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Gelatinization and retrogradation characteristics of Korean rice cake in the presence of citric acid

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Abstract The effect of citric acid on rice starch gelatinization and low-temperature (4 ºC) storage was studied in order to produce rice cake with a lower retrogradation rate. A citric acid solution in the ratio of 0, 0.5, 1.0, and 1.5% (w/w) of the water used during production was utilized. The gelatinization properties, gel strength, thermal properties, and texture analysis were evaluated to determine the retrogradation rate. The result showed that acid hydrolysis occurred in samples treated with citric acid. Thus, increasing citric acid decreased gelatinization temperature (58.63±1.98 to 45.84±1.24 ºC). The moduli of elasticity increased with increasing citric acid concentration, indicating an increased gel strength. Thermal analysis of starch showed that the onset, peak, and conclusion temperatures of retrogradation were increased significantly with the storage period and decreased with citric acid concentration. After 72 h of low-temperature storage (4 ºC), the retrogradation rate was lowest in the rice cake with 1.5% citric acid solution, with an increased ratio of 12.01 to 13.60% compared to the control sample, with a ratio of 12.99 to 43.54%. This shows a high retrogradation rate in the control sample. Additionally, sensory properties and retrogradation ratio suggest that the addition of 1.0% citric acid solution during rice cake production is efficient in retarding the retrogradation without an adverse effect on the rice cake modeling and acceptance.

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Introduction

Rice is one of the world's most important food crops, with more than half of the world's population relying on it as their primary caloric source [1]. Many popular oriental foods, such as rice pasta, rice noodles, rice starch, and rice cakes, are rapidly growing home meal replacements (HMR) in the market space due to their ease of access and short re-cooking periods [2]. The physical properties of starch are influenced by the amylose/amylopectin ratio [3]. During gelatinization, the starch granules swell and form gel particles. In general, the swollen granules are enriched in amylopectin, while the linear amylose diffuses out of the swollen granules and makes up the continuous phase outside the granules [3]. Amylose has been proposed to act as a restraint to swelling [4]. Besides, the structure of starch gels is dependent on various factors, such as the structure of swollen starch granules, the amount of amylopectin and amylose leached out of granules, heating conditions, and added material properties, and the gel structure eventually influences the texture and the mechanical properties of starch gels [5].

Starch retrogradation, in addition to gelatinization, is an important physicochemical phenomenon in cooked rice or rice-cake products. The term retrogradation refers to the changes that occur in gelatinized starch upon cooling [5,6]. The cooling process after gelatinization implies fully reversible recrystallization of amylopectin and partially irreversible recrystallization of amylose. Thus, the structural changes are directly correlated to the changes in the texture and the mechanical properties of starch gels [7,8]. With retrogradation, the firmness or rigidity of starch gel increases significantly [9,10].

The retardation of retrogradation in starch has been studied using various methods, including hydroxypropylation and starch hydrolyzation [11-13]. This process involves modifying the starch structure to enhance its cooking properties, gelling ability, flow characteristics, and freeze-thaw stability or to achieve other objectives such as retarding retrogradation [14,15]. Among these methods, starch hydrolyzation by adding an acid such as citric acid is widely used for food starch because it increases paste viscosity and preserves paste properties and texture during cold storage [16-18]. Citric acid is a natural organic acid with a multi-carboxylic structure, which makes it useful as a starch crosslinking agent [19,20]. It is non-toxic, highly soluble, and has antioxidant and mild reaction properties. Citric acid can modify the composition, structure, and function of starch through hydrolysis and esterification. This largely depends on factors such as temperature, concentration, processing time, and the ratio of amylose to amylopectin in the starch [19,20]. It has been shown to affect the physical and chemical properties, paste texture, structure, thermal behavior, rheology, and digestibility of talipot palm starch, causing a decrease in relative crystallinity from 16.35 to 3.06% [19]. The acid increases the hydrophilic nature of the starch and weakens the bonds within the granules, allowing for easier gelatinization and improved stability of the starch paste or gel [21]. However, excess starch hydrolyzation in the presence of acid can negatively impact rice cake production by causing the dough to become too sticky due to excess moisture available for gelatinization, leading to the need for increased energy and time in molding or extruding it into the desired shape.

