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Purification of glucomannan from salep: Part 1. Detailed rheological characteristics

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ABSTRACT

The aim of this study was to investigate the effects of different extraction temperatures (25, 55 and 85 °C) on the rheological characteristics of glucomannan (GM) purified from salep. GM was isolated using the following method: extraction of GM with water, removal of impurities by centrifugation and precipitation of GM with ethanol. The extraction yield of GM was approximately 45% for all products. Higher GM (95%) and lower starch (3%) contents were obtained for GM25. GM25 showed higher apparent and intrinsic viscosity, storage and loss modulus and lower activation energy than GM55 and GM85. The addition of different salts and sugars did not affect viscosity due to the non-polyelectrolyte behavior of GM. Gel behavior of purified samples appeared at pH 9. The usage of salep in milk was reduced by purification. These results are potentially useful for widening the applications for salep and for furthering research and development of GM.

1. Introduction

Salep is made from plant tubers from the *Orchidaceae* family and is a good source of glucomannan (GM), which is composed of linear chains consisting of glucose and mannose connected by β-(1 → 4) glycosidic bonds. The tubers are usually grown in eastern Mediterranean countries. For commercial purposes, the other major GM source is konjac, which has long been used China and Japan. After boiling in water, the tubers are dried and then ground to produce salep powder (Yasar, Kahyaoglu & Sahan, 2009). Salep has been used in different application such as a traditional beverage and a stabilizer for hard-serve ice cream, drinks and medicines (Farhoosh & Riazi, 2007; Kurt, Cengiz & Kahyaoglu, 2016; Kurt & Kahyaoglu, 2014). The edible film properties (Kurt & Kahyaoglu, 2014), emulsion stabilizing behavior (Georgiadis et al., 2012) and effect of storage stability of salep (Ayar, Sert & Akbulut, 2009) have also been reported.

Clearer and more stable solutions resulting from elimination of impurities make purification studies essential (Razmkhah, Mohammadifar, Razavi & Ale, 2016). The procedure for obtaining salep powder, as described above, does not include any purification process, and the resulting powder is used directly in formulations. However, salep contains impurities such as starch, protein and ash, which result in decreased quality. The composition of salep has been reported as 56.1% glucomannan, 36.31% starch, 4.60% protein and 2.07% ash (Kurt & Kahyaoglu, 2015). The effectiveness of increasing glucomannan content to 75% with a simple ethanol treatment was reported in our previous study (Kurt & Kahyaoglu, 2015), which showed that the viscosity of salep could be increased approximately 5-fold compared to the native extracted material. However, impurities could reduce the quality.

In recent years, the methods for extraction and purification of GM from konjac have been widely studied and developed (Chua et al., 2012). In the literature, konjac glucomannan has been explored in more purification studies than salep, providing glucomannan preparations ranging from 52.7% to 92.9% (Chua et al., 2012), from 74.13 to 90.63% (Wang et al., 2014) and 92.69% purity (Harmayani, Aprilia & Marsono, 2014) with purification. However, salep has been the subject of a limited number of studies aiming to increase its quality, and there has been no study to increase the glucomannan content of salep above 90%, so the current study can be considered novel in this regard.

In this study, salep powder was mixed with water in specific ratios, temperatures and times to obtain GM extracts. Then, insoluble matter was removed by centrifugation, and GM was precipitated with ethanol. The method for collecting GM plays an

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important role because the functional properties of the end product are related to the process parameters. In this study, we aimed to purify glucomannan from salep using different extraction procedures (25, 55 and 85 °C), and functional analyses of the extraction procedures was performed, including detailed rheological analysis. The effects of pH, salt and sugar on the flow characteristics of purified samples were also evaluated. Structural characterization experiments will be included in another manuscript.

2. Materials and methods

The native salep powder (10% moisture) purchased from a local market (Kastamonu, Turkey) was used as a raw material for purification. All chemicals used in this study were of analytical reagent grade. The ethanol, sodium hydroxide, 3-5-dinitrosalicylic acid (DNS), sulfuric acid, acetone, sodium chloride, calcium chloride, sucrose, lactose were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). The formic acid and pH buffer solutions were obtained from Merck (Darmstadt, Germany).

2.1. Glucomannan extraction from salep powder

Ten grams of native salep powder was stirred in 1 L of distilled water for 1 h at three different temperatures (25, 55 and 85 °C), followed by centrifugation (5000 rpm, 10 min) to remove insoluble material. The supernatants obtained from each extraction were mixed with absolute ethanol at a ratio of 1:1 (v/v) to precipitate of glucomannan. The resultant pellets were washed successively with absolute ethanol and acetone and then subsequently dried with a forced air dryer (Mikrotest, Turkey) at 45 °C overnight. The dried samples were milled, sieved, stored in an airtight bottle at room temperature and coded according to the extraction temperature: GM25, GM55, GM85 and NS (native salep). Five independent extractions were performed for each temperature.

