

# Effect of Fermented Lettuce Extract on Starch Retrogradation in Korean Rice Cake, *Garaetteok*

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**Abstract:** Starch retrogradation, i.e., reassociation or recrystallization of gelatinized starch, affects the flavor and texture of food. To prevent starch retrogradation, various methods such as replacement of chemically modified starch and use of hydrolytic and branching enzymes or additives have been studied. However, the relationship between fermented products and starch retrogradation has been not studied. Here, we investigated the effect of fermented lettuce extract on the retrogradation of Korean rice cake, *garaetteok* using Rapid Visco Analyzer (RVA), differential scanning calorimetry (DSC), texture analysis, and scanning electron microscopy (SEM). Fermented lettuce extract reduced peak viscosity, trough viscosity, breakdown, and setback of rice flour, as determined using the RVA. DSC revealed reduced onset temperature, peak temperature, and enthalpy change of cakes prepared from flour treated with fermented lettuce extract during storage compared with those of an untreated control. The change in the hardness of the treated rice cakes during storage, as determined via texture analysis, was smaller than that in the control. The surface of the fermented lettuce extract treated-rice cakes was found to be less cracked and porous than that of the control, as determined via SEM. Collectively, these findings showed that starch retrogradation during storage was retarded in fermented lettuce extract treated-rice cakes compared with untreated rice cakes. Therefore, these results suggested that fermented lettuce extract prevents starch retrogradation, improves food quality, and could be used as a food additive to retard starch retrogradation in the food industry.

**Keywords:** fermented lettuce extract, starch retrogradation, retrogradation retardation, rice cake

## 1. INTRODUCTION

Rice cake is a representative traditional food from Asia. It is made by mixing rice flour with water and adding sugar or salt according to preference. *Garaetteok*, the most popular rice cake in Korea, is prepared by steaming and extruding [1]. It is very soft, sticky, pliable, and chewy. However, during storage, its texture quickly becomes hard because of starch retrogradation.

Starch retrogradation is a process of reassociation of gelatinized starch into an ordered structure and recovery of some crystalline features [2]. It is a critical factor in food processing and industrial applications. Various food properties, such as the type and amount of starch, the content and structure of amylose or amylopectin, moisture content, storage temperature, and additives affect starch retrogradation [3,4,5,6].

Starch retrogradation affects food quality, such as flavor, texture, and digestibility [2]. Therefore, there is considerable interest in methods that inhibit this process. For example, as one solution, some raw material may be replaced with chemically modified starch containing new functional groups [7]. As another example, starch structure is modified by treatment with hydrolytic and branching enzymes [8,9]. Most studies in the field consider the use of additives, such as polyphenols, sugar alcohols, oligosaccharides, hydrocolloids, and emulsifiers, and using resistant starches to retard starch retrogradation [10,11,12].

Microbial fermentation of vegetables and fruits increases the content of various ingredients, such as polyphenols and enzymes [13,14], in the fermented products. Hence, it is possible that fermented products could retard starch retrogradation. However, to date, few studies have focused on the effect of fermented extracts on starch retrogradation. It has been known that lettuce cultivated in Korea has various polyphenols [15], and fermented lettuce extract as a food additive

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increases the levels of total polyphenols in starchy food [16]. In addition, fermented lettuce extract might be used as potential food additives in rice cakes for diabetics due to its antidiabetic effect [16]. Therefore, in the current study, we investigated the effect of fermented lettuce extract on starch retrogradation in *garaetteok* using Rapid Visco Analyzer (RVA), differential scanning calorimetry (DSC), texture analysis, and scanning electron microscopy (SEM).

## 2. MATERIALS AND METHODS

### 2.1. Rice cake, garaetteok

In a preliminary study, we investigated the optimal concentration for starch retrogradation by comparing rice cakes treated with 0.1%, 0.5%, and 1.0% (w/w) of fermented lettuce extract (prepared as described in section 2.2). Using RVA, DSC, texture analysis, and SEM, we found that the effect of retardation of starch retrogradation in rice cakes containing 0.5% of fermented lettuce extract showed a tendency to be high compared with those of untreated rice cakes and rice cakes treated with other concentrations of fermented lettuce extract during storage (data not shown). The rice cake was prepared with fermented lettuce extract in January 2021 by adding 0.5% (w/w) of fermented lettuce extract to rice flour, which was milled via a wet method using a roll grinder after soaking 1 kg of rice (*Oryza sativa* L., Korea in 2019) in 1 kg water for 12 h. The mixture was steamed for 1 h in boiling water and the rice cake was obtained using a noodle maker with a round-shaped nozzle (25 mm in diameter). The size of the cakes was 25 mm (diameter) × 200 mm (length). After wiping the moisture from the surface, the rice cakes were stored at 4°C for 0, 3, 5, and 7 days.