Therefore, the objective of this study was to evaluate the influence of citric acid concentration on the gelation and retrogradation retardation process of rice cake by examining the viscoelastic properties, thermal properties, and textural characteristics during steaming and at low-temperature storage (4 °C), respectively.

Materials and Method

Materials

Rice flour (*Oryza sativa*) with a moisture content of 16.38% was purchased from Nongshim Co., Ltd. (Seoul, Korea). The amylose to amylopectin ratio of Korean rice varieties typically ranged from 18.8 to 21% for amylose and 68.1 to 73.3% for amylopectin [21]. Other ingredients, such as salt and anhydrous citric acid were purchased from a local market (Chuncheon, Korea).

Preparation of rice cake

Rice flour (1000 g), salt of 1.5 g, and anhydrous citric acid dissolved proportionally in 600 mL of distilled water based on the desired concentration of a citric acid solution (0.5, 1.0, and 1.5% w/w) were used to prepare the rice cake with a final moisture content of 53.62% measured at the point of extrusion. The rice flour and salt were first dried-mixed for 1 min, followed by the addition of citric acid solution with a subsequent mixing for 15 min in a dough mixer (Model SZM-20, Xuzhong Machinery Co., Ltd., Guangzhou, China). The paste was then placed in an electric steaming machine (Model KRRSB 10W, Koresta, Seoul, Korea) for gelatinization at 80 °C for 40 min. Finally, the rice dough was cooled at room temperature for 10 min and then extruded using a lab-scale extrusion machine (Shandong Luerya Machinery Co., LTD., Shandong, China) to form a cylindrical rice cake measuring 20 mm in diameter. The extruded rice cake samples were collected from the extrusion machine and placed into a pan filled with 15 °C water for 5 min to cool, then vacuum-packed. All samples maintained the vacuum packing condition prior to all analyses. A control sample with pH 6.2 made from distilled water (600 mL) and rice flour (1000 g) was used as a standard for evaluation. The mixing ratio of citric acid and rice flour is shown in Table 1. The maximum concentration of citric acid, 1.5% (w/w), was determined by the preliminary studies to maintain the processing characteristics of rice flour.

Thermal properties of rice paste

The thermal properties of rice paste were examined using a differential scanning calorimeter (Discovery DSC, TA Instruments, New Castle, DE, USA) according to Hirashima et al. [12]. The rice paste was pre-weighed and placed into the DSC stainless-steel pan, hermetically sealed, and weighed to an accuracy of±0.01 mg. Another empty pan that was hermetically sealed was used as a reference pan during the analysis. The sample was heated over a temperature range of 25 to 80 °C at a heating rate of 10 °C/min in a standard DSC cell that was flushed with nitrogen at a rate of 50 mL·min^{-1} for all runs. From the thermal curves presented by the TA Instrument software (Trios, TA Instruments, New Castle, DE, USA), the onset temperature (T_O), peak temperature (T_P), conclusion temperature of amylopectin gelatinization (T_C), and gelatinization enthalpy (ΔH_G) could be obtained.

To investigate the retrogradation analysis of the rice cake

Table 1 Rice flour and citric acid mixing ratio

Sample	Citric acid concentration (w/w% distilled water)	Sample code name
(A) Rice flour	0	Control
(B) Rice flour + citric acid solution	0.5	RCA-0.5
	1.0	RCA-1.0
	1.5	RCA-1.5

during low-temperature (4 °C) storage for 72 h, a rice cake weighing 10 to 15 mg was placed into the DSC stainless pan, and the samples were heated over a temperature range of 25 to 180 °C at a heating rate of 10 °C/min. From the thermal curve, retrogradation enthalpy (ΔH_R) was obtained, and the retrogradation ratio was estimated using Eq. (1) [22]:

$$\text{Retrogradation ratio} = \frac{\Delta H_R}{\Delta H_G} \quad (1)$$

where ΔH_G is the gelatinization enthalpy of rice cake and ΔH_G is the enthalpy of reheated retrograded rice cake. All analysis was carried out in five replicates.

