國立臺灣海洋大學食品科學系碩士班 專題討論書面報告

A Comparative Study of Enzymatic and Chemical Pathways Strategies to Prevent Retrogradation of Rice Cake Product

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報告日期 : 2025 年 11 月 26 日

内容	時間控制	表達能力	投影片	書面資料
40%	10%	30%	10%	10%

指導老師	:	老師
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2025/11/26

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Abstract

The retrogradation phenomenon of starch is mainly a primary challenge in the production of rice cake (RC) as it is causing a rapid decrease in quality and reduces shelf-life period of the RC that leads to consumer acceptability decrease. This report compares the retrogradation retarding mechanisms of maltogenic α-amylase (MA), glycerol (GLY), and sucrose stearate (sucrose fatty acid ester; SE). MA was incorporated into the RC with concentration ranging from 1.5×10⁻³ U/g to 9.5×10⁻³ U/g in the rice flour. The optimal concentration was concluded in 4.0×10⁻³ U/g after account the sensory acceptability test. Long amylopectin chains were hydrolyzed by MA into shorter fragments (DP \leq 9) and leads to gelatinization enthalpy decrease, reduced setback viscosity, and maintaining the RC softness during the 7-days storage period at 4°C. When GLY (1%, 5%, and 10%) and SE (0.1%, 0.3%, and 0.5%) were added to the RC, the most pronounced inhibition observed at 5% GLY and 0.3-0.5% SE. GLY weakened the hydrogen bonding and delayed recrystallization, while SE formed starch-lipid complexes that were obvious with the Vtype crystal pattern that hindered the recrystallization of starch molecules. Both additives show the decrease in initial hardness and reduced amylopectin melting enthalpy. Overall, these agents were working with different mechanics in the molecular level and giving positive results of retard the retrogradation process success. It is also evident that the changes in the physical attribute are not always concurrent with the definition of retrogradation in the molecular level

1. Introduction

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Retrogradation is an phenomenon attributed to starch, a primary molecular component of rice that reassociates and recrystallizes over time (Reddy et al., 1994). Rice cakes (RC) and its varieties is a traditional food developed since ancient times and mainly consumed across Asian countries. Generally, RC were usually made of rice flour and water, usually mixed with other ingredients depending on the variety. Fresh RC generally has a sticky and soft texture; however, it tends to easily undergo the starch retrogradation process and makes the RC products as dry, dehydrated, and hard products. This affects the overall quality and shortens its shelf-life, especially during storage and commercial period (Guo et al., 2015). To prevent this, there are common methods used for anti-retrogradation for starch-based foods: moisture control, temperature control, and additives (Wang et al., 2015). In this report, additive addition was chosen as the method to inhibit starch retrogradation. From the broad choices of anti-retrogradation agent, maltogenic α-amylase (MA), sucrose stearate (sucrose fatty acid ester, SE), and glycerol (GLY) were chosen. The objective of this report is to compare different mechanism actions of different retrogradation inhibitor agents that are incorporated to RCs products to maintain the quality of products during storage. The main interest of these studies are the physical quality and molecular characteristics of the rice cakes. The digestibility and sensory evaluation were conducted for MA study to further evaluate the optimal concentration to overall end-product quality. The kinetics of retrogradation were conducted to explore the retrogradation phenomenon and understand the factors that directly influence the rate.

