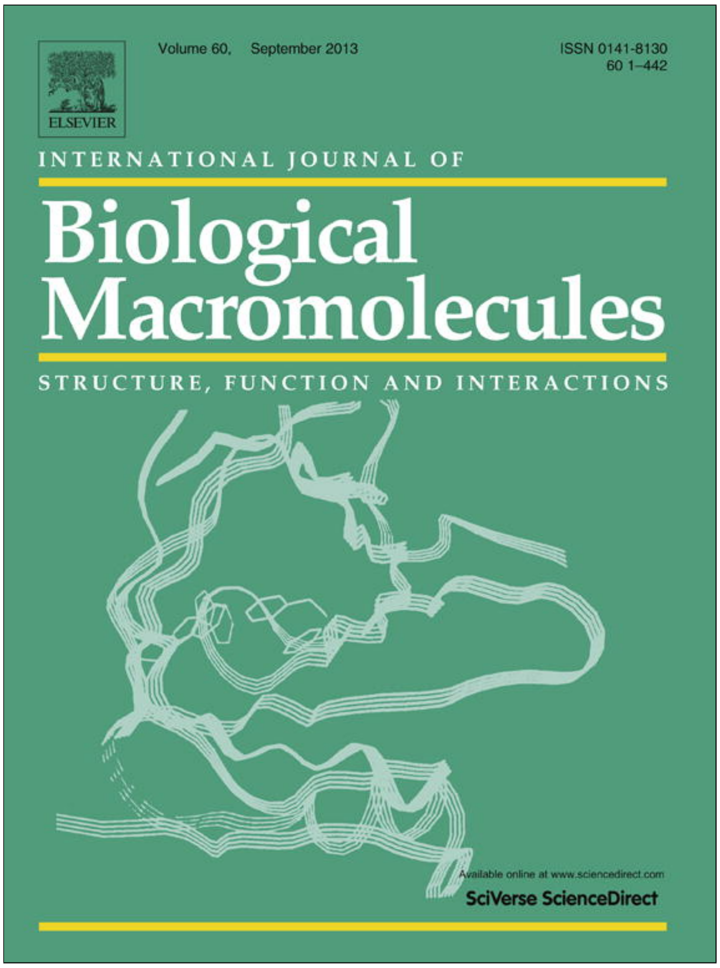

Volume 60, September 2013
ISSN 0141-8130
60 1–442

INTERNATIONAL JOURNAL OF
Biological Macromolecules
STRUCTURE, FUNCTION AND INTERACTIONS

International Journal of Biological Macromolecules 60 (2013) 422–426

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

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

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ARTICLE INFO

Article history:
Received 11 January 2013
Received in revised form 4 April 2013
Accepted 11 April 2013
Available online 18 April 2013

Keywords:
4-α-Glucanotransferase
Rice flour
Unfrozen water
Noodle
Freeze–thaw cycling

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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http://dx.doi.org/10.1016/j.ijbiomac.2013.04.032

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.

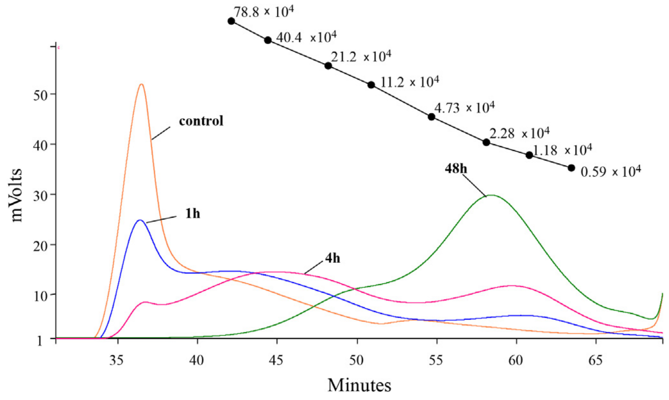

Fig. 1. Molecular weight distributions of 4αGTase-treated rice flours.