Viscoelastic properties of rice paste

The viscoelastic properties of the rice paste were measured using a dynamic rheometer (Discovery Hybrid Rheometer HR-3, TA Instruments, New Castle, DE, USA) with a flat geometry having a 40 mm diameter. The rice paste was placed in a 2 mm gap between the flat geometry and the Peltier plate at 25 °C. The perimeter was covered with a thin layer of silicone oil to prevent sample dehydration during the experiment. A stress sweep test was performed to determine the linear viscoelastic region (LVR) for the paste. Based on the result of the stress sweep, a frequency of 1 Hz and a strain of 0.4% within the LVR were selected and used in the temperature sweep test [12]. The sample temperature was regulated through a Peltier plate controlled by a cooling system (ThermoCube 220-500, Solid State Cooling systems, NY, USA). This system circulates water to maintain the desired temperature of the sample during the analysis process. Following an initial equilibration of samples for 2 min at 25 °C, the temperature ramp at 5 °C/min was conducted to an endpoint of 80 °C. The elastic modulus (G′) and viscous modulus (G″) were carried out in triplicate.

Texture properties of rice cake during low-temperature (4 °C) storage

The texture profile analysis of vacuum-packed rice cake stored at 4 °C and humidity of 65% for 72 h was performed using a texture analyzer (TA-XT Plus, Stable Microsystems Ltd., Surrey, UK). The analyzer was linked to a computer that recorded the data via a software program (Stable Micro Systems Software, Stable Microsystems Ltd.). A two-compression cycle at 60% strain was applied to rice cake with a diameter of 20 mm and a height of 15 mm. A cylindrical probe with a 25 mm diameter was used. The texture analyzer test speed was set at 1mm/sec. An average of at least 10 replicates was considered to determine the respective values of hardness, adhesiveness, and chewiness during a 72 h storage at 4 °C.

Sensory evaluation

Rice cake samples treated with citric acid aqueous solutions of different concentrations were evaluated for sensory properties following the method of Feng et al. [23]. The evaluation was conducted with a 15-member panel in the age range of 20 to 30 years and familiar with rice cake properties. Sensory parameters, including color, sourness, and hardness, were evaluated using a quantitative descriptive test focused on attribute scoring from 1 (low intensity) to 7 (high intensity). The sensory attributes consisted of color (lighter to darker), sourness (not sour to pronounced sour taste), and hardness (soft to hard). To determine the overall consumer acceptability of rice cakes, a seven-point hedonic scale (1 indicated extreme dislike, 4 indicated neither like nor dislike, and 7 indicated extreme like) was used. The size of samples offered for sensory tests was 20 mm in diameter and 30 mm in height. After each evaluation, the panelists rinsed their mouths with warm water and waited for at least 5 min before the next test.

Statistical analysis

All experiments were repeated at least three times. A one-factor ANOVA analysis with SPSS v19.0 (IBM Corp., Armonk, NY, USA) and the average differences were further evaluated by the Tukey test using a 95% confidence interval. Trends were considered significant when the means of compared sets differed at $p<0.05$ (Student's t-test).

Results

Effect of citric acid on gelatinization and gel strength of rice flour

The influence of citric acid on rice flour gelation temperature and enthalpy measured during thermal analysis are presented in Figure 1 and Table 2. As shown in Figure 1, the thermal characteristics of the rice flour indicated an endothermic reaction with an increasing enthalpy value as the citric acid concentration increased. The results showed that the onset temperature (T_O), peak temperature (T_P), and conclusion temperature (T_P) of gelatinization for rice paste treated with citric acid significantly decreased ($p<0.05$) while the gelatinization enthalpy (ΔH_G) increased with increasing citric acid concentration (Table 2). These results agree with those reported by Wang et al. [24], who reported that onset and peak gelatinization temperature of corn starch decreases significantly with an increase in hydrochloric acid concentration. The result for the temperature dependence gel strength of rice dough is presented in Figures 2a and 2b, as storage modulus (G′) and loss modulus (G″), respectively. The values of G′ were greater than those of G″ for all the samples examined, which showed that the rice dough has more elastic characteristics than the viscous state. The moduli value increased with an increase in citric acid concentration.

