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Effect of Cooking Method and Additives on the Freeze-Thaw Stability of Mung Bean Starch Paste for Preparation of *Omija-eui*

Hyun Jeong Han, Keum-Il Jang[1], In Young Bae, Kwang Yeon Lee, Seung Hyun Koo, Mi Kyung Kim, Soo Jin Jun, and Hyeon Gyu Lee*

Department of Food Science and Nutrition, Hanyang University, Seoul 133-791, Korea

[1]*Department of Food Science and Technology, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea*

Abstract Optimum conditions for the freeze-thaw stability (FTS) of mung bean starch (MBS) paste as a main ingredient in *omija-eui* were investigated. For the optimization of the paste preparation condition, the FTS of MBS prepared by boiling in a shaking water bath (BMSW) or by pressure-cooking in an autoclave (PCMA) were analyzed using a response surface methodology (RSM). In addition, the effects of various additives such as gums, sugars, and emulsifier were evaluated on the FTS of MBS paste prepared under optimal conditions. The predicted maximal FTS of MBS paste prepared by the PCMA method (73%) was higher than that of the paste prepared by the BMSW method (36%). In case of additives, gellan gum and sodium alginate effectively prevented the syneresis of MBS paste in the BMSW method and in the PCMA method, respectively. The use of a fructose fatty acid ester as an emulsifier decreased syneresis in a dose-dependent, while the addition of sugars accelerated syneresis. Consequently, MBS paste for *omija-eui* preparation may be efficiently prepared by adding sodium alginate and fructose fatty acid ester under the optimal conditions of 4.3% MBS content, 121°C heating temperature, and 89°C cooling temperature by pressure-cooking in an autoclave.

Keywords: mung bean starch paste, freeze-thaw stability, boiling, autoclave, additives

Introduction

Porridge, typically prepared from boiled rice, maize, wheat, and other starchy cereals in water, is a traditional dish consumed in Asia since ancient times. Because porridge is easily consumed and digested, it is usually served as an accompaniment to other dishes or as a stand-alone meal for the ill, elderly, or infants (1,2). In Korea, *eui* is very similar to a fine, watery porridge and is prepared using starch such as mung bean, adlay, sorghum, arrowroot, and lotus root (3-5). Among them, *omija-eui* is a type of porridge made by boiling mung bean starch (MBS) with *omija* (the berries of *Schizandra chinensis*) extract, honey, or other ingredients (3).

The main ingredient in the preparation of *omija-eui*, mung bean starch (MBS), has high gelation ability and special texture such as special flavor, springiness, and chewiness (6,7). The gelation and the texture of MBS paste are affected by the composition (amylose, water, protein), and their content, molecular weight, storage temperature, and additives (8). When the starch paste is repeatedly frozen and thawed during storage, the hydrogen bonds of water in the paste become unstable and less cohesive. As a result, dehydration from the paste and association among the amylose molecules lead to syneresis (9) and then the gel structure destructed through the phase change of water and the wave of heat energy (10). Specially, MBS paste which contained high amylose and protein content exhibited higher degree of syneresis than corn and rice starch during the freeze-thaw cycle (11). Therefore, in order to improve the quality of *omija-eui*, it is need to investigate the optimal conditions for MBS paste preparation to minimize syneresis.

Food additives such as gums, sugars, and emulsifiers influence the quality of foods prepared with starches. Gums modulate the rheological characteristics of starch-containing foods, improving viscosity and decreasing the change in viscosity during cold storage (12). Funami *et al.* (13) reported that gum prevented the re-crystallization of amylose and amylopectin in corn starch, which retarded the retrogradation of corn starch during long-term storage. Mali *et al.* (14) found that gum addition decreased syneresis and minimized the structural degradation of yam starch during the freeze–thaw cycle. Although freeze-thaw stability (FTS) improved with increasing gum concentration (15), the mechanism of action is different by the types of gums. During repeated freeze-thaw cycles, both xanthan gum and guar gum increased FTS; paste viscosity of starch gel decreased by xanthan gum, while increased by guar gum (16). Alginate prevented the re-crystallization of starch during the freeze-thaw cycle and stabilized the starch gel structure due to the relative reaction between water molecules and starch chains (17). When sugars were added to starch-containing foods, the viscoelasticity of the gel structure increased by swelling during the gelation of starch suspension, and the viscoelasticity of starch paste increased in proportion to sugar content (18). Kim (19) reported that sugar addition to gel starch decreased free water adsorption from the outer environment in the amorphous area, stabilizing the crystallization area; thus, retrogradation was controlled by preventing the re-crystallization of amylopectin. In addition, oligosaccharide (20) and sucrose (21) are effective additives for preventing the retrogradation of starch. On the other hand, sucrose

