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Rheological properties and structural characterization of salep improved by ethanol treatment

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ABSTRACT

Glucomannan is the main and important constituent of salep. The other contents are called as impurities. In this study, the removal of them was achieved by ethanol treatment to increase salep quality (40%, 50%, 60%, and 70%). The highest glucomannan and the lowest impurities contents were succeeded with the 40% ethanol treatment (SF40). Apparent viscosity of SF40 increased about 5 folds as compared with native salep. SF40 also had higher intrinsic viscosity [η] and lower critical concentration (C^*) than other samples. The improved molecular entanglement between glucomannan chains were demonstrated with increased Berry number $Cx[\eta]$. The storage (G') and loss (G'') moduli spectra of SF40 were found higher within the whole range of frequency and temperature. This simple ethanol process could be used as a promising modification method for improving the properties of salep.

1. Introduction

Glucomannans are neutral polysaccharides composed of linear chains consist of glucose and mannose connected by β-(1 → 4) glycosidic bonds. Two glucomannans having major commercial importance are konjac glucomannan (KGM) from *Amorphophallus* konjac and salep glucomannan from the *Orchidaceae* family. Salep derived from the tubers has long been used as a traditional beverage and food additives in Eastern Mediterranean countries. As a hydrocolloid, salep forms viscous solutions when dissolved in water as many polysaccharide gums obtained from plant tissues due to the higher molecular weight. Salep has been found less applications and studies as compared with KGM up to now. The edible film properties (Kurt & Kahyaoglu, 2014), emulsion stabilizing properties (Georgiadis et al., 2012) and the effect on storage stabilities of salep (Ayar, Sert, & Akbulut, 2009) have just been considered in addition to extensively reported rheological properties of ice cream and beverages (Karaman, Yilmaz, & Kayacier, 2011; Kaya & Tekin, 2001; Kayacier & Dogan, 2006; Yasar, Kahyaoglu, & Sahan, 2009). The collected plant tubers are washed and boiled in water, dried and ground to obtain salep powder. The reason of taking less attention may be attributed to the poor qualities of salep due to the including impurities such as starch, protein and ash as a result of direct usage.

The constituents of ten different species of salep were reported by Tekinsen and Guner (2010) as glucomannan 17.7–54.6%; starch 5.44–38.7%; protein 3.11–4.95%; ash 0.95–2.87%. They stated that the main polysaccharide content of salep is glucomannan which shows the negative relationship between starch contents. The glucomannan content more than 40% is considered as high quality. In the same report *Orchis italica* was suggested as a higher quality due to the higher glucomannan contents and glucomannan/starch ratio. The contents of salep may vary with nurture, collection period and region even the same species. Therefore obtaining higher quality salep not only depends on species but also collected region because salep is a wild terrestrial orchids. In addition, the variation of glucomannan and starch in a wide range depending upon species causes difficulties to obtain salep with a higher quality.

Another way to acquire higher quality salep may be subjected on hand salep to the process in order to increase glucomannan percentage or glucomannan/starch ratio independently from species. The simple and feasible approach for increasing glucomannan ratio of KGM was reported by the authors (Xu et al., 2014a, 2014b). The enhancement was achieved by removing of the impurities existing in konjac flour. The constituents except glucomannan are considered as impurities. The main impurity is starch which affects the purity and quality of salep such as reducing viscosity (Yoshimura, Takaya, & Nishinari, 1998). Therefore it is expected that decreasing

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starch ratio and removing other impurities such as protein and ash content may increase the quality of product due to the possible enhancement of interaction between glucomannan chains.

The principle of aimed process in this study is that glucomannan molecules possess better swelling ability and become insoluble whereas the impurities dissolves in ethanol/water system due to the dependence of solubility to the dielectric constant of solution (Xu et al., 2014a). Moreover, the ethanol treatment is the basic step for purification and clarification processes of the gums before extraction of polymer with solvent. The different ethanol concentrations were applied in the different purification studies such as 40% (Xu et al., 2014a) and 50% (Chua et al., 2012) for konjac flour, 70% (Georgiadis et al., 2012) for salep flour, and absolute ethanol (Jahanbin, Moini, Gohari, Emam-Djomeh, & Masi, 2012) for *Acanthophyllum bracteatum* roots. Therefore, ethanol concentration is the parameter which is needed to be determined for removing of impurities in a higher ratio. To the best of our knowledge there are no reports about effect of ethanol concentration on purifying gums and ethanol treatment to increase glucomannan/starch ratio of salep. The ethanol treatment was conducted at 78 °C in this study based on the published reports (Xu et al., 2014a, 2014b). They demonstrated higher removal of impurities at 78 °C which was called as azeotropy point of ethanol-water solutions.

The present study was undertaken to increase glucomannan contents of salep with suitable ethanol concentration in order to obtain it with a higher quality. The novelty of the study is that it is the first report for elevating the properties of salep with a simple method.