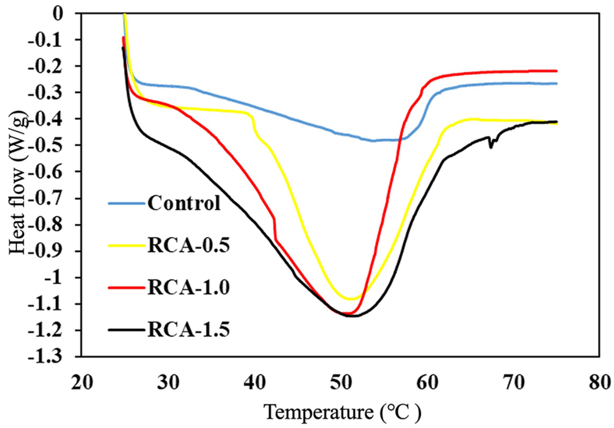

Fig. 1 Thermal curve of the mixture of rice flour and citric acid

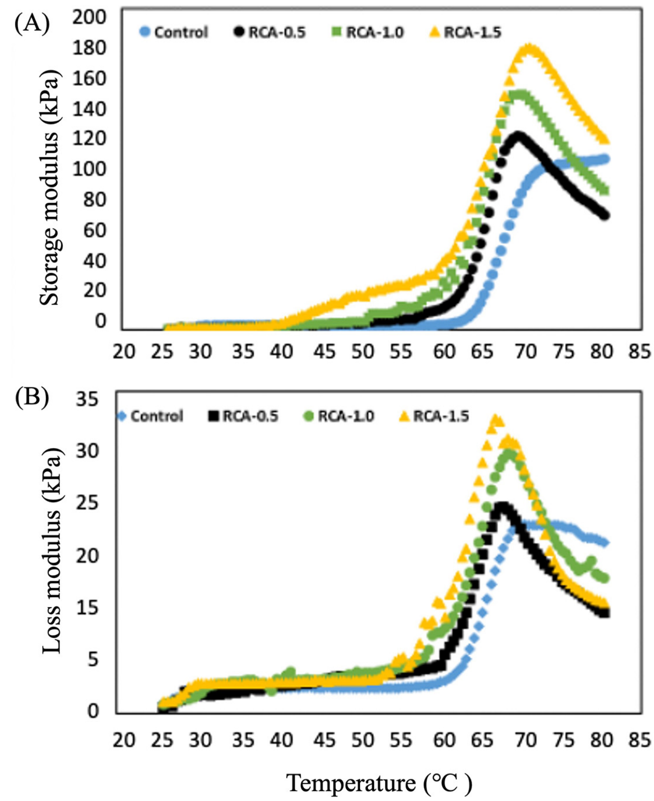

Fig. 2 Viscoelastic properties of rice paste as influenced by citric acid (A) Storage modulus (G′), and (B) Loss modulus (G″)

Texture properties of rice cake during 72 h storage

The changes in the texture properties of vacuum-packed rice cake stored at low-temperature (4 °C) and 65% humidity as influenced by citric acid concentration are presented in Table 3. From the data shown, the hardness of the rice cake increased significantly (p <0.05) for the control sample as the storage period increased (595.97±81.35 to 5226.01±569.07 g). However, for the rice cake samples treated with citric acid, the hardness ratio decreased as the citric acid concentration increased. Thus, RCA-1.5 did not experience any significant changes in hardness until after 48 hours of storage. On the other hand, the control sample showed a sharp rise in hardness value from the start of storage (0 h) to 48 h of storage. The adhesiveness and chewiness of the rice cake also dropped significantly with storage time. When compared based on the citric acid concentration ratio, both adhesiveness and chewiness increased as the concentration of citric acid increased.