*Corresponding author: Tel: +82-2-2220-1202; Fax: +82-2-2292-1226
E-mail: hyeonlee@hanyang.ac.kr
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Table 1. Coded level of 3 factors used in CCD to optimize the syneresis of MBS paste prepared with BMSW and PCMA methods

Variables of BMSW	X_i	-1	0	+1	ΔX	Variables of PCMA	X_i	-1	0	+1	ΔX
MBS content (%)	X_1	40	45	50	5	MBS content (%)	X_1	3	4	5	1
Heating temperature (°C)	X_2	50	55	60	5	Heating temperature (°C)	X_2	110	115	120	5
Heating time (min)	X_3	20	25	30	5	Cooling temperature (°C)	X_3	70	80	90	10

fatty acid ester used as an emulsifier inhibits the retrogradation of starch (20). Nuessli *et al*. (22) reported that the addition of an emulsifier to potato starch resulted in weak gel structure formed by the reaction between branch chains of amylopectin and the emulsifier. Kim and Shin (8) noted that the addition of an emulsifier prevented the retrogradation of rice starch during storage.

The purposes of this study are 1) to investigate optimum conditions for the method of MBS paste preparation by boiling in a shaking water bath (BMSW) or by pressure-cooking in an autoclave (PCMA) using response surface methodology (RSM), and 2) to evaluate the effects of various additives, such as gums, emulsifiers, and sugars, on the FTS of MBS paste prepared under optimal conditions.

Materials and Methods

Materials Mung bean starch (MBS) was prepared with powdered mung beans, which had been cultivated in Cheorwon province of Korea, using the alkali method described by Joo and Chun (23), with some modifications. Guar gum, locust bean gum, glucose, and sucrose were purchased from Sigma-Aldrich (St. Louis, MO, USA), and gellan gum, sodium alginate, and maltose were obtained from Wako Pure Chemical Industries (Tokyo, Japan), Kanto Chemical Co., Inc. (Tokyo, Japan) and Fluka Chemie GmbH (Tokyo, Japan), respectively. Fructose fatty acid ester used as an emulsifying agent was purchased from Ilshinwells Co., Inc. (Seoul, Korea).

Preparation of MBS pastes via BMSW and PCMA In the BMSW method, MBS paste was prepared by boiling off 40-50% of the paste water content at 50-60°C for 20-30 min in a shaking water bath. In the PCMA method, MBS paste was prepared by autoclaving off 3-5% of the paste water content at 110-120°C for 30 min and then cooling at 70-90°C for 30 min in a shaking water bath.

Freeze-thaw stability (FTS) FTS was measured using the method described by Lee *et al.* (17) and Deetae *et al.* (24) with some modifications. To compare FTS in MBS preparations obtained via the BMSW and PCMA methods, each preparation was subjected to a single freeze-thaw cycle consisting of 20 hr frozen storage at −20°C, followed by 4 hr storage at room temperature. The samples were then centrifuged at 4,000×g for 10 min. The free liquid was decanted, and the weight of the remaining gel was determined. The ratio of free liquid weight to the initial sample weight (expressed as a percentage) was used as a measure of syneresis. And the FTS was then obtained from the calculated ratio using the following equation:

$$\text{FTS}\ (\%) = 100 - S \quad (1)$$

where S is the syneresis (%).

