Thus, the target yield could be reached in a short time when the heating temperature was high. When designing a manufacturing procedure, a balance of reaction temperature and time is important.

Development of a manufacturing procedure for indigestible polysaccharide production.

A kneading machine was used to obtain the indigestible polysaccharides on an industrial scale (Fig. 3). Using a mixture of 3 kg of activated carbon and 100 kg of glucose syrup on a dry basis, the reaction was carried out using a kneading machine. The barrel of a kneading machine was heated to 180–230°C under reduced pressure. The reaction mixture was solubilized in water to adjust the concentration to about 30% (w/w). The reaction mixture was decolorized by passing it over an activated carbon filter and deionized by passing through an ion-exchange resin. The purified reaction mixture was evaporated, and spray-dried. A total of 84.6 kg of polysaccharide powder containing 81.6% indigestible fraction was obtained. The fraction containing 81.6% of indigestible components was termed the resistant glucan mixture (RGM).

The reaction mixture was treated with α-amylase and glucoamylase in an attempt to obtain the indigestible fraction. After enzymatic treatment, the content of the indigestible fraction to the total saccharide was 78.0%. After purification (decolorized and deionized), the low molecular weight digestible saccharides such as di- or monosaccharides were chromatographically separated from reaction mixture, and then the higher molecular weight saccharides was evaporated and spray-dried. The content of the indigestible fraction was 97.9%, and the yield of the indigestible fraction compared with the weight of the starting material was 62%. We termed this indigestible fraction resistant glucan (RG). The dietary fiber content (as dried solid) in RGM and RG was 80 to 82%, except for sample C.
measured by the AOAC2001.03 method was 81.7 and 99.7%.

HPLC chromatogram of RGs.
The composition of the starting material (glucose syrup), RGM, the reaction mixture after 48 h of treatment with α-amylase and glucoamylase, and RG were analyzed by HPLC (Fig. 4). The RGM was composed of continuously distributed polymers of mono- to polysaccharides (Fig. 4 (b)). After 48 h of treatment with enzymes (Fig. 4 (c)), the glucose content (described as "1" in Fig. 4 (c)) increased by 6%, although the content of polysaccharides larger than a DP 3 were only slightly decreased from those of RGM. This indicated that RGM resisted α-amylase and glucoamylase catalyzed hydrolysis. As shown in Fig. 4 (d), mono- and disaccharides were almost completely removed by chromatography. HPLC analysis of maltodextrin (data not shown), indicated that the components were separated according to their DP while the components of RG were not clearly separated. These results indicated that the structure of RG was highly branched.

Properties of RGM and RG.
The properties of RGM and RG are described in Table 2. RGs have higher solubility in water (solutions of 70% (w/w) or greater at 25°C) than other carbohydrates such as pectin and maltodextrin.

The saccharide content with a DP 3 or more in RGM and RG was 88.7 and 99.8%, respectively. RGM contained 3.3% of 1,6-anhydro-D-glucose. The dietary fiber content (as dried solid) in RGM and RG was 81.7 and 99.7%, respectively. The viscosities of RGM and RG were similar to that of low-viscosity dietary fibers such as polydextrose and indigestible dextrin.

RGM and RG were shown not to possess any reverse mutation inducing activity in five bacterial strains (E. coli WP2uvrA, S. typhimurium TA98, TA100, TA1535, and TA1537) with or without S9 metabolic activation. The LD₅₀ values for RGM and RG in male and female rats were higher than 10,000 mg/kg body weight per day indicating that RGM and RG were safe substances for food.

Digestibility of RGs in vitro.
An in vitro digestion experiment was carried out to examine the resistance of RG to successive digestion by salivary amylase, artificial gastric juices, pancreatic amylase, and intestinal mucosal enzymes (Table 3). Maltodextrin was only minimally digested by the artificial gastric juice but a total of 84.3% of the maltodextrin was digested by the combined action of salivary amylase, and pancreatic amylase and intestinal mucosal enzymes. Indigestible dextrin was digested 0.8% by salivary amylase, 2.7% by pancreatic amylase, and 13.8% by intestinal mucosal enzymes. RGM and RG were not digested by salivary amylase and artificial gastric juice. RGM and RG were slightly digested by pancreatic amylase, 0.6 and 0.1%, respectively, and by intestinal mucosal enzymes, 7.8 and 5.1%, respectively. The hydroly-

Table 2. Properties of RGM and RG.