### 2.2. Fermented lettuce extract

The fermented lettuce extract was provided by HumanEnos LLC (Wanju-gun, South Korea). It was produced as described previously [16], with modification. Briefly, fresh lettuce purchased from a local market was sterilized with ozonized water for 20 h, dried for 24 h, and ground with a cutting mill (KM tech, Icheon, South Korea). The material was sieved to obtain particle sizes less than 4 mm in diameter. *Bacillus subtilis* (KCTC 1201BP) was cultured on the ground lettuce mixed with distilled water in a 1:9 (w/v) ratio at 37°C for 15 ~ 25 days under aerobic conditions. After fermentation, the supernatant was separated from the solids using ultrafiltration, sterilized, and used as the fermented lettuce extract.

### 2.3. Rapid Visco Analyzer (RVA)

The pasting profile of rice flour was determined using RVA

4500 (PerkinElmer, Waltham, MA). Briefly, 1 g of rice flour was immersed in 10 g of water in an RVA canister and analyzed as per manufacturer's recommendations. The RVA temperature was increased from 45°C to 95°C, the temperature maintained for 19 min, and then cooled to 50°C, and the temperature maintained for 9 min. Trough viscosity (TV), peak viscosity (PV), breakdown (BD), and setback (SB) were determined from the RVA profile [2].

### 2.4. Differential scanning calorimetry (DSC)

Thermal characteristics of *garaetteok* were analyzed using a differential scanning calorimeter (DSC250, TA Instruments, Milford, MA). Samples were heated from 30°C to 80 °C at a heating rate of 5 °C/min, and the thermal curve obtained. The onset temperature, peak temperature, and enthalpy change were then calculated from the thermal curve [17<XREF>].

### 2.5. Texture analyzer

*Garaetteok* hardness was measured using a texture analyzer (TA.XT 2i-25, Stable Micro Systems, Godalming, United Kingdom). *Garaetteok* pieces (10 × 10 × 10 mm<sup>3</sup>) were placed on a sample plate. Data were acquired by compressing the sample twice with a probe (P/5, 5 mm) using a pre-test speed of 2.00 mm/s, test speed of 1.00 mm/s, post-test speed of 1.00 mm/s, and a distance of 2.00 mm of the original sample. The measurements were repeated five times to obtain an average value.

### 2.6. Scanning electron microscopy (SEM)

The microstructure of rice cake surface was evaluated via SEM (SU3800, Hitachi, Tokyo, Japan). After the sample was completely dried, it was coated with a gold thin film in a vacuum evaporator. The samples were observed using an acceleration voltage of 10 kV under 100× and 500× magnification.

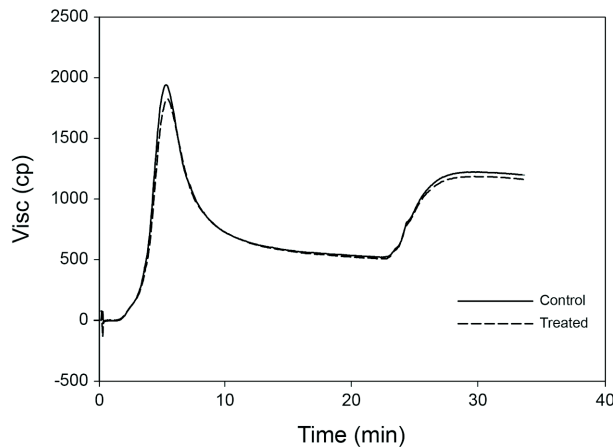
### 2.7. Statistical analysis

The data were analyzed using Student's *t*-test in STATISTICA (Version 7.1, StatSoft, Tulsa, OK). The significance threshold was set at 0.05. All experiments were performed in triplicate for each condition tested.