Optimal conditions for MBS paste preparation The optimal conditions for MBS paste preparation to minimize syneresis were determined using the RSM. To ascribe the effect of various factors on the response surface in the region of investigation, a central composite design (CCD) with 3 factors at 3 levels was performed. The investigated factors were MBS content, heating temperature, and heating time in the BMSW method, and MBS content, heating temperature, and cooling temperature in the PCMA method (Table 1). A 3-dimensional graph was obtained from the calculated response surface using the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_{ii}X_i + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j + \sum_{i=1}^{3}\beta_{ii}X_i^2 \quad (2)$$

where Y is the response, β_0 is the constant coefficient, and X_i (i=1-3) are the coded settings for the 3 factors. The β terms are the calculated parameter estimates for each main effect, interaction, and quadratic effect. Data were processed for Eq. 2 using Statistical Analysis Software (SAS, Version 8.0, SAS Institute Inc., Cary, NC, USA) to obtain the interaction between the process variables and the response. The analysis of results from each design produced a predictive model of the response as a function of the process factors.

Effect of additives To determine the effect of additives on the FTS of MBS paste, gums, sugars, and emulsifiers were added to optimal MBS pastes prepared via the BMSW or PCMA method. After 0.1, 0.3, and 0.5%(w/v) addition of guar gum, locust bean gum, gellan gum, or sodium alginate, the addition of a fructose fatty acid ester as the emulsifying agent, and the addition of 10, 30, and 50%(w/v) of sucrose, maltose, or glucose, the FTSs for each additive were analyzed by measuring the resultant change in syneresis.

Statistical analysis The results were expressed as the mean±standard deviation (SD) and subjected to analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS, Version 12.0, 2004; SPSS Inc., Chicago, IL, USA). Significant differences between sample means were determined at the $p<0.05$ level using Duncan's multiple comparison test. All experiments were performed in triplicate.