2. Effects of maltogenic α -amylase on physicochemical properties and edible quality of rice cake

This study aims to discover whether MA can modify rice flour that is described as a complex system during the fermentation process to improve the quality of RCs. RCs were made according to the traditional production method with four concentrations $(1.5 \times 10^{-3} \text{ U/g}, 4.0 \times 10^{-3} \text{ U/g}, 7.0 \times 10^{-3} \text{ U/g}, \text{ and } 9.5 \times 10^{-3} \text{ U/g MA})$ and one control were set for this study. RCs were stored for 7 days in plastic packaging in the refrigerator (4°C). The molecular weight distribution result was shown in Figure 1 and Table 1. The molecular weight of RCs after treatment didn't show any typical bimodal characteristic curve. Table 1 elaborates the data further where M_n and M_w of RCs show a downward trend except for 4.0×10^{-3} U/g. M_p value of RCs that were treated with 4.0×10^{-3} U/g and 9.5×10⁻³ U/g increased. The ratio of M_w/M_n and M_z/M_n did not change significantly suspected due to the difference in composition between rice flour and starch. The chain length distribution results were presented in Table 2. It shows the amylose content ranging from 27-29%. 9.5×10^{-3} U/g samples were the highest (29.7%) and the sample contains 28.1% amylose. This result showed that MA did not act on amylose significantly. Figure 2 shows that addition of MA leads to the increase in shorter chains with degree of polymerization (DP) ≤ 9 . The shorter chains cannot participate in the

formation of double-helix formation that leads to the disruption of the crystalline structure. Table 3 shows the DSC analysis data. The gelatinization temperature decreased as the concentration increased. The unlikeliness of the long side branch of amylopectin to from a stable double helix crystal that lowers the gelatinization temperature that might have be the reason of this result. The gelatinization enthalpy (ΔH) also decreases as the concentration increases, with a 9.5×10⁻³ U/g sample showing that the ΔH dropped to 0.84 J/gram. The drop in value of enthalpy is the sign that the retrogradation effectively inhibited. Pasting properties of RCs were presented in Figure 2 and Table 4, it can be concluded that the parameters measured were all significantly lower in the MA treated samples compared to the control sample. Lower setback value indicates a trend of retrogradation resistance (Suklaew et al., 2020). The *in vitro* analysis (Figure 3) also showed that the content of rapidly digestible starch (RDS) decreased and slowly digestible starch (SDS) increased. The decrease in RDS and decrease in SDS are indication that the MA reduced the digestion rate of RC and the amount of resistance starch remains unchanged.

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The texture of RCs were analyzed after storing in the refrigerator for 0, 1, 3, 5, and 7 days and the data is presented in Table 5. The hardness of the MA-treated RCs showed an insignificant increase and increase in chewiness value. This result indicates that MA plays a role in keeping and increases the quality of RC across the storing value. Maltooligosaccharide (G1-G5) production of RC were also analyzed (Figure 4). G1 and G2 production were decreased and increased across the MA treated RCs compared to the sample. Notable production of G1-G5 content can be seen in a significant increase in higher concentration of sample, G2 content increased with the degree of starch hydrolysis by MA. It was previously reported that the accumulation of maltose during lactic acid fermentation demonstrated a negative effect on the fermentation process (Okano et al., 2007). Fresh RC samples were given out for sensory assessment and scored based on the parameter on Table 6. Table 7 elaborates the data that have been gathered from the panelist. overall sensory acceptability for 1.5×10⁻³ U/g and 4.0×10^{-3} U/g samples were higher compared to the rest. 1.5×10^{-3} U/g sample was the best within the viscosity and chewiness category and 4.0×10^{-3} U/g sample was the best within the hardness category. Higher concentration of MA treated RC samples were described as 'increased in sweetness that destruct the balance between the sweet and sour flavor profile.' The sample from 9.5×10^{-3} U/g sample also showcased the collapse of the structure and it happened probably due to the inhibited fermentation due to increasing G2 production. This study concluded that the optimum addition of MA to this specific type of rice cake is 4.0×10^{-3} U/g after combining the technical and sensory analysis results.