2.2. Physicochemical analyses

Glucomannan contents of native and purified samples were determined using the 3,5-DNS method, which has been previously reported to be the most reliable and accurate method of the phenol-sulfuric acid and enzymatic colorimetric assays for konjac glucomannan. Starch contents of samples were determined by using total starch assay kit (Megazyme), which was detailed in our previous study (Chua et al., 2012).

The cellulose and lignin contents of samples were determined by the methods proposed by Slavutsky and Bertuzzi (2014) according to the recommended protocol using a Fiber Analyzer (ANKOM Technology Fiber Analyzer Model 220, USA). In this method hemicellulose contents were also determined which indicated the glucomannan content of samples.

2.3. Rheological properties

Analyses of the rheological properties of samples were performed using rheometer (HAAKE Mars III; Thermo Scientific, Germany) equipped with a Peltier heating system in a cone and plate configuration (diameter: 35 mm, cone angle: 2°, gap size: 0.105 mm).

2.3.1. Steady-shear flow behavior of glucomannan

Different concentrations of the samples (0.5, 1.0, 1.5, and 2.0%) were prepared in distilled water with stirring for 1 h using a magnetic stirrer at 85 °C. For each test, samples were allowed to equilibrate for 2 min at the desired temperature (5, 25, 45 and 65 °C). Samples were sheared continuously at a rate ranging from 0 to 300 s^{-1} in 3 min for fitting the data to the Ostwald-de Waele model (Eq. (1))

$$\tau = K \cdot \dot{\gamma}^n \quad (1)$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency coefficient (Pa.s^n), and n is the flow behavior index (dimensionless).

2.3.2. Dynamic viscoelastic properties of glucomannan

Frequency sweep tests were carried out for a frequency range of 0.1–100 Hz at 1 Pa within the linear viscoelastic range (LVR). Stress sweep measurements were carried out in the range of 0.01–100 Pa at a frequency of 1 Hz to determine LVR. The frequency sweep test was performed for different concentrations (1.0, 1.5, 2.0%) at 25 °C.

The temperature sweep measurements were performed at constant stress and different frequencies of 0.2 Pa and 1, 5 and 10 Hz, over a temperature range of 10–90 °C at a heating rate of 3 °C min^{-1}. The test was performed at 2% (w/v) concentration.

2.3.3. Intrinsic (limiting) viscosity [η] of glucomannan

The intrinsic viscosity [η] of purified glucomannans was determined in distilled water using a stock solution (0.5 g/dL) prepared at 80 °C for 1 h. Serial dilution was conducted with the solvent up to 0.01 g/dL to determine relative viscosity (η_{rel}) ranging from 1.2 to 2.0. Each measurement was performed at 25 °C. The shear rate applied was within the range of 10-100 1/s, and each step (multiples of 10 s^{-1}) lasted 10 seconds. The Newtonian viscosity was converted to the relative viscosity ($\eta_{rel} = \eta/\eta_s$; η_s: solvent viscosity) and specific viscosity ($\eta_{sp} = \eta_{rel}-1$).

Stock solutions of GM25 were also prepared with two different salts (NaCl and $CaCl_2$) at different concentrations (25 mM and 50 mM) and different concentrations (5 and 10%) for the sugars sucrose and lactose. Tanglertpaibul and Rao's equation was used to obtain the [η] value (Tanglertpaibul & Rao, 1987).

(2) $\eta_{rel} = 1 + [\eta]C$

2.3.4. pH effect on the viscoelastic properties of glucomannan

The solutions were prepared in different pH buffer solutions (pH 9, pH 7 and pH 4) for NS and GM25 at 80 °C for 1 h. Steady and dynamic rheological analyses were performed at 0.5% gum concentration.

2.3.5. Rheological properties of glucomannan in milk

Steady and dynamic (stress and frequency sweep tests) analyses were performed for native and purified salep (extracted at 25 °C, GM25) dissolved in milk and stirred for 1 h at 85 °C. The measurements were done at 25 °C. The temperature sweep test was also conducted as detailed Section 2.4.2 to observe gelation temperature which is possible for GM25 at 10 Hz.

2.4. Statistical analysis

SPSS software (Version 16.0, SPSS Inc., Chicago, USA) was used to analyze the data. The means were compared by Tukey's test at a 5% level of significance using analysis of variance (ANOVA).

3. Results and discussion

3.1. Extraction yields and physicochemical analyses

The effects of temperature on GM extraction yield are summarized in Table 1. The yield ranged between 43.64 and 44.92%. Increasing the extraction temperature did not affect extraction yield significantly ($p > 0.05$). Therefore, extraction of GM at 25 °C