## 3. RESULTS AND DISCUSSION

### 3.1. Pasting profile of rice flour

The RVA profiles of the control and rice flour treated with fermented lettuce extract are shown in Fig. 1. The RVA parameters (TV, PV, BD, and SB) were different between the samples (Table 1). The BD and SB values of the control flour were



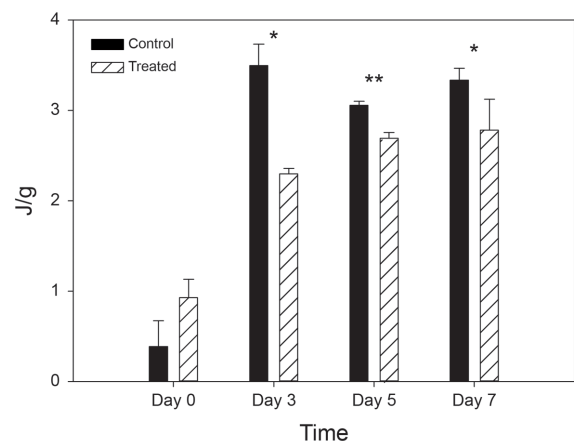
**Fig. 1.** RVA profiles of rice flour, either untreated (Control) or after treatment with 0.5% (w/w) fermented lettuce extract (Treated). The profiles are representative of three replicate measurements.

higher than those of the treated flour ( $p < 0.05$ ). The TV and PV values of treated flour showed a decreasing trend compared to those of control. BD, defined as the difference between peak viscosity and minimum viscosity during the holding phase, has been used as an indicator of starch pasting stability [18]. The BD value was 1419 cP for the control and 1316 cP for the treated flour, indicating that fermented lettuce treated-rice flour decreases the ability of starch granules to withstand resistance to heating and shear stress during food processing or cooking [19]. In addition, SB, defined as the difference between peak and final viscosity, is associated with amylose retrogradation or gelling ability. Lower SB values showed a lower tendency of starch retrogradation [20]. The SB value was 702 cP for the control and 677 cP for the treated flour. The higher SB value in

the control flour indicated faster retrogradation than that of the treated flour. These observations indicate that the rice flour treated with fermented lettuce extract retards the retrogradation of rice starch, thus potentially affecting the quality of food.

### 3.2. Starch retrogradation in garaetteok

DSC thermogram analysis is used to follow the melting endotherm of recrystallized amylopectin during starch retrogradation [21]. The gelatinization onset and peak temperatures, and gelatinization enthalpy of *garaetteok* depending on the pretreatment of rice flour with fermented lettuce extract and with storage at 4°C are shown in Table 2 and Fig. 2, accordingly.



**Fig. 2.** Enthalpy of rice cakes prepared from untreated flour (Control) or flour treated with 0.5% (w/w) fermented lettuce extract (Treated), after storage at 4°C for 0, 3, 5, and 7 days. Data are shown as the mean + standard deviation and are representative of three replicate measurements. Significant difference set as \*  $p < 0.05$  and \*\*  $p < 0.01$ .

**Table 1.** Trough viscosity (TV), peak viscosity (PV), breakdown (BD), and setback (SB) of rice flour<sup>1</sup>, untreated (Control) or after treatment with 0.5% (w/w) fermented lettuce extract (Treated), as determined by Rapid Visco Analyzer

	TV (cP)	PV (cP)	BD (cP)	SB (cP)
Control	544 ± 32	1987 ± 64	1442 ± 32 <sup>a</sup>	707 ± 7 <sup>a</sup>
Treated	504 ± 7	1835 ± 14	1331 ± 21 <sup>b</sup>	674 ± 4 <sup>b</sup>

<sup>1</sup> The data are averages from three replicates measurements and are shown as the mean ± standard deviation. Values followed by different superscript letters within the same column are significantly different ( $p < 0.05$ ).

**Table 2.** The onset and peak temperatures of rice cakes<sup>1</sup> made from untreated flour (Control) or flour treated with 0.5% (w/w) fermented lettuce extract (Treated), after storage at 4°C for 0, 3, 5, and 7 days

		Day 0	Day 3	Day 5	Day 7
Onset temperature (°C)	Control	36.26 ± 0.45 <sup>a</sup>	41.06 ± 0.20	40.43 ± 0.37 <sup>a</sup>	41.18 ± 0.30 <sup>a</sup>
	Treated	39.68 ± 2.09 <sup>b</sup>	40.63 ± 1.08	39.61 ± 0.51 <sup>b</sup>	39.23 ± 1.42 <sup>b</sup>
Peak temperature (°C)	Control	54.67 ± 6.74	53.93 ± 0.91 <sup>a</sup>	52.31 ± 0.50	53.20 ± 0.29 <sup>a</sup>
	Treated	53.39 ± 1.08	52.60 ± 1.06 <sup>b</sup>	53.13 ± 1.77	50.54 ± 0.50 <sup>b</sup>