3. Starch Retrogradation in Rice Cake: Influences of Sucrose Stearate and Glycerol

Korean rice cakes is a staple in South Korea and generally sold as fresh and generally cannot be sold after 24 hours of showcase storage at room temperature (Song

(RGLY) and SE added was 0.1%, 0.3%, and 0.5% (RSE). The rice cakes were packed in vacuum sealed packaging and stored under 25°C for 14 days. The hardness data of the rice cakes and results were presented in Figure 5. It can be seen that depending on the concentration of GLY and SE were incorporated, they can both reduce the firming rate of rice cakes up to five days before reaching the maximum hardness compared to the control group; alas addition of GLY and SE doesn't reduce the maximum hardness. GLY works by penetrating to the amorphous region and is able to reduce the firmness of starchy foods by blocking the aggregation of starch chains or providing mobility for molecules to move within the polymer structure (Baik & Chinachoti, 2002). The addition of SE shows the positive result in slowing down the firming rate. SE worked by contributing to the cross-linking effects helps to slow down the retrogradation in the long run (Meng et al., 2014). In Figure 6A, native rice flour showed a typical A-type crystal pattern, fresh RCs showed an overall amorphous pattern, and retrograded RCs showed typical B-type crystal pattern. Different intensity of peaks can be seen between the control and treated RCs, where the latter's peaks are relatively lower compared to the former and can be concluded that addition of GLY and SE reduced the crystallization of rice cakes. SE sample have a broad shoulder around 13°, which is a typical V-type crystal pattern and it's shown when starch molecule interacts with other molecules like fatty acid, forming a complex and alter the stable double helical structure of starch to single helix and hence retard the retrogradation (Putseys et al., 2010). The relative crystallinity of RCs is highly dependent on the concentration of additives added (Figure 6B, 6C). RCs with GLY (RGLY) overall have the relative crystallinity reduced immediately while RCs SE (RSE) showed an effect after 5 days of storage. Based on this result it can be an indication that retrogradation of rice cake in macro and micro level did not always happen at the same time. The Tg' of RGLYs are significantly lower than the control (-3.38°C). While the initial Tg' were lowered, it is inevitable to lower the Tg' during retrogradation, and RGLY10 even showing a dramatic increase in Tg' and suspected due to the anti-plasticizing effects (Figure 7A, 7D). SE addition didn't show any notable difference in T_g'. The control rice cake showed gradual decrease in ΔH_i, with the presence of GLY and SE in the system, the stabilization occurs after 5 days storage, demonstrating the ability of GLY and SE to slow down the retrogradation process (Figure 7B, 7E). ΔH_r of RCs are also decreased after addition of additives (Figure 7C, 7F). SE is able to adhere to the surface of the amylopectin molecules and interrupts the distribution of water and interaction with amylopectin side chains through hydrogen bonding. From a previous study, it is reported that 0.2% of SE effective to retard starch gels retrogradation (Katsuta et al., 2002). In this study, 0.1% of SE didn't effectively retard the retrogradation and suggested that a certain amount of emulsifier necessary to retard the starch retrogradation. Solid-state ¹H NMR transverse relaxation time (T₂) analysis shows that the values of all samples decreased with increasing

storage time (Figure 8). This strongly indicated that proton mobility in the rice cake changed from more mobile state to less mobile state. Though the decrease of T_2 is a common phenomenon and the initial value of T_2 was altered compared to the control sample, the addition of GLY and SE in this study did not retard the decrease of T_2 value across the storing period.

Avrami equation were used to investigate the retrogradation kinetics of starch. All of the data that have been gather will be substituted to the Avrami mathematical model (Avrami, 1939) and after inserting all of the data that has been obtained (Figure 9, Table 7), it can be seen that it is well suited for the basic retrogradation indicators (XRD and ΔH_r) where it yields a high coefficient of determination (R²) ranging from 0.94 to 0.99. Where hardness, T_g ', and ΔH_i value ranging from 0.81 to 0.96. T_2 value yields a much lower and wider range of R₂, ranging from 0.48 to 0.82. With such gap, R² value from T₂, hardness, T_g', and ΔH_i indicates that using Avrami equation is not suitable for describe the retrogradation process. To define accurately, empirical modelling is used to deduct the retrogradation kinetics of starch in complex starch systems (Lin et al., 2001). This equation used to describe the data by using the values of the independent variables to predict the values of the dependent variable. Figure 10 shows the R² value as a graph plot after the parameter value substituted to the exponential rise to maximum equation and the data value is shown in Table 8. All of the R² increased in all cases where the range of value starts from 0.85 to 0.99. With this, it can be said that exponential rise to the maximum equation can be an alternative to determine retrogradation kinetics when the Avrami equation is not suitable.

