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Physicochemical functionality of 4-α-glucanotransferase-treated rice flour in food application

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ABSTRACT

The physicochemical properties of 4-α-glucanotransferase (4αGTase)-modified rice flours were examined by measuring the molecular weight distribution, moisture sorption isotherm, and melting enthalpy of ice crystals. The results obtained by measuring the moisture sorption isotherm and melting enthalpy of ice crystals revealed that 4αGTase-modified rice flours had high water binding capacity than that of control rice flour. When the textural properties of noodles containing 4αGTase-treated rice flours after freeze–thaw cycling were measured by texture profile analysis, the textural properties of control noodle deteriorated. However, those of noodle with 4αGTase-modified rice flours were retained. For the melting enthalpy of ice crystals formed within cooked noodles, 4αGTase-treated rice flour showed similar effect to sucrose for reducing the melting enthalpy of ice crystals, however, the texture and taste of noodle with sucrose was undesirable for consuming. 4αGTase-treated rice flour appeared to have good potential as a non-sweet cryoprotectant of frozen product.

1. Introduction

Starches have been used as stabilizers, thickeners, and gel-forming agents in many food products because they can produce highly viscous dispersions and/or gels, depending on the concentration and temperature conditions. However, in the food industry, the use of native starches is limited by their lack of stability under the temperature, shear, pH, and refrigeration conditions that are commonly applied to processed foods [1]. Therefore, starches are often chemically, physically, or enzymatically modified, to allow the resultant pastes to withstand the heat, shear, and acidity associated with particular processing conditions and to introduce specific functionalities. In particular, the use of carbohydrate enzymes for starch modification is of increasing interest to researchers. By changing the type of enzyme, the substrate, and the reaction conditions, the enzymes can be employed to produce glucose polymers with controlled molecular size and structure. Thus, this approach can be used to manufacture novel glucose polymers [2–5].

4-α-Glucanotransferase (4αGTase) is known to modify starch polymers by attacking α-1,4-glucosidic bonds to transfer α-glucan chains from donor α-glucan molecules to the non-reducing end of acceptor α-glucans by forming a new α-1,4-glucosidic linkage. This process is called 'disproportionation' [6–8]. This enzyme can use high molecular weight starch molecules as both a donor and acceptor and catalyzes the transfer of long α-1,4-glucan chains, or even a highly branched cluster unit of amylopectin [3]. Several studies have reported the formation of a thermoreversible starch gel after the treatment of rice starch with thermostable 4αGTase from *Thermus scotoductus*, due to the reduction of long chain amylose and the modification of the amylopectin side chain [2,9]. These studies suggest that 4αGTase-modified starches have considerable potential in many industrial applications.

Among many applications, in this study, we focused on the application of 4αGTase-modified rice flour to the frozen food product. In our preliminary experiments, we discovered that 4αGTase-treated rice starch or flour had lower water activity at the same moisture content compared with non-treated rice starch or flour. Freezing is one of the most effective methods for food preservation. Low temperature not only protects the food product from microbiological spoilage but also slows down the rates of other degradative biochemical reactions. In spite of the many advantages however, freezing cause certain undesirable changes in food. To protect the food products from undesirable changes and improve the technological properties of frozen product, cryoprotective substances are often used. The most effective cryoprotectants are

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carbohydrates, such as sucrose, sorbitol, maltodextrins and polydextrose. However, their application is limited by their sweetness. Therefore, we hypothesized it might be possible to substitute 4αGTase-modified rice starch or flour for carbohydrate cryoprotectants as a low-sweetness cryoprotectant in frozen food.

The objectives of this study were (1) to investigate the possibility that 4αGTase-treated rice flour could be used as cryoprotectant in food and (2) to evaluate the possible cryoprotective effect of 4αGTase-treated rice flour on the freeze–thaw stability of frozen noodles during frozen storage.

2. Materials and methods

2.1. Materials

Rice flour and wheat flour were provided by Samlip General Foods (Siheung, Korea). Commercial salt and sugar were purchased from a local market. The 4-α-glucanotransferase isolated from *Thermus aquaticus* was kindly provided by the Food Enzymology Laboratory, Department of Food Biotechnology, Seoul National University (Korea).

2.2. Production of modified rice flour using 4αGTase

Rice flour dispersions were prepared by suspending rice flour in distilled water (5%, w/v) and gelatinized in a boiling water bath with continuous stirring for 30 min. The pastes were cooled and incubated at 75 °C with 4αGTase (5 U/g, dry basis) for different time periods (1, 3, and 48 h). The reaction was terminated by boiling the mixtures for 10 min. Five volumes of ethanol were added to the reaction mixture for precipitation. The precipitant was removed after centrifugation at 3703 × *g* for 20 min and dried at room temperature. The dried samples were ground and sieved through a 45-mesh sieve.

2.3. Molecular weight distribution analysis

After the rice flour paste reacted for 1, 3, and 48 h with the enzyme, the 4αGTase-treated rice flour samples (120 mg) were hydrated with 1.2 ml water and then dispersed in 10.8 ml dimethyl sulfoxide (DMSO). The suspensions were stirred with heating in a boiling water bath for 1 h and then stirred mechanically for 24 h at 25 °C. A 5 ml aliquot of flour dispersion (1%, w/v) was mixed with five volumes of ethanol (25 ml) for precipitation. The ethanol-precipitated starch was separated by centrifugation at 11,305 × *g* for 10 min and mixed with acetone. After centrifugation, the starch pellet was redissolved in 10 ml boiling water and stirred for 20 min in a boiling water bath. The hot samples were filtered using a 5.0 μm disposable membrane filter and injected into a high-performance size-exclusion chromatography (HPSEC) system. The HPSEC system consisted of a pump (Prostar 210, Varian, Inc., Palo Alto, CA, USA), an injection valve with a 100 μl sample loop (Rheodyne 7072, Cotati, CA, USA), a differential refractive index detector (Prostar355, Varian), and two SEC columns (G5000 PW, 7.5 mm × 600 mm and G3000 PW, 7.8 mm × 300 mm; Tosoh Co., Tokyo, Japan). The columns were maintained at room temperature. The flow rate of the mobile phase (50 mM $NaNO_3$) was constant at 0.4 ml/min.

2.4. Measurement of moisture sorption isotherm

4αGTase-treated rice flours (0.5 ± 0.001 g) were placed in disposable aluminum dishes (57 mm diameter) inside desiccators at 25 °C. Each desiccator had different saturated salt solutions (LiCl, K_2CO_3, NaBr, NaCl, KCl). Water activity of different saturated salt solutions ranged between 0.11 and 0.83. The samples were weighed and allowed to equilibrate for approximately 10 days until there was no discernible weight change (±0.001 g). The weight loss or gain was measured as the percentage of moisture desorbed or adsorbed based on the initial sample weight. Each experiment was conducted in triplicate.

Table 1
Formula of noodle containing modified rice flour (unit: g).

	WF	RF	Substituent	Salt	Water
RF:WF = 1:1[a]	20	20.0	0.0	0.8	13.20
1-h-5%[b]	20	19.0	1.0	0.8	12.74
1-h-10%[c]	20	18.0	2.0	0.8	13.17
3-h-5%[d]	20	19.0	1.0	0.8	12.74
3-h-10%[e]	20	18.0	2.0	0.8	13.17
48-h-5%[f]	20	19.0	1.0	0.8	12.74
48-h-10%[g]	20	18.0	2.0	0.8	13.17

[a] Wheat flour noodle containing rice flour at the ratio 1:1.
[b] noodle containing 4αGTase-treated rice flour for 1 h at the level of 5%.
[c] Noodle containing 4αGTase-treated rice flour for 1 h at the level of 10%.
[d] noodle containing 4αGTase-treated rice flour for 3 h at the level of 5%.
[e] Noodle containing 4αGTase-treated rice flour for 3 h at the level of 10%.
[f] Noodle containing 4αGTase-treated rice flour for 48 h at the level of 5%.
[g] Noodle containing 4αGTase-treated rice flour for 48 h at the level of 10%.

2.5. Melting enthalpy of ice crystals

The amount of unfrozen water was determined using a differential scanning calorimeter (DSC; Pyris Diamond DSC, Perkin Elmer, Waltham, MA, USA) equipped with an intracooler (Perkin Elmer) and a nitrogen gas purge. Flour samples of known water content were weighed in a large-volume DSC pan (Perkin Elmer 319-1605) and water was added to make a final moisture content of 59%. The sample pan was equilibrated for 5 h at room temperature before analysis. The sample pan was cooled from 20 to −40 °C at a rate of 2 °C/min and heated from −40 to 130 °C at a rate of 5 °C/min to measure the melting enthalpy of the ice crystals before gelatinization. After the first scanning, the sample was cooled to 0 °C, cooled again from 0 to −40 °C at a rate of 2 °C/min, and then reheated from −40 to 130 °C at a rate of 5 °C/min to measure melting enthalpy of the ice crystals after gelatinization.

For the melting enthalpy of ice crystals formed within noodle dough and cooked noodle containing sucrose, maltodextrins and 4αGTase-treated rice flours, dough samples were weighed in a DSC pan and pan was cooled to −20 °C at a rate of 2 °C/min and heated from −20 to 20 °C at a rate of 5 °C/min.

2.6. Noodle preparation

Wheat flour, rice flour, and the enzymatically modified rice flours were premixed in a mixer (Kitchen Aid, 5K5SS) using speed 1 for 30 s, and then a salt solution was added to make a final moisture content of 40 wt%. Noodle recipes are shown in Table 1. Noodles containing 4αGTase-treated rice flours as cryoprotectant were prepared by replacing the rice flour with 4αGTase-treated rice flours at levels of 5 and 10% of the total rice flour used. Mixing was continued for 4 min at speed 1. The dough was allowed to rest in a wrap at room temperature for 1 h to distribute the water uniformly throughout the flour particles. The dough was then passed through noodle machine (Dong Nam, Korea) rollers with a 2 mm gap. It was then folded and passed through the rollers three more times for sheeting. Finally, the width and thickness of noodle strand was 6 and 2 mm, respectively.

Noodles (20 g) were cooked in 200 mL boiling distilled water for 4 min and immediately rinsed with cold water. Rinsed noodles were dewatered on a wire mesh for 2 min, weighed, and then used for further study.