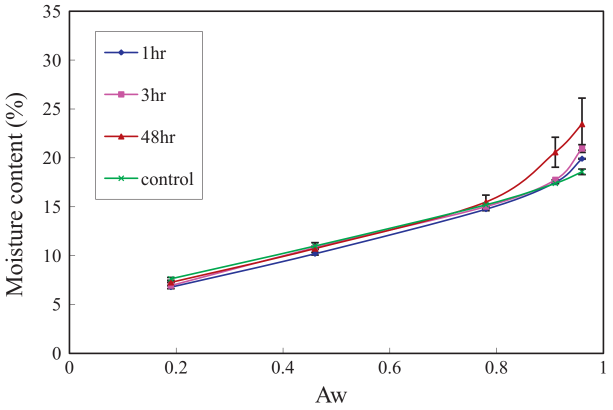

Fig. 2. Sorption isotherm of 4αGTase-treated rice flours.

2.7. *Noodle texture after freeze–thaw cycles*

The textural properties of the noodles were measured using a texture analyzer (TA-XT2i, Stable Microsystems, Surrey, UK). Cooked noodles were frozen at −20 °C overnight and then thawed for 60 min at room temperature. This freeze–thaw process was repeated twice. The frozen–thawed noodles were heated in 200 mL of boiling distilled water for 1 min and immediately rinsed with cold water. Noodle samples were evenly cut to 3 cm lengths, and individual noodle strand (30 mm × 10 mm, 3 mm thickness) was placed on the platform of the texture analyzer. The texture profile analysis (TPA) was performed at a pre-test, test, and post-test speed of 1.0 mm/s using an aluminum cylinder probe (Ø50 mm) and 70% strain.

2.8. *Statistical analysis*

The results were processed by the SPSS software (V.19.0 for Windows, SPSS, Chicago, IL, USA) using a one-way analysis of variance and then Duncan's multiple-range test or Turkey test. The resultant data were considered to be significant at $p < 0.05$.

3. Results and discussion

3.1. *Molecular weight distribution of 4αGTase-treated rice flours*

After the rice flour paste reacted for 48 h with the enzyme, the changes in the molecular weight distribution of 4αGTase-treated rice flours were analyzed using HPSEC. The time points at which the molecular distribution was altered significantly were selected for further study. The selected time points were 1, 3, and 48 h during hydrolysis; the molecular distributions are shown in Fig. 1. As the enzyme treatment time increased, the first fraction (elution volume, Ve: 35–40 ml) in the elution profile, which corresponds to amylopectin macromolecules, decreased in height, whereas the second (Ve: 40–52 ml) and third fraction (Ve: 53–65 ml) gradually [3,5]. When the rice flour was treated with 4αGTase for 48 h, the first and second fractions almost disappeared and the enzymatic product was composed of polymers, the sizes of which were hundred to thousand times smaller than that of the native starch. 4αGTase catalyzes a well-characterized disproportionating reaction in which a glucan moiety is transferred from one α-1,4-glucan molecule to another [2,3,7]. Previous research revealed that 4αGTase could attack inner chains of amylopectin (probably long B or C chains) as well as outer chains (A or short B chains) by disproportionation, resulting in the production of amylopectin clusters of reduced size with rearranged branch chains [3]. These modified amylopectin clusters might contribute to the formation of thermoreversible gel as suggested previously [2]. Therefore, we propose that the second and third fractions in the profile were created after treatment with 4αGTase, by the formation of intermediate *Mw* amylopectin clusters with rearranged branch chains. In the following experiments, 1-, 3-, and 48-h-modified rice flours were used to examine cryoprotective effect of 4αGTase-treated rice flour.

3.2. *Sorption isotherm of modified rice flour*

A water sorption isotherm represents the relationship between the water adsorbed or desorbed within food and its water activity (Aw) or the related humidity (RH) of air at a constant temperature and under equilibrium conditions. Many other applications of sorption isotherms are useful for food technologists, for example, in drying process modeling, the design and optimization of driers, food shelf-life prediction, modeling moisture changes within food during storage, and food packaging selection [10–12]. The sorption isotherms obtained for 4αGTase-treated rice flours at 25 °C are shown in Fig. 2. For Aw values lower than around 0.8, the equilibrium moisture content of 4αGTase-treated rice flours was not different from the control. However, for Aw values higher than around 0.8, the equilibrium moisture content of 4αGTase-treated rice flours was higher than that of the control, and the moisture content of rice flours treated for 48 h was the highest, indicating that the 4αGTase-treated rice flours could hold more moisture compared with the control at an Aw > 0.8. We postulate that structural changes, such as the exposure of active sites or hydrophilic groups, might have occurred during enzyme hydrolysis, which facilitated an association with water. The association between 4αGTase-treated rice flours and water therefore increased at Aw > 0.8 after enzyme hydrolysis. Another possible reason might be that formation of relatively bulky starch chains ($Mw \sim 10^5$) with a reorganized chain distribution within the 4αGTase-treated rice flours after enzyme hydrolysis.