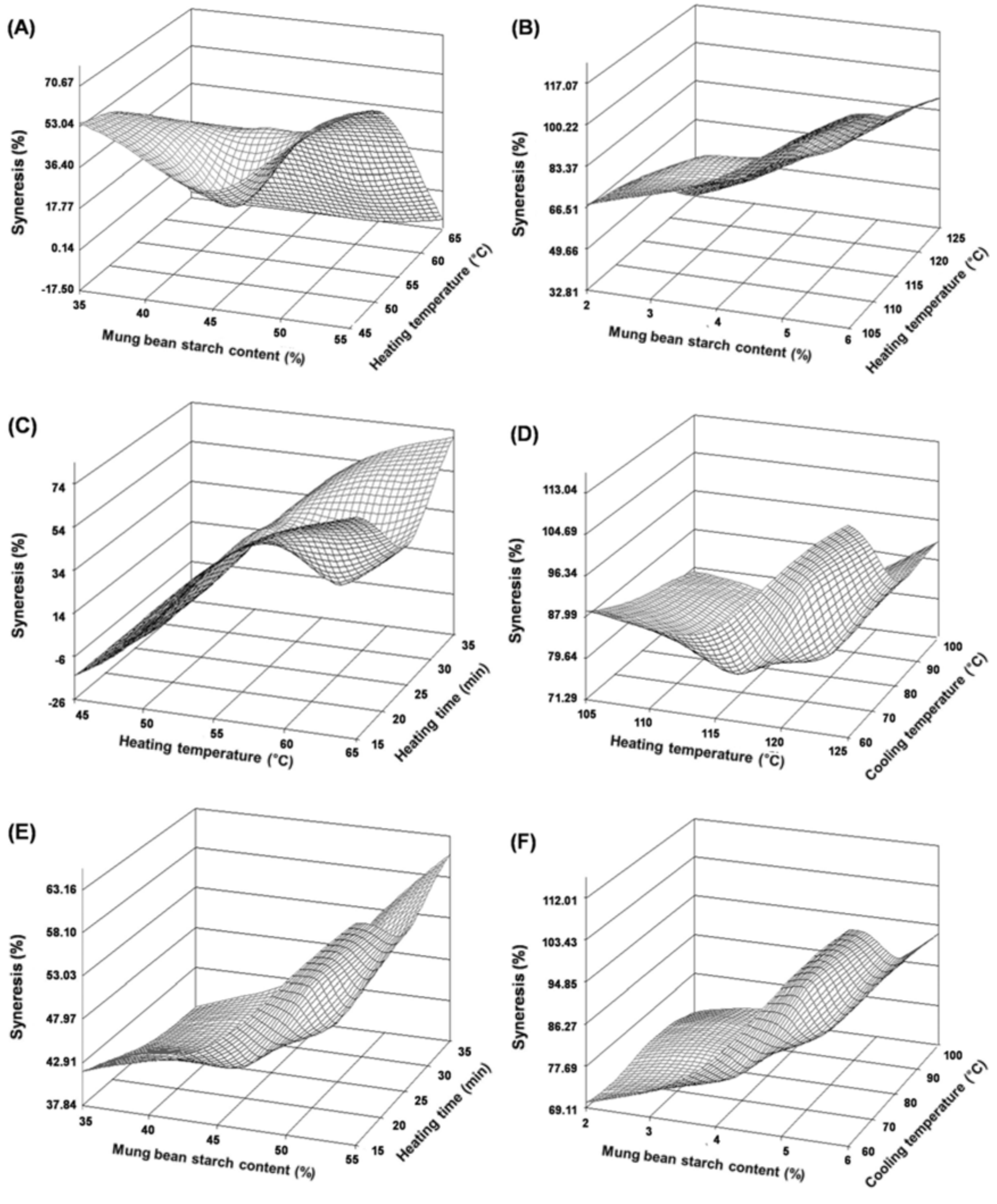

Fig. 1. A 3-dimensional response surface showing the effect of MBS content and the heating temperature (A and B), the heating temperature and the heating time (C), the heating temperature and the cooling temperature (D), MBS content and the heating time (E), and MBS content and the cooling temperature (F) on syneresis of MBS pastes for *omija-eui* prepared with MBSW (left) and PCMA (right) methods.

Results and Discussion

Response surface analysis and maximal FTS for MBS paste A central composite design, with 3 settings for each of 3 factors, was performed to minimize syneresis. FTS were processed for Eq. 1. In the BMSW method, the settings for each factor were as follows: MBS content (% w/v), 40(-1), 45(0), and 50(+1); heating temperature (°C), 50(-1), 55(0), and 60(+1); heating time (min), 20(-1), 25(0), and 30(+1). In the PCMA method, the settings for each factor were as follows: MBS content (%, w/v), 3(-1), 4(0), and 5(+1); heating temperature (°C), 110(-1), 115(0), and 120(+1); cooling temperature (°C), 70(-1), 80(0), and 90(+1). Both models showed goodness-of-fit [coefficient of determination (R^2)=0.93 for BMSW and 0.94 for PCMA], meaning that 93 and 94% of the variability, respectively, can be accounted for by the polynomial regression equation given below:

Table 2. Predicted optimum conditions for the FTS response of MBS paste prepared with BMSW and PCMA methods

Response[2)]	Variable[1)]						Expected syneresis values	Expected FTS values
	X_1		X_2		X_3			
	Coded	Uncoded	Coded	Uncoded	Coded	Uncoded		
Y_1	0.12	45.5%	-0.28	54.5°C	0.03	25.14 min	64%	36%
Y_2	0.33	4.3%	-0.58	112°C	0.92	89°C	27%	73%

[1)] X_1 and X_2 were the concentration of MBS and the heating temperature, respectively; X_3 was distinguished the heating time on Y_1 and the cooling temperature on Y_2.
[2)] Y_1 and Y_2 were the freeze-thaw syneresis of mung bean starch paste prepared with BMSW and PCMA methods, respectively.