Retrogradation of rice cake

The changes in retrogradation properties of rice cake samples (control, RCA-0.5, RCA-1.0, and RCA-1.5) during low-temperature (4 °C) storage were studied by analyzing the melting endotherm of recrystallized amylopectin by thermal analysis and results are presented in Table4. The enthalpies (ΔH) for melting of the control sample (1184.4±17.6 to 353.4±4.9 J/g) are lower than those of citric acid-treated samples at all storage periods, inferring that the starch molecules in the control sample are in a more orderly pattern [25,26]. As shown in Table 4, as the storage period increases, the enthalpy values decrease for all samples, with a greater decreasing ratio in the control sample. Therefore, from the initial storage period until till the 72 h of storage, the control sample had the highest ΔH reduction (1184.4±17.6 to 353.4±4.9 J/g), followed by RCA-0.5 (1407.1±14.2 to 997.7±8.1 J/g), RCA-1.0 (1539.1±14.7 to 1178.1±10.5 J/g) and RCA-1.5 (1592.8±13.6 to 1405.9±14.9 J/g). From this result, the onset, peak, and conclusion temperature decreased with increasing storage period. However, there were no significant differences in the onset temperature of the control and RCA-0.5 samples at every storage time until 72h, indicating that the modification of starch structure at a citric acid concentration level of 0.5% is not significant. This is in agreement with the report of Karlsson and Eliasson [27], who reported no

Table 2 Gelatinization parameters of rice paste treated with citric acid obtained from the thermal analysis

	Gelatinization parameters			
	T_O (°C)	T_P (°C)	T_C (°C)	ΔH_G (J/g)
Control	46.23±0.79^a	58.63±1.98^a	60.00±1.18^a	153.87±5.94^c
RCA-0.5	41.64±1.22^b	51.12±1.43^b	54.53±1.09^b	181.36±6.37^{ab}
RCA-1.0	33.78±1.35^c	47.29±1.67^c	50.01±1.39^c	189.75±4.88^a
RCA-1.5	31.69±1.82^c	45.84±1.24^c	49.43±1.01^c	191.21±5.34^a

Values are means ± SD with different superscripts (a-c within the same column are significantly different (p <0.05))

Table 3 Texture properties of rice cake during low-temperature (4 °C) storage for 72 h

Parameters	Storage period (h)	Sample			
		Control	RCA-0.5	RCA-1.0	RCA-1.5
Hardness (g)	0	595.97±81.35^{c}	493.15±63.23^{c}	377.28±46.78^{c}	293.37±34.57b^{c}
	24	2939.13±283.41^{b}	778.09±122.16^{b}	521.19±158.63^{c}	301.54±62.81b^{c}
	48	4574.82±402.75^{a}	1915.84±164.87^{a}	956.23±227.37^{b}	389.72±65.51^{b}
	72	5226.01±569.07^{a}	2251.49±334.46^{a}	1321.19±275.92^{a}	577.33±45.26^{a}
Adhesiveness (g)	0	352.27±54.12^{a}	543.34±73.01^{a}	635.09±84.41^{a}	689.76±88.52^{a}
	24	116.55±38.72^{b}	440.64±86.27^{a}	599.71±99.39^{a}	612.71±74.92^{a}
	48	32.39±11.97^{c}	212.07±36.68^{b}	381.81±59.15^{b}	471.56±99.39^{ab}
	72	18.51±7.68^{c}	77.77±41.64^{c}	173.13±38.47^{c}	259.79±42.12^{c}
Chewiness (g)	0	467.67±71.19^{d}	388.25±37.82^{c}	206.17±25.92^{d}	196.16±18.73^{d}
	24	2406.99±190.69^{c}	898.07±194.41^{b}	573.39±44.46^{c}	307.24±61.42^{c}
	48	3575.34±406.75^{b}	1404.28±266.47^{a}	915.58±72.69^{b}	683.79±17.73^{b}
	72	6855.75±890.31^{a}	1646.81±272.73^{a}	1174.96±61.12^{a}	941.63±53.31^{a}

Values are means ± SD with different superscripts (a-d within the same column of similar properties being significantly different (p <0.05)).