<table>
<thead>
<tr>
<th></th>
<th>RGM</th>
<th>RG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>White powder</td>
<td>White powder</td>
</tr>
<tr>
<td>Solubility</td>
<td>Water-soluble</td>
<td>Water-soluble</td>
</tr>
<tr>
<td>Taste/odor</td>
<td>Slightly sweet/odorless</td>
<td>Slightly sweet/odorless</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>1.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Dietary fiber (%)</td>
<td>81.7</td>
<td>99.7</td>
</tr>
<tr>
<td>Sugar composition (%)</td>
<td>≥ DP 3 (%)</td>
<td>88.7</td>
</tr>
<tr>
<td>DP 2 (%)</td>
<td>5.9</td>
<td>0</td>
</tr>
<tr>
<td>DP 1 (%)</td>
<td>5.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Color (IU)</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Average molecular weight</td>
<td>1900</td>
<td>2400</td>
</tr>
<tr>
<td>DE</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Viscosity (mPa·s)b</td>
<td>7.3</td>
<td>8.4</td>
</tr>
</tbody>
</table>

*Measured using an enzyme-HPLC method (AOAC2003.01 method).

bViscosity of 30% (w/w) solution measured at 20°C.
Glc high branched glucan. The composition of glucosidic was 16.8% in RG indicating it was a 1,2-, 1,3,4-, 1,2,3-, and 1,3,6-linked Glc while the atoms were observed at 5.5 ppm, C NMR of RG (JSTAGE) (

**Table 3.** Hydrolysis rate (%) of maltodextrin and water-soluble dietary fibers in vitro.

<table>
<thead>
<tr>
<th></th>
<th>Maltdextrin</th>
<th>RGM</th>
<th>Polydextrose</th>
<th>Indigestible dextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary amylase</td>
<td>22.4</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>Artificial gastric juice</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pancreatic amylase</td>
<td>5.0</td>
<td>0.6</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Intestinal mucosa enzymes</td>
<td>56.9</td>
<td>7.8</td>
<td>5.1</td>
<td>6.8</td>
</tr>
<tr>
<td>Total hydrolysis rate</td>
<td>84.3</td>
<td>8.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Increment of reducing sugar measured by the Somogyi-Nelson method.*

**Table 4.** Structural analysis of RG.

<table>
<thead>
<tr>
<th>Glucosidic linkages (%)</th>
<th>Maltdextrin</th>
<th>RG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonreducing end Glcp</td>
<td>6.4</td>
<td>38.6</td>
</tr>
<tr>
<td>1,2-linked Glcp</td>
<td>0</td>
<td>7.1</td>
</tr>
<tr>
<td>1,3-linked Glcp</td>
<td>0</td>
<td>2.2</td>
</tr>
<tr>
<td>1,4-linked Glcp</td>
<td>88.9</td>
<td>11.5</td>
</tr>
<tr>
<td>1,6-linked Glcp</td>
<td>0</td>
<td>21.4</td>
</tr>
<tr>
<td>1,3,4-linked Glcp</td>
<td>0</td>
<td>0.7</td>
</tr>
<tr>
<td>1,2,3-linked Glcp</td>
<td>0</td>
<td>0.9</td>
</tr>
<tr>
<td>1,3,6-linked Glcp</td>
<td>0</td>
<td>5.2</td>
</tr>
<tr>
<td>1,4,6-linked Glcp</td>
<td>4.8</td>
<td>10.1</td>
</tr>
<tr>
<td>Others(a)</td>
<td>0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*Others” may also contain 1,3,4,6-, 1,2,3,6-, and 1,2,4,6-linked Glcp.

sis rate of RG was almost the same as that of polydextrose and indigestible dextrin. These results indicated that RG reaches the large intestine similar to polydextrose and indigestible dextrin.

**Structural analyses of resistant glucan.**

From the spectra of 1H and 13C NMR of RG (JSTAGE Supplementary Material Fig. S1), signals for the α-anomeric atoms were observed at δH 4.9–5.5 ppm, δC 95–100 ppm, while the β-anomeric atoms were detected at δH 4.4–4.7 ppm, and δC 103–105 ppm. The ratio of the α to β anomers in RG was about 1:1, as determined from the integration ratio of the 1H NMR signals.

The results from the methylation analysis of RG are shown in Table 4. The 1-6 linkage predominated. The 1-3, 1-2, 1-3, 1-2, 1-3-6, and 1,3,6-linked Glcp were all detected in RG, while they were not observed in maltdextrin. The total amount of glucosidic linkages of 1,3,4-, 1,2,3-, 1,3,6-, and 1,4,6-linked Glcp was 16.8% in RG indicating it was a highly branched glucan. The composition of glucosidic linkages in RG was similar to that in polydextrose. (3)

Our results indicate that RG is resistant to the action of enzymes in the upper digestive tract and provides a safe source of soluble dietary fiber. Our future research will focus on investigating the physiologic functions of RG and the reaction mechanism of activated carbon in sugar polycondensation.

REFERENCES