4. Conclusion

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It is evident that starch retrogradation in rice cakes can be effectively delayed through both enzymatic and physiochemical approaches. The MA study demonstrated that enzymatic hydrolysis plays a critical role in controlling starch retrogradation, by cleaving long amylopectin side branches into shorter ones and prevented double-helix formation and starch recrystallization. This resulted in lower gelatinization enthalpy, setback viscosity, and texture hardening after storage, ultimately maintaining a softer rice cake texture. The optimal concentration of 4.0×10⁻³ U/g provided the best balance between texture, storage stability, and sensory quality. In contrast, the GLY/SE study explores the physicochemical mechanism of retrogradation. GLY acted as a plasticizer that inserted its molecules between the starch chains, reducing hydrogen bonding, and increasing molecular mobility. SE is an emulsifier and able to form a lipid complex with starch molecules that physically blocks crystal formation and stabilizes the amorphous starch regions. Both of these agents reduced crystallinity and amylopectin melting enthalpy, leading to delayed hardening and improved shelf-life quality. Based on the data processed by mathematical model, it can be said retrogradation is not always happening at the same time with observable changes in the physical properties of the products. Retrogradation can be said as a complex phenomenon and cannot be said just because the changes are based on one single parameter alone.

GRAPHS AND TABLES

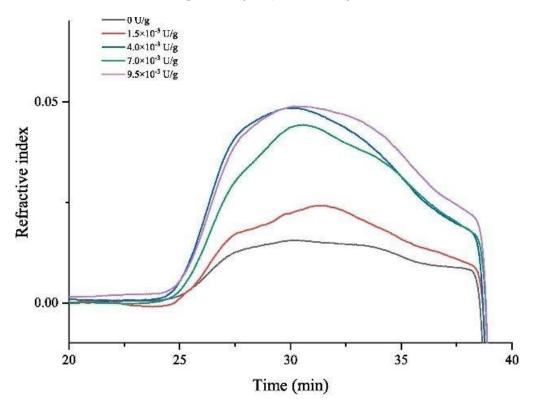


Figure 1 Molecular weight distribution profiles of RC treated with MA. The samples were treated with 0, 1.5×10 -3 U/g, 4.0×10 -3 U/g, 7.0×10 -3 U/g, and 9.5×10 -3 U/g MA.

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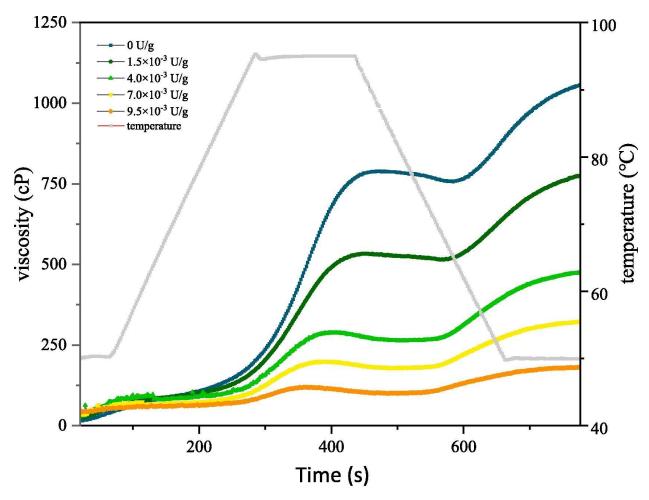
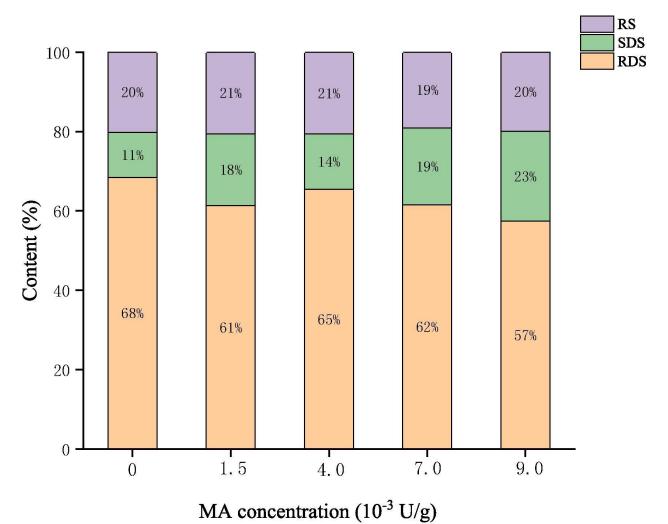