Table 4 Changes in thermal properties of rice cake under low-temperature (4 °C) storage condition

Storage period (h)	Sample	T_O (°C)	T_P (°C)	T_C (°C)	ΔH_R (J/g)	Retrogradation ratio (%)
0	Control	129.12±1.21^{a}	131.05±0.87^{a}	136.75±1.21^{a}	1184.4±17.6^{d}	12.99
	RCA-0.5	127.15±1.34^{a}	129.76±1.03^{b}	133.34±1.76^{b}	1407.1±14.2^{c}	12.89
	RCA-1.0	125.18±0.57^{b}	127.55±1.75^{b}	130.27±0.98^{c}	1539.1±14.7^{b}	12.33
	RCA-1.5	124.02±0.48^{c}	128.31±1.09^{b}	129.58±0.98^{c}	1592.8±13.6^{a}	12.01
24	Control	129.84±1.48^{a}	136.54±1.01^{a}	138.62±1.49^{a}	904.3±9.8^{d}	17.02
	RCA-0.5	128.36±0.98^{a}	131.47±2.14^{b}	135.12±1.51^{b}	1384.5±15.8^{c}	13.09
	RCA-1.0	125.91±1.09^{b}	128.06±0.78^{c}	130.34±1.17^{c}	1487.6±16.2^{b}	12.76
	RCA-1.5	125.26±1.45^{b}	128.27±1.15^{bc}	129.88±0.99^{d}	1558.3±13.8^{a}	12.27
48	Control	134.17±2.01^{a}	141.23±1.68^{a}	147.88±1.81^{a}	617.3±7.1^{d}	24.93
	RCA-0.5	131.42±1.55^{a}	134.89±1.98^{b}	137.91±1.83^{b}	1159.2±15.4^{c}	15.65
	RCA-1.0	128.14±1.63^{ab}	130.56±0.89^{c}	131.77±1.31^{c}	1335.9±12.1^{b}	14.20
	RCA-1.5	127.76±0.98^{b}	129.73±1.34^{c}	130.66±1.14^{c}	1497.3±12.4^{a}	12.77
72	Control	137.56±1.18^{a}	143.48±1.23^{a}	148.39±2.24^{a}	353.4±4.9^{d}	43.54
	RCA-0.5	132.38±1.68^{b}	136.54±1.39^{b}	141.49±1.34^{b}	997.7±8.1^{c}	18.18
	RCA-1.0	129.47±1.11^{c}	131.45±1.07^{c}	132.82±1.22^{c}	1178.1±10.5^{b}	16.10
	RCA-1.5	126.71±1.34^{d}	131.28±1.28^{c}	133.09±1.29^{c}	1405.9±14.9^{a}	13.60

Values are means ± SD with different superscripts (a-d within the same column for the same storage day are significantly different (p <0.05))

changes in the onset temperature of potato retrogradation with storage time. However, the peak and conclusion temperatures for the rice cake samples were significantly (p <0.05) different at all storage periods (Table 4).

Sensory evaluation

The sensory evaluation of freshly prepared rice cake treated with citric acid is presented in Table 5. Citric acid, due to its astringent sour taste influenced the flavor properties of the rice cake. Thus, rice cake sourness increased with increasing citric acid concentration, with sample RCA-1.5 having the sourest taste, and less preferred. However, the control sample and RCA-0.5 had no significant difference in the sourness and were equally liked. Consequently, in correlation to the texture and rheological properties measured, citric acid influences the hardness of the rice cakes by the modification of the starch molecules; thus, rice cake samples with citric acid had a soft texture. On the contrary, too soft rice cake sample resulted in increased sticky properties, which is not desired by evaluators. Therefore, increasing the citric acid concentration to 1.5% (RCA-1.5) resulted in a too-soft rice cake sample and was neither liked nor disliked. Furthermore, as shown in Table 5, citric acid had no significant influence on the sample color. Consequently, all samples had a high overall acceptability score (>4) except RCA-1.5, which scored below average due to its sourness and its increased sticky properties.