2. Materials and methods

2.1. Materials

Dried and finely ground salep roots were purchased from a supplier in Kastamonu, Turkey. All analytical grade chemicals were used as obtained without any further purification.

2.2. Preparation of ethanol washed salep flour

Salep dispersions (1:6) were prepared in a series of ethanol solutions (40, 50, 60, 70% v/v) under constant stirring at 600 rpm, 78 °C for 2 h, followed by centrifugation (5000 × *g*, 15 min, 25 °C). The each resultant pellet (precipitates) was washed by ethanol with the same concentration used at the first step and subsequently dried with a forced air dryer (Mikrotest, Turkey) at 45 °C overnight. The dried samples were milled, sieved and stored in an airtight bottle at room temperature until used in the experiments. The products were coded as SF40, SF50, SF60, SF70 and the control sample (native) was coded as SF.

2.3. Physicochemical analyses

2.3.1. Moisture, ash and protein contents

The moisture and ash content were analyzed by using gravimetric methods at 105 and 500 °C, respectively; Kjeldahl method was performed for protein by the applying the nitrogen conversion factor of 5.7 (AOAC, 2000).

2.3.2. Glucomannan and starch contents

Determination of glucomannan (GM) content was conducted by using 3,5 DNS method which previously reported as the most reliable and accurate method within the phenol-sulphuric acid and enzymatic colorimetric assays for konjac glucomannan (Chua et al., 2012). 0.2 g salep flour was stirred in 0.1 M formic acid–sodium hydroxide buffer 4 h at room temperature and subsequently centrifugated (4500 × *g*, 40 min, 25 °C) to obtain salep glucomannan extraction (T_0). 5.00 ml supernatant was hydrolysated for 1.5 h with 3.0 M sulfuric acid in a boiling water bath. 6.0 M sodium hydroxide was added to cooled mix, followed by diluted with deionized water to obtain the salep glucomannan hydrolysate (T). The extraction and hydrolsate samples (2 ml) were subjected to colorimetric reaction with 3.5 DNS (1.5 ml) for spectrophotometric analysis (using deionized water as a blank). Absorbance was measured at 550 nm and used to calculate glucose content by means of standard curve of glucose. All tests were carried out in triplicate, and GM percentage calculated according to following equation:

$$\text{GM content}\,(\%) = \frac{5000f(5T - T_0)}{m} \quad (1)$$

where

- f = correction factor for mannose sugar; determined using the glucose:mannose ratio (1:3.3) was determined using a glucomannan assay kit purchased from Megazyme International Ireland Ltd. (catalog number: K-GLUM) and gradient of the constructed standard curves for glucose and mannose as followed

$$f = \left[\frac{1}{4.3} + \left(\frac{3.3}{4.3} \times \frac{0.3364}{0.3092}\right)\right] = 1.067$$

- T = glucose content of SGM hydrolysate (mg)
- T_0 = glucose content of SGM extraction (mg)
- m = mass of SF (200 mg).

Total starch assay kit purchased from Megazyme International Ireland Ltd. (catalog number: K-TSTA) was used to determine starch content of salep samples. 0.1 g sample in glass test tube was wetted 80% (v/v) ethanol by stirring with vortex mixer. The mix was incubated with 3 ml thermostable α-amylase in a boiling water bath for 6 min and 0.1 ml amyloglucosidase at 50 °C for 30 min, respectively followed by centrifugation (3000 rpm for 10 min). The supernatant, D-glucose standard and deionized water (reagent blank) for each 0.1 ml were subjected to colorimetric reaction with GOPOD reagent (3 ml) for spectrophotometric analysis (50 °C for 20 min). The absorbance was read at 510 nm against reagent blank for sample and D-glucose control. All tests were carried out in triplicate, and starch percentage calculated according to following equation:

$$\text{Starch}\,\% = \Delta_A \times \frac{F}{W} \times 90 \quad (2)$$

where

- Δ_A = absorbance value of sample solution against reagent blank

$$F = \frac{100(\mu\text{g of glucose control})}{\text{Absorbance for D-glucose control}}$$

- W = the weight of the sample (100 mg)

2.4. Rheological properties

Aqueous solution of 1.0% (w/v) of native and ethanol washed samples were prepared by careful dispersion in deionized water under constant stirring at 1300 rpm, 85 °C and 30 min. Steady and dynamic shear measurements for samples were performed using rheometer (HAAKE Mars III; Thermo Scientific, Germany) equipped with a Peltier heating system. The samples were allowed to equilibrate between cone and plate configuration (diameter: 35 mm, cone angle: 2°, gap size: 0.105 mm) for 2 min before testing to allow residual stresses to relax. All the steady, viscoelastic (dynamic) properties and intrinsic viscosity were determined at 25 °C. Each measurement was repeated three times.