Figure 2 Pasting properties curves of RC treated with MA at dosage of 0, 1.5×10^{-3} U/g, 4.0×10^{-3} U/g, 7.0×10^{-3} U/g, 9.5×10^{-3} U/g, respectively.



MA concentration (10⁻³ U/g) Figure 3 The in vitro digestibility of RC treated with MA at dosage of 0, 1.5×10^{-3} U/g, 4.0×10^{-3} U/g, 7.0×10^{-3} U/g, 9.5×10^{-3} U/g, respectively.

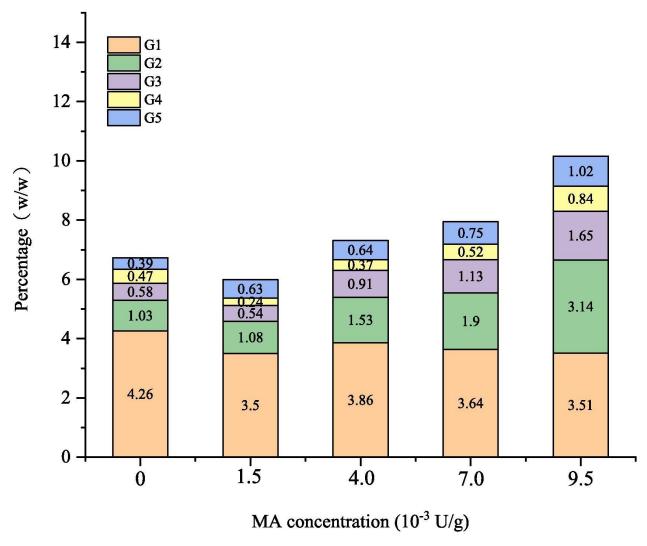


Figure 4 Distribution of oligosaccharides in RC treated with 0, 1.5×10^{-3} U/g, 4.0×10^{-3} U/g, 7.0×10^{-3} U/g, 9.5×10^{-3} U/g.

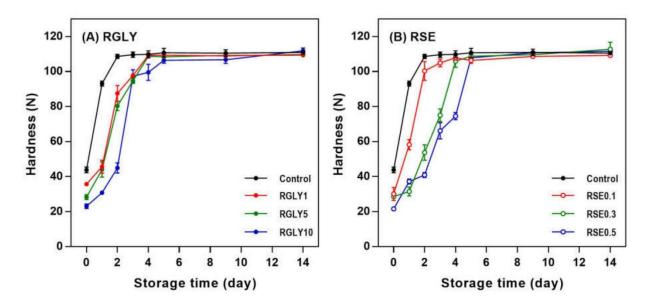


Figure 5 Changes in hardness of rice cakes with glycerol (A) or sucrose fatty acid ester (B) during retrogradation. RGLY: rice cakes with glycerol; RSE: rice cakes with sucrose fatty acid ester.