Table 5 Sensory evaluation scores for rice cake treated with citric acid

Sample	pH	Sourness	Hardness	Color	Overall acceptance
Control	6.20±0.01	6.9±0.2^{a}	6.0±0.3^{a}	6.4±0.2^{a}	6.2±0.4^{a}
RCA-0.5	5.87±0.01	6.5±0.3^{a}	6.5±0.2^{a}	6.2±0.1^{a}	6.3±0.3^{a}
RCA-1.0	4.64±0.01	4.8±0.4^{b}	5.3±0.2^{b}	6.1±0.2^{a}	5.6±0.2^{b}
RCA-1.5	3.91±0.01	3.9±0.4^{c}	4.1±0.2^{c}	6.2±0.2^{a}	2.9±0.3^{c}

Values are means ± SD with different superscripts (a-c within the same column are significantly different (p <0.05))

Discussion

Generally, starch granules possess a crystalline order that serves as the basic underlying factor that influences the functional properties, such that when the granules collapse or are modified, the crystalline order within the granules displays an irreversible change in the physical properties [28]. During the exposure of starch to sufficient moisture in the presence of heat, gelatinization begins from the hilum of the granules and then a rapid swelling of the periphery; thus, gelatinization occurs initially at the amorphous regions because hydrogen bonding is weekend at this area [29]. The decrease of the gelatinization temperature as the concentration of acid increased, shown in Table 2, could be a result of the changes in the molecular structure in the starch granule such as the extent of branching in the amylopectin chain, and reordering of the crystalline structure after acid hydrolysis [30]. In terms of the influence of the pH on the gelatinization mechanism, as shown in Table 5, studies have shown that the pH of some starch, including yam [31], corn, potato, and rice starch [31,32], does not influence the initial, peak, and final gelatinization temperature. Instead, variations in enthalpy were attributed to the corrosion of the amorphous regions of the granules by the acidic medium facilitated by heating that reveals the crystalline regions [32].

The reason for the insignificant difference in the enthalpy value and gelatinization temperature of RCA-1.0 and RCA-1.5 is not well established, but it is believed that a further increase in citric acid concentration beyond 1.0% has no significant effect on the modification of the rice starch. On the other hand, the control sample had a higher gelatinization completion temperature (60±1.18 ^{o}C) resulting from a high degree of crystallinity, which provided structural stability and makes the granule more resistant towards gelation [27].

The significant increase in the moduli of rice dough treated with citric acid might be attributed to the difference in the amount of branching in the amylopectin chain due to acid hydrolysis [30]. In relation to the gelatinization temperature assessed through thermal analysis, G' and G" steadily increased until reaching the gelatinization temperature, at which point there was a sudden spike in all samples, with a more significant increase observed in samples with higher citric acid content. The implication of this lies in the increasing sticky properties of rice dough. This is because citric acid forms a stronger hydrogen-bond interaction with the starch. Thus, its adhesive properties are increased [34]. This was observed during the extrusion and molding of the rice cake as rice dough got stuck in the extrusion machine for sample RCA-1.5 (result not shown). An interesting observation was also found in the G' and G" after heating the sample beyond 65 ^{o}C, where both moduli values decreased as the concentration of citric acid increased from 0.5 to 1.5%. However, the control sample did not show any significant changes in the moduli at this temperature level (65 ^{o}C). The variation observed in the samples treated with citric acid may be due to the citric acid weakening the structural integrity of the rice starch, causing it to lose stability when subjected to a certain level of shear stress. This is in line with previous findings on corn starch treated with citric acid, which showed that the addition of acid led to the hydrolysis of glucose chains [35].

Retrogradation is mainly a crystallization process. It arises because of the strong tendency for hydrogen-bond formation between hydroxyl groups on adjacent starch molecules [30]. Starch retrogradation consists of two processes: gelation of amylose solubilized during gelatinization and amylopectin recrystallization within the gelatinized granules, which takes place on cooling and during storage [35,36]. During storage, gelatinized starch molecules associate with an ordered structure. Since rice cakes used in this study contains very low amylose and very high amylopectin, amylopectin recrystallization occurs readily. Hence, the rice cakes showed high retrogradation enthalpy after storage at a low temperature (4 ^{o}C) especially occurring within 24-48 h of storage [2,11]. In addition, the lower enthalpies (ΔH) for melting observed in the control sample compared to samples treated with citric acid was due to the surface moisture generated by retrograded rice starch. Retrograded rice starch generates moisture on the surface, and, the energy is transferred easily in the presence of water, so less energy was required to melt the retrograded starch (especially in the control sample). The retrogradation ratio is calculated using Eq. (1). The citric acid solution had a positive correlation in retarding the rate of retrogradation, such that the RCA-1.5 sample had about four (4) times lesser retrogradation rate compared to the control sample. Furthermore, the retrogradation ratio decreased with increasing citric acid and storage periods (Table 4). Therefore, both the storage period and the citric acid concentration were factors influencing the retrogradation of rice cake.