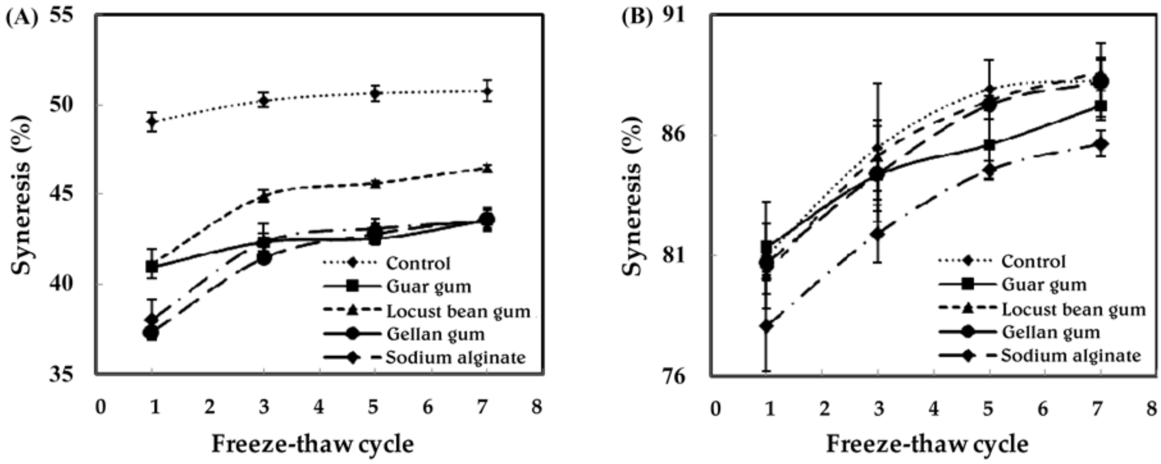

Fig. 2. Effect of gums (0.3%, w/v) for syneresis of MBS pastes prepared with BMSW (A) and PCMA (B) with increase of freeze-thaw cycles.

$$\text{BMSW: } Y=33.73-17.52\ X_3-30.34\ X_2^2 \quad (3)$$
$$\text{PCMA: } Y=77.57-7.32\ X_1-4.54\ X_2+4.46\ X_1X_3 \quad (4)$$

where Y is syneresis, and X_1, X_2, and X_3 are the coded settings for MBS content, heating temperature, and heating time (BMSW) or cooling temperature (PCMA), respectively. The analysis revealed that heating temperature affected syneresis in BMSW, and that MBS content affected syneresis in PCMA. The 3-dimensional graph obtained from the calculated response surface is shown in Fig. 1.

The optimal conditions for minimal syneresis were identified by canonical analysis. For 2 models, the canonical equation for each factor was shown as:

$$\text{BMSW: } Y=36.08+12.44\ X_1+11.1\ X_2+30.31\ X_3 \quad (5)$$
$$\text{PCMA: } Y=72.76+5.62\ X_1+0.33\ X_2+2.72\ X_3 \quad (6)$$

where Y is syneresis and X_1, X_2, and X_3 are the coded settings for MBS content, heating temperature, and heating time (BMSW) or cooling temperature (PCMA), respectively. In the BMSW method, the fitted surface was minimal at 45.5% for MBS content, 54.5°C for heating temperature, and 25 min 14 sec for heating time, yielding predicted minimal syneresis of 64% and maximal FTS of 36%. In the PCMA method, the fitted surface was minimal at 4.3% for MBS content, 121°C for heating temperature, and 89°C for cooling temperature, yielding predicted minimal syneresis of 27% and maximal FTS of 73% (Table 2). Thus, our results indicate that the PCMA method is more suitable for preparing MBS paste as a main ingredient of *omija-eui* than the BMSW method.

Effect of gum Changes in syneresis during repeated freeze-thaw cycles were examined after adding 0.3% gum (i.e., guar gum, locust bean gum, gellan gum, or sodium alginate) to MBS pastes prepared under optimal conditions using the BMSW or PCMA method (Fig. 2). In both methods, gelation and syneresis increased with increasing number of freeze-thaw cycles, and the structure of the MBS paste slowly deteriorated. These results are consistent with a previous report by Choi and Oh (25), which demonstrated that stored MBS paste became firmer and crumbled more easily as a result of increased syneresis and decreased adhesion and cohesion. When gum was hydrated, water coherence increased and the re-binding of amylose and amylopectin was inhibited, thus retarding starch retrogradation (15). In another study, gum addition prevented the retrogradation of amylose in starch paste and inhibited the sponge phenomenon as a result of binding between the gum and amylose and a subsequent decrease in syneresis (26). Therefore, our results confirm that the addition of various gums decreases syneresis in MBS paste prepared via both methods.