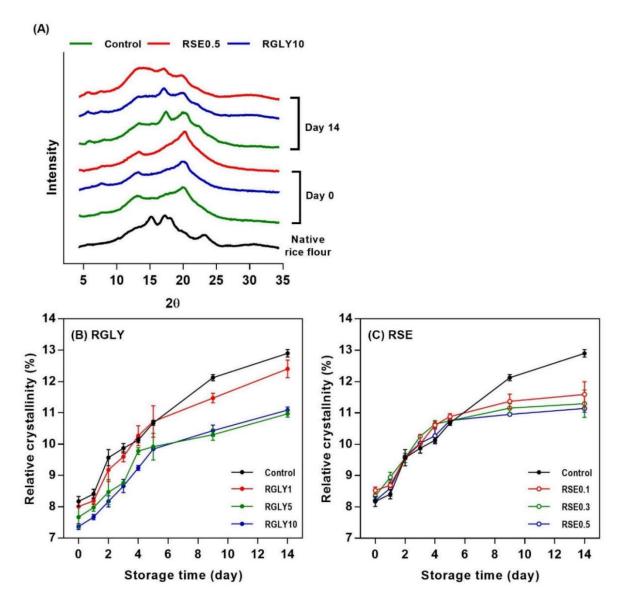


Figure 6 X-ray diffraction patterns of fresh and retrograded rice cakes (A), and changes in relative crystallinity of rice cakes with glycerol (B) and sucrose fatty acid ester (C) during retrogradation.

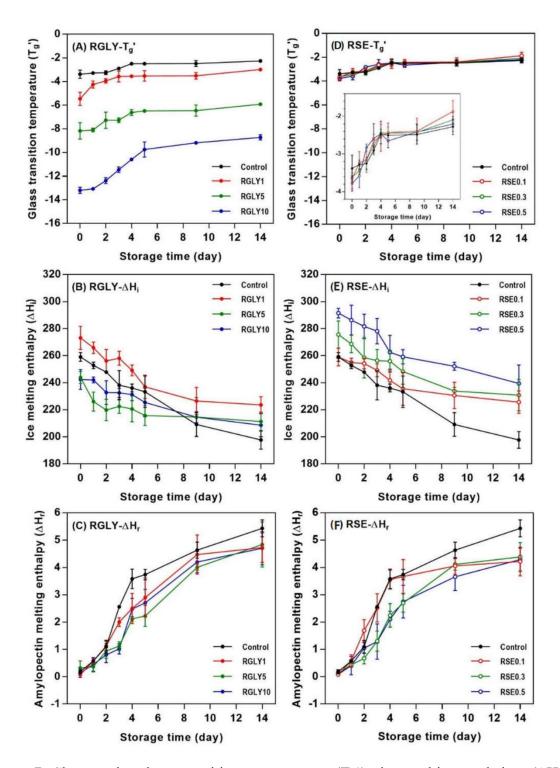


Figure 7 Changes in glass transition temperature (T_g) , ice melting enthalpy (ΔH_i) , and amylopectin melting enthalpy (ΔH_r) of rice cakes with glycerol (A–C) and sucrose fatty acid ester (D–F) during retrogradation, respectively.

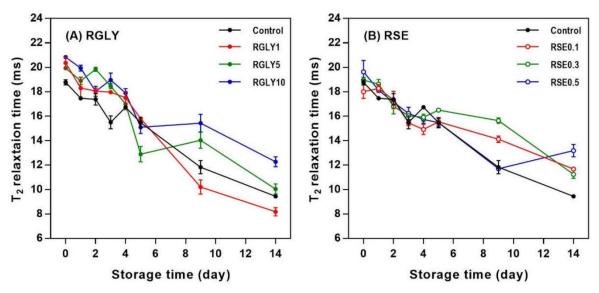


Figure 8 Change of 1H NMR transverse relaxation time (T₂) of rice cakes with glycerol (A) and sucrose fatty acid ester (B) during retrogradation.