3.3. *Melting enthalpy of ice crystals*

The melting enthalpy of the ice crystals that formed in rice flour, wheat flour, and 4αGTase-treated rice flours were measured using DSC. Typical DSC curves for ice crystal melting enthalpy of 4αGTase-treated rice flour for 48 h are shown in Fig. 3. During heating of frozen flour in the first and second scan, endothermic peaks were detected between −5 and 10 °C. Other flours also showed a similar DSC curve to that of 48-h-treated rice flour and from DSC curves of flours (data not shown), ice crystal melting enthalpy of wheat, control rice and modified rice flours were obtained (Table 2).

Freezable water is defined as water that can be separated within the sample matrix. When freezable water is sufficiently cooled, the melting enthalpy of ice crystals can be detected by DSC. In the

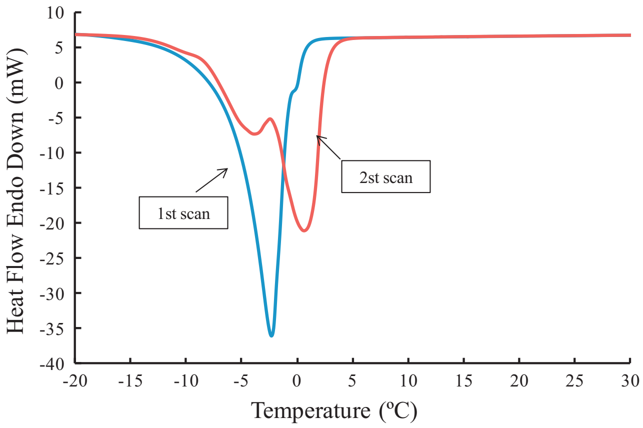

Fig. 3. Ice crystal melting enthalpy of 4αGTase-treated rice flour for 48 h.

first and second scan, non-treated rice and wheat flours had higher melting enthalpies than 4αGTase-treated rice flours. The decrease in the melting enthalpy became larger as the enzyme treatment time increased. This result indicated that 4αGTase-treated rice flours had a lower quantity of freezable water than non-treated flours. This result might have been caused by the relatively high water binding capacity of the 4αGTase-treated rice flours, and was in good agreement with the moisture sorption isotherm. These results implied that 4αGTase-treated rice flours potentially could be used as effective food additives to improve freeze–thaw stability.

3.4. *Textural properties of noodles containing 4αGTase-treated rice flours*

The purpose of this experiment was to investigate the applicability of 4αGTase-treated rice flours as cryoprotectants in frozen cooked noodles. As explained above, 4αGTase-treated rice flours had a relatively high water binding capacity compared to non-treated rice flour, which led to an increased amount of bound water and a lower Aw. Therefore, we expected that the partial substitution of normal flour with 4αGTase-treated rice flours could improve the freeze–thaw stability of frozen cooked noodles.

To investigate the stability of noodles containing 4αGTase-treated rice flours following freeze–thaw cycling, noodles were prepared with 4αGTase-treated rice flours and the textural properties were measured by TPA test (Table 3). A successful frozen convenience food must offer a significant time savings to the consumer. For this reason, the raw noodles were cooked prior to being frozen and were reheated in boiling water for only 1 min after the freeze–thaw cycle. Before the freeze–thaw cycling, the hardness, adhesiveness, springiness, and chewiness of noodles containing 4αGTase-treated rice flours were lower than those of noodles prepared with non-treated rice flour. After repeated freeze–thaw cycling, the textural parameter values of control noodles decreased, however, those of noodles containing 4αGTase-treated rice flours increased. This result suggested that structure of control noodle was disrupted during the freeze–thaw cycling, whereas noodles containing 4αGTase-treated rice flours could retain the hardness, springiness and chewiness even after freeze–thaw cycling.