The decline in the hardness characteristics of the control sample shows the influence of retrogradation taking effect in the rice cake, which was observed as an increase in hardness and reduction in adhesiveness. This has been reported to be rapid at low-temperature (4 °C) storage, especially during the initial 24 to 48 h, after which the increasing rate declines [2]. The slow-increasing ratio in the hardness of RCA-0.5, RCA-1.0, and RCA-1.5 was a result of the acid hydrolysis of starch, which caused the samples to remain soft during cold storage. This result was consistent with the report of von Borries-Medrano et al. [37], and it showed that citric acid inhibited the starch chain association by weakening the bonding formed between the starch molecules. Thus, the hardening of the rice cake can be effectively retarded using citric acid. For the RCA-0.5, RCA-1.0, and RCA-1.5, the initial hardness value decreased with increasing citric acid concentration. This is because acid hydrolysis causes modification to the starch molecule and, thus, exposes them for easy absorption of moisture and a quicker gelatinization during steaming based on the concentration of citric acid used. The adhesiveness indicates sample surface characteristics, and it depends on a combined effect of adhesive and cohesive forces [38]. Usually, the decrease in adhesiveness is one of the distinct features of starch gel during storage-induced retrogradation [2,11]. As presented in Table 3, all samples showed a reduction in adhesive properties during low-temperature (4 °C) storage. However, the samples treated with citric acid remained stickier than the control samples at the beginning and during storage periods. Furthermore, rice cake sample adhesiveness decreased significantly (p <0.05) with the storage period. The control sample indicated a higher decreasing value than in the RCA-0.5, RCA-1.0, and RCA-1.5. This correlates with the result of rice cake hardness and, thus, shows the ability of citric acid to retard the retrogradation in the samples. The chewiness property of the rice cake, which quantifies the stimulating energy required to masticate the sample to a steady state for swallowing, reduces with increasing citric acid concentration. Generally, all the properties measured (hardness, adhesiveness, and chewiness) are dependent on the level of retrogradation, and it decreases with an increase in citric acid concentration.

The present study demonstrated the influence of citric acid in retarding the rate of retrogradation in rice cake. The result of the gelatinization temperature suggests that acid hydrolysis of rice starch causes the changes in the molecular structure of starch granules; thus, acid-treated rice flour had a relatively low gelatinization temperature (45.84±1.24 °C) compared to the control sample (58.63±1.98 °C). These, however, gave rise to an increased storage modulus as the citric acid concentration increased. The implication of this was found in the increased sticky properties, which prevented easy extruding and molding of rice cake, as rice dough attaches to the extrusion rod. However, the result of the texture profile analysis indicated the slowest increasing ratio of hardness and decreasing ratio of adhesiveness in RCA-1.5 with the highest citric acid concentration. These two properties (i.e., hardness and adhesiveness) imply a reduction in the retrogradation rate. Furthermore, the enthalpy of melting of amylopectin in the thermal analysis showed the least value (353.4±4.9 J/g) in the control sample as against RCA-1.5 (1405.9±14.9 J/g) at the 72 h storage period at 4 °C due to the smallest requirement of amylopectin melting energy as retrogradation has fully taken place. Also, the retrogradation ratio measured showed that with 1.5% citric acid, it is possible to retard the retrogradation rate in rice cake by more than 4 times of the control sample. Based on the sensory analysis and retrogradation retardation by citric acid, RCA-1.0 is most preferred, with a high level of acceptance by consumers and a significant reduction in the rate of retrogradation without influencing the molding ability of the rice cake.

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Conflict of interest Hereby, all authors declare that there is no conflict of interest in this manuscript.

Ethical statement Ethical approval for the involvement of human subjects in this study was granted by Kangwon National University Ethics Committee, Reference number KWUIRB-2017-05-002-001, 2017/06/22.

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