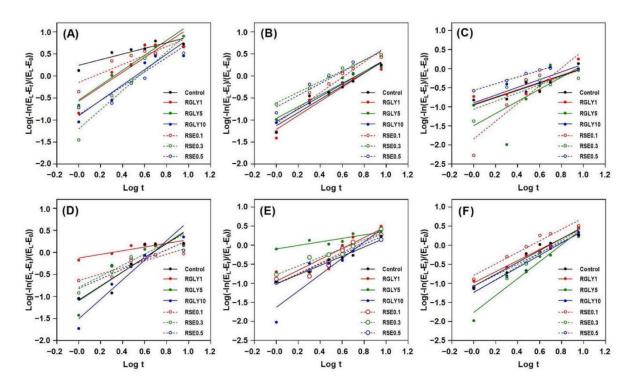


Figure 9 Retrogradation kinetics of rice cakes with glycerol or sucrose fatty acid ester using Avrami equation; hardness (A), relative crystallinity (B), 1H NMR transverse relaxation time (C), glass transition temperature (D), ice melting enthalpy (E) and amylopectin melting enthalpy (F).

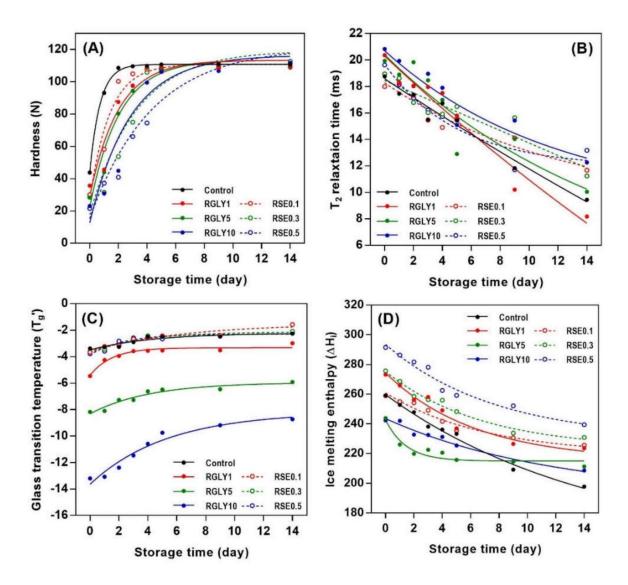


Figure 10 Retrogradation kinetics of rice cakes with glycerol or sucrose fatty acid ester using exponential rise to maximum equation; hardness (A), ¹H NMR transverse relaxation time (B), glass transition temperature (C), and ice melting enthalpy (D).

Table 1 Parameters of molecular weight distribution of samples treated with different concentrations of MA¹.

Samples (10 ⁻ ³ U/g)	Mn (10 ⁶ g/ mol)	Mw (10 ⁶ g/ mol)	Mp (10 ⁶ g/ mol)	Mw/Mn	Mz/Mn
control	$\begin{array}{l} 3.63 \pm \\ 0.10^{\mathrm{b}} \end{array}$	$4.64 \pm 0.13^{ m b}$	$\begin{array}{l} \textbf{4.12} \pm \\ \textbf{0.12}^{\textbf{b}} \end{array}$	$1.28 \pm \\0.01^a$	$1.84 \pm 0.02^{ m c}$
1.5	$\begin{array}{l} 3.50 \pm \\ 0.13^{c} \end{array}$	4.56 ± 0.08^{c}	$\begin{array}{c} \textbf{2.96} \pm \\ \textbf{0.04}^{\textbf{d}} \end{array}$	$\begin{array}{l} \textbf{1.30} \pm \\ \textbf{0.01}^{\text{a}} \end{array}$	2.19 ± 0.01^{a}
4.0	$\begin{array}{l} \textbf{4.07} \; \pm \\ \textbf{0.12}^{\text{a}} \end{array}$	5.15 ± 0.11^{a}	$\begin{array}{l} \textbf{4.43} \pm \\ \textbf{0.01}^{\text{a}} \end{array}$	$\begin{array}{l} 1.27 \pm \\ 0.03^a \end{array}$	$\begin{array}{l} \textbf{1.84} \pm \\ \textbf{0.04}^{c} \end{array}$
7.0	3.54 ± 0.02^{c}	$4.62 \pm 0.09^{ m b}$	$\begin{array}{l} 3.61 \pm \\ 0.03^{c} \end{array}$	$\begin{array}{l} 1.30 \pm \\ 0.01^a \