In both methods, gum addition increased the FTS of MBS paste, and syneresis decreased with increasing gum concentration (data not shown). In the BMSW method, the greatest decrease in syneresis was observed after adding 0.3% gellan gum, followed by sodium alginate, guar gum, and finally locust bean gum (Fig. 2A). In the PMCA method, the greatest decrease in syneresis was observed after adding 0.3% sodium alginate, followed by guar gum, gellan gum, and locust bean gum (Fig. 2B). Furthermore,

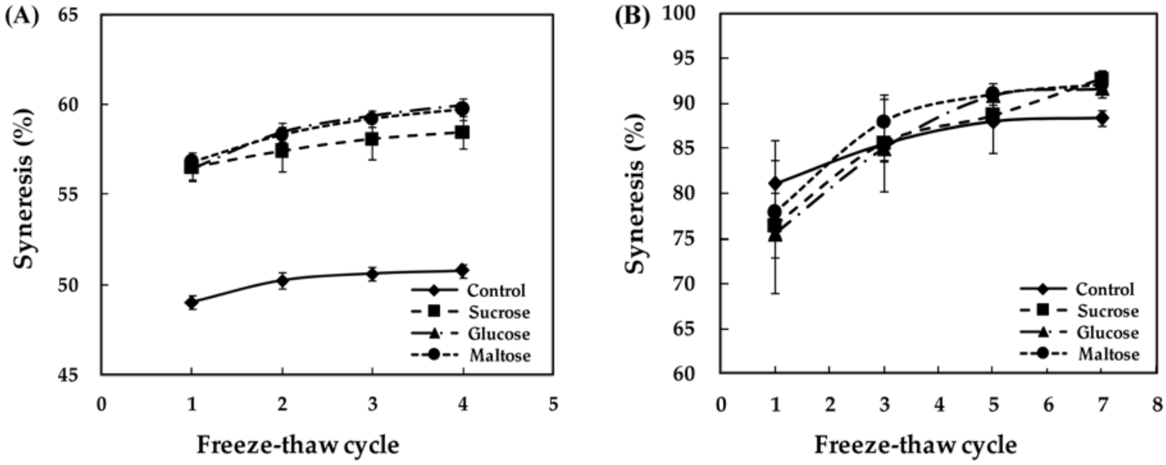

Fig. 3. Effect of sugars (30%, w/v) for syneresis of MBS pastes prepared with BMSW (A) and PCMA (B) with increase of freeze-thaw cycles.

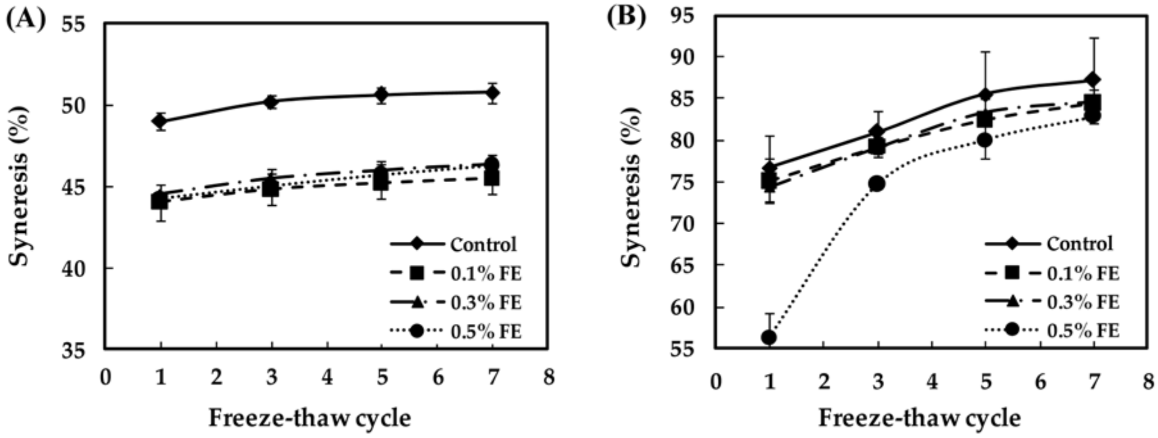

Fig. 4. Effect of fructose fatty acid ester (FE) for syneresis of MBS pastes prepared with BMSW (A) and PCMA (B) with increase of freeze-thaw cycles.