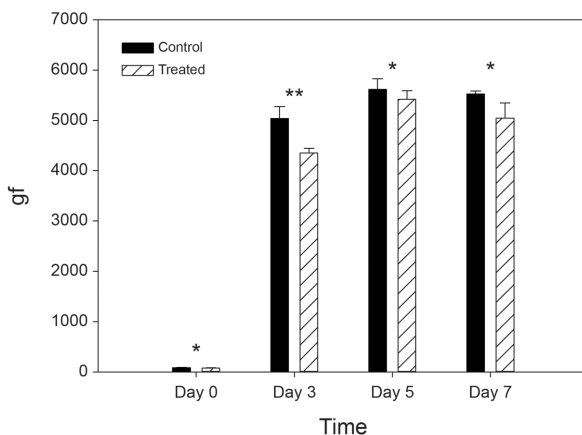
<sup>1</sup> The data are averages from three replicates measurements and are shown as the mean ± standard deviation. Values followed by different superscript letters within the same column are significantly different ( $p < 0.05$ ).

The onset temperature, a temperature of collapse of the starch microstructure [22], is affected by hydrogen bonding and starch structure, and indicates the degree of starch retrogradation related to the destruction of amylose complex [23,24]. The onset temperature of *garaetteok* exhibited a decreasing trend when the cake was prepared from flour pretreated with fermented lettuce extract compared to that of the control in each storage time, indicating that various components of the fermented extract reduced the moisture transfer and loss inside the rice cake. The peak temperature, a measurement of the melting temperature of starch crystallites, showed a similar tendency. The peak temperature of rice cakes prepared from flour treated with the fermented extract was lower than that of the control, revealing that the various components of the fermented extract reduced the starch chain interactions [25].

The enthalpy is driven by a collapse or cleavage of amorphous starch chains (e.g., in a rice cake) and provides the thermal properties for and irregular coagulation, hydrogen bonding between water and starch molecules, and the bonding between protein and starch [26]. The change in enthalpy increased from 0.38 to 3.33 J/g and from 0.93 to 2.78 J/g for rice cakes prepared from the control and treated flour, respectively, after 7 days of storage (Fig. 2), indicating that the fermented lettuce extract retarded the retrogradation of rice cakes. Most likely, micelles were not being formed because the various components of the fermented lettuce extract competitively combined with water and starch [23].

### 3.3. Hardness of *garaetteok*

Starch retrogradation refers to the crystallization or ordering of



**Fig. 3.** Hardness of rice cakes prepared from untreated flour (Control) or flour treated with 0.5% (w/w) fermented lettuce extract (Treated), after storage at 4°C for 0, 3, 5, and 7 days. Data are shown as the mean + standard deviation and are representative of five replicate measurements. Significant difference set as \* $p < 0.05$  and \*\* $p < 0.01$ .

gelatinized starch from an amorphous state. Hardness, one of the typical physical properties for measuring starch refrigeration, has been used a direct indicator associated with starch retrogradation. Generally, the hardness of rice cakes was increased by starch retrogradation during storage [27].