A variety of different physicochemical processes occur during the freezing process, including ice formation, freeze concentration, and phase transitions [13,14]. Because of the growth of ice crystals and moisture migration during frozen storage, it was expected that noodle quality would deteriorate after freeze–thaw cycling. When cooked noodles are cooled to temperatures at which the water crystallizes, several physicochemical processes occur that can affect noodle texture. First, ice crystals may physically penetrate and disrupt the structure, resulting in decreased textural parameter values [15]. Second, water redistribution, which is caused by the formation of ice crystals during frozen storage, may affect noodle texture [16]. The redistribution of water results in water-rich and water-dry areas, which may also produce undesirable texture.

The textural properties of noodles containing 4αGTase-treated rice flours implied that their physical structure was not deteriorated after repeated freeze–thaw cycling and these results might be partly attributed to the increased amount of bound water, which increased the volume of the unfrozen aqueous phase in the noodle and decreased the tendency for the structure of noodle to be disrupted during freezing. Besides, it was postulated that hard gel-forming property of 4αGTase-treated rice starch might contribute to the textural integrity of noodles [17]. However, further investigation should be needed to clarify this effect.

3.5. *Comparison of efficacy in cryoprotecting frozen noodle*

In this section of experiment, to compare the cryoprotective effect of 4αGTase-treated rice flour to other cryoprotective substance, such as sucrose and two types of maltodextrin containing different dextrose equivalents (DEs) (*i.e.*, DE 12 and DE 20), the melting enthalpy of the ice crystals of noodle dough and cooked noodle containing 4αGTase-treated rice flour and cryoprotective substances was measured using DSC. In the preparation of noodle dough, rice flour was replaced with sucrose, maltodextrins, and 4αGTase-treated rice flour at levels of 5% of the total rice flour used (Table 1).

The result about melting enthalpy of ice crystals formed within noodle dough is shown in Table 4. Frozen noodle dough containing sucrose, maltodextrins and 4αGTase-treated rice flour showed lower melting enthalpy of the ice crystals than that of control frozen noodle dough and the degree of lowering was big in the noodle dough containing 4αGTase-treated rice flour. This result indicated that 4αGTase-treated rice flour was more effective for increasing unfrozen water within frozen noodle dough than other added substances.

Fig. 4 shows the hardness value of cooked noodle containing sucrose, maltodextrins and 4αGTase-treated rice flour measured by TPA test. Hardness of cooked noodle containing two types of maltodextrin and 4αGTase-treated rice flour was similar to that of control noodle, however, cooked noodle containing sucrose showed lower hardness value and more soft texture compared to control noodle.

For the melting enthalpy of ice crystals formed within cooked noodles, noodles containing sucrose and 4αGTase-treated rice flour showed lower level than other noodles (Table 4). This indicated that the amount of unfrozen water, *i.e.*, bound water increased with added 4αGTase-treated rice and effect of 4αGTase-treated rice flour on the decreasing the enthalpy was similar to that of sucrose.

Table 2
Ice crystal melting enthalpy of wheat, rice and modified rice flours (J/g).

	Rice flour	Wheat flour	4αGTase-treated rice flour		
			1 h	3 h	48 h
1st scan	284 ± 3.5	272 ± 2.8	259 ± 5	254 ± 2	243 ± 4
2nd scan	266 ± 5.1	266 ± 3.6	255 ± 1.1	235 ± 3.4	226 ± 3

Table 3
The changes of textural properties of noodles after repeated freeze–thaw cycling.