after gum addition, MBS paste prepared using the BMSW method showed less syneresis than that prepared via the PCMA method, exhibiting more effective for MBS preparation. Kwon *et al*. (9) reported that the FTS of rice paste containing alginate and gellan gum, which had a small, uniform, and stable structure upon scanning electron microscopy, was lower than the FTS of other gum-added rice pastes. Starch paste prepared with locust bean gum showed a greater degree of syneresis than paste prepared with guar gum during repeated freeze-thaw cycles because the binding ratio of galactose to mannose differed between locust bean and guar gum (17). Alginate, which is an ionic linear copolymer of homopolymeric blocks of (1-4)-linked β-D-mannuronate and its C-5 epimer α-L-guluronate covalently linked in different sequences or blocks, inhibited the crystallization of adjacent water by virtue of its ionicity and inhibited dehydration as a result of strong binding among polysaccharide chains (27). Thus, our results demonstrate that gellan gum and sodium alginate maximize the FTS of MBS paste when using the BMSW and PCMA methods, respectively.

Effect of sugar Changes in syneresis during repeated freeze-thaw cycles were examined after adding 30% sugar (i.e., glucose, sucrose, or maltose) to MBS pastes prepared under optimal conditions using the BMSW or PCMA method (Fig. 3). In the BMSW method, starch syneresis increased with the addition of a sugar additive. Glucose and maltose addition resulted in greater acceleration than sucrose. However, in the PCMA method, sugar additives decreased syneresis during the initial freeze-thaw cycles (1-3 cycles), followed by a marked increase in syneresis during subsequent cycles. In the previous study examining the relationship between sugar addition and starch retrogradation, sucrose accelerated the retrogradation of rice starch, glucose and fructose accelerated the retrogradation of corn starch, and fructose accelerated the retrogradation of wheat starch (28). Kohyama and Nishinari (29) reported that the enthalpy for gelation of sweet potato starch was initially increased because the energy for dissolution of starch crystals was more required by bridges between starch branches and sugar chains elongated with increased sugar content. Germani *et al*. (30) reported that sucrose addition increased starch retrogradation to a greater degree than fructose addition because the interaction between amylopectin and sucrose was hindered by the high molecular weight of sucrose and the presence of more equatorial-OH groups on the glucose residue. Based on these studies and our results, we concluded that sugar is ineffective as an additive to improve the FTS of MBS paste.

Effect of fructose fatty acid ester The addition of an emulsifier and a surfactant into starch prevented the crystallization of amylose as a result of complex formation between these additives and amylose and amylopectin; thus, emulsifiers and surfactants retard starch retrogradation (31). Therefore, we analyzed the effect of a fructose fatty acid ester (FE) on syneresis during repeated freeze-thaw cycles. In the BMSW method, FE addition decreased syneresis compared to pure MBS paste, but no difference was observed with additional freeze-thaw cycles or changes in fructose fatty acid ester content (Fig. 4A). In contrast, 0.5% fructose fatty acid ester addition to MBS prepared via the PMCA method decreased syneresis, whereas pastes containing 0.1 or 0.3% fructose fatty acid ester showed a similar degree of syneresis, compared to pure paste, and increasing syneresis with increasing number of freeze-thaw cycles (Fig. 4B). These results are consistent with the previous report by Choi and Oh (32), in which the addition of sucrose fatty acid ester decreased syneresis in starch paste. Kulp and Ponte (33) reported that fatty acids and esters retarded the retrogradation of starch through complex formation of these surfactants with amylose and amylopectin, thus preventing starch crystallization. Our results suggest that 0.5% fructose fatty acid ester is appropriate as an additive for MBS paste in *omija-eui* preparation, because it decreased syneresis in pastes prepared via both the BMSW and PMCA methods.

In conclusion, the optimum conditions for the preparation of MBS paste with improved FTS were investigated using different preparation method and various additives. The FTS of MBS paste prepared by the PCMA method (73%) was more stable than that of the paste prepared by the BMSW method (36%). In case of additives, gellan gum and sodium alginate effectively prevented the syneresis of MBS paste and the use of a fructose fatty acid ester as an emulsifier decreased syneresis in a dose-dependent. However, the addition of sugars accelerated syneresis. Thus, MBS paste with ameliorated FTS for *omija-eui* may be efficiently prepared by the PCMA method in combination with additives such as gellan gum, sodium alginate, and fructose fatty acid ester.

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