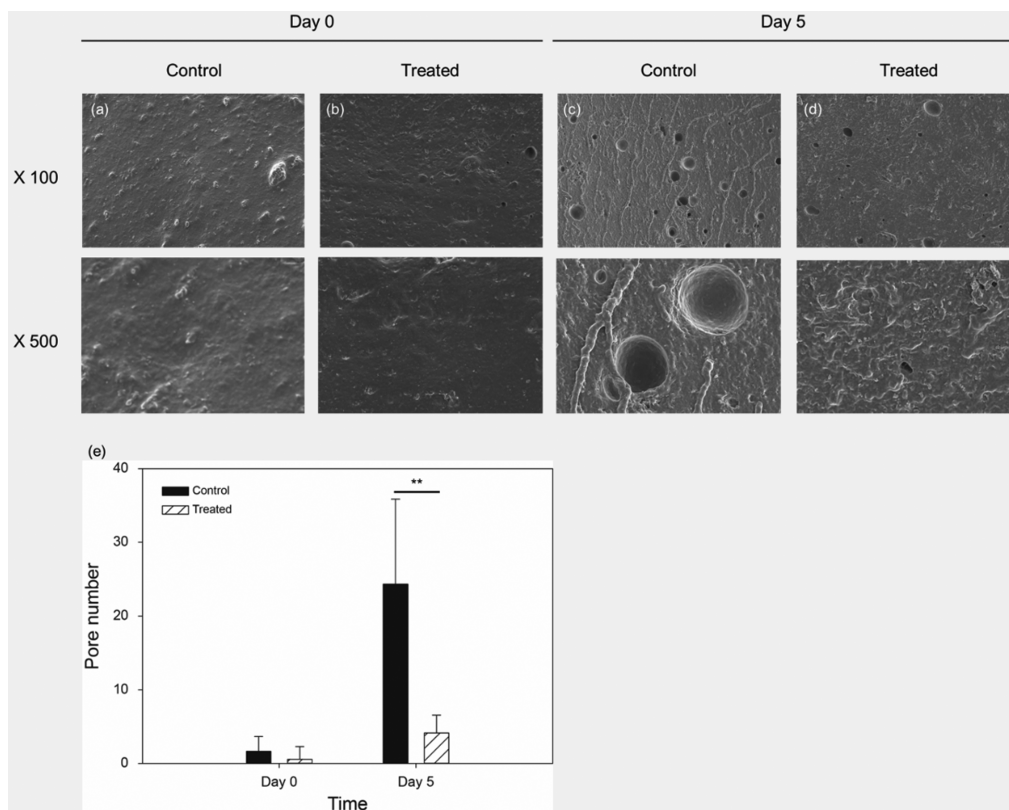
Changes in the hardness of *garaetteok* over time are shown in Fig. 3. The hardness of *garaetteok* prepared using untreated rice flour increased from 81 to 5521 gf during 7 days of storage, whereas that prepared with rice flour treated with fermented lettuce extract increased from 72 to 5045 gf. As the storage time increased, the hardness of all samples increased due to evaporation of moisture. However, the hardness of the untreated rice cakes was higher than that of the treated rice cakes during storage ( $p < 0.05$ ). These observations indicate that the fermented extract retards the retrogradation of rice cakes because of the binding of various components of the fermented extract to water or the presence of various enzymes in the fermented extract.

### 3.4. Morphological properties of *garaetteok*

SEM has been used for differentiating microstructures formed by the reassociation or recrystallization of dispersed starch molecules [2]. SEM images (magnification of 100 and 500 $\times$ ) of the rice cakes upon storage are shown in Fig. 4. On day 0, the microstructure of the control and treated-flour rice cakes was similar (Fig. 4(a) and Fig. 4(b)). However, major changes were apparent after 5 days of storage at 4°C (Fig. 4(c) and Fig. 4(d)). The formation of cracks and the number of pores on the rice cake surface in the control *garaetteok* were more visible and 2.3 times higher, respectively, than those in rice cakes prepared with flour treated with fermented lettuce extract (Fig. 4(e)), probably because of the loss of water capacity and reduced syneresis during storage [28,29]. In addition, the surface of control cakes was solid and compact, indicating high crystallinity [30]. The difference in structure is associated with hardness [31]. These observations indicate that the fermented lettuce extract retards the retrogradation of rice cakes.

## 4. CONCLUSION

In the current study, we investigated the retrogradation properties of rice cakes prepared with flour pretreated with fermented lettuce extract using RVA, DSC, texture analysis, and SEM. When fermented lettuce extract was added to the rice cakes, during storage, the TV, PV, BD, and SB values (as determined from the RVA profile); onset temperature, peak temperature, and the change in enthalpy (as determined via DSC); and hardness (as determined using the texture analyzer) were reduced



**Fig. 4.** Scanning electron micrographs of rice cakes prepared from untreated flour (Control) or flour treated with 0.5% (w/w) fermented lettuce extract (Treated). (a) Untreated-flour rice cake on day 0. (b) Treated-flour rice cake on day 0. (c) Untreated-flour rice cake after 5 days of storage at 4°C. (d) Treated-flour rice cake after 5 days of storage at 4°C. SEM images are shown at 100 and 500× magnifications. The images are representative of SEM replicates. (e) Quantification of the number of pores from the SEM images (100×) of rice cakes prepared from untreated flour (Control) or flour treated with 0.5% (w/w) fermented lettuce extract (Treated), after storage at 4°C for 0 and 5 days, respectively. Significant difference set as \*\*  $p < 0.01$ .

compared to the control. In addition, less cracks and pores were observed on the surface of the treated-flour rice cake than on the surface of the control (as determined via SEM). These observations showed that fermented lettuce extract inhibits starch retrogradation and can improve food quality. It can therefore be used as a potential additive in the food industry for retardation of starch retrogradation.

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