	Before freeze–thaw cycling				After 2-time freeze–thaw cycling			
	Hardness (g)	Adhesiveness (g s)	Springiness	Chewiness	Hardness (g)	Adhesiveness (g s)	Springiness	Chewiness
WF:RF 1:1	1898 ± 85^{bc}	-392 ± 20^{a}	0.92 ± 0.01^{c}	1099 ± 46^{b}	1784 ± 254^{abc}	-347 ± 159^{a}	0.82 ± 0.07^{bc}	870 ± 255^{bc}
1-h-5%	1765 ± 165^{abc}	-337 ± 86^{a}	0.90 ± 0.03^{c}	985 ± 153^{c}	1840 ± 371^{abc}	-387 ± 176^{a}	0.88 ± 0.07^{c}	970 ± 312^{c}
3-h-5%	1890 ± 190^{abc}	-358 ± 94^{a}	0.88 ± 0.05^{c}	1028 ± 158^{c}	1966 ± 256^{bc}	-411 ± 148^{a}	0.83 ± 0.13^{c}	977 ± 323^{c}
48-h-5%	1809 ± 295^{abc}	-330 ± 110^{a}	0.87 ± 0.05^{c}	973 ± 212^{c}	1941 ± 162^{bc}	-350 ± 19^{a}	0.85 ± 0.04^{c}	978 ± 155^{c}
1-h-10%	1602 ± 106^{a}	-128 ± 45^{b}	0.73 ± 0.03^{ab}	682 ± 95^{ab}	1819 ± 40^{abc}	-403 ± 16^{a}	0.85 ± 0.03^{c}	909 ± 57^{bc}
3-h-10%	1677 ± 240^{ab}	-137 ± 56^{b}	0.66 ± 0.03^{a}	597 ± 87^{a}	1805 ± 251^{abc}	-451 ± 72^{a}	0.89 ± 0.04^{c}	967 ± 227^{c}
48-h-10%	1702 ± 59^{ab}	-124 ± 15^{b}	0.70 ± 0.03^{a}	680 ± 50^{ab}	2046 ± 47^{c}	-490 ± 125^{a}	0.86 ± 0.08^{c}	1032 ± 146^{c}

Values followed by the same letter in the same textural property are not significantly different ($p < 0.05$).

Table 4
Ice crystal melting enthalpy of noodle dough and cooked noodle containing different cryoprotectants (J/g).

	Control	Sucrose	MD (DE12)	MD (DE20)	4αGTase-treated rice flour
Dough	96.3 ± 0.57	91.9 ± 0.57	95.2 ± 1.06	91.7 ± 0.57	87.9 ± 0.99
Cooked noodle	162.2 ± 13.5	146.7 ± 12.4	156.0 ± 14.5	154.6 ± 13.5	148.5 ± 11.5

MD: maltodextrin.

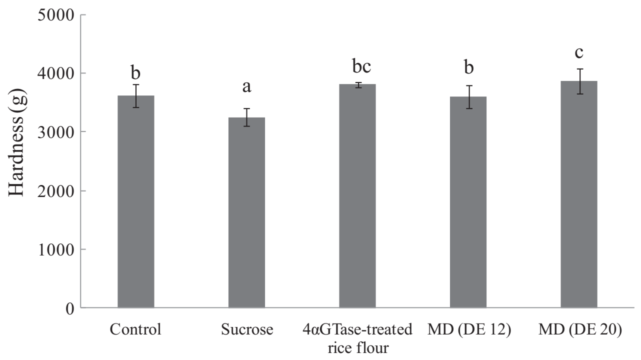

Fig. 4. Hardness of cooked noodles prepared with various cryoprotecting substances. Values indicated by the same letters are not significantly different ($p < 0.05$).

However, as explained above, noodle containing sucrose showed lower hardness and more soft texture than control noodle after cooking and sucrose imparted a considerably sweet taste to noodle, thus there would be limitation for application of sucrose to frozen noodle [18]. There have been many efforts to find non-sweet additives with cryoprotective effects that equal those of the low molecular weight sugars and polyols. For this reason, 4αGTase-treated rice flours or starch would be useful as a food cryoprotectant. The favorable properties of 4αGTase-treated rice flours or starch, such as high water binding capacity, low sweetness, low viscosity, and low calorie content, may improve its usefulness in frozen food products.

4. Conclusions

As a result of structural changes of 4αGTase-treated rice flours after enzyme hydrolysis, water binding capacity of 4αGTase-treated rice flours increased compared to control rice flour. Subsequently, increased water binding capacity improved textural properties of noodles containing the 4αGTase-treated rice flours after repeated freeze–thaw cycling. The effect of 4αGTase-treated rice flours for reducing freezable water within frozen noodle dough and noodle was similar to that of sucrose, however, the texture of noodle containing sucrose became soft and sweet after cooking. Thus, this study suggested that 4αGTase-treated rice flours have good potential as a cryoprotectant for noodle, and its low sweetness would be advantage over sucrose for food processing.

Acknowledgement

This research was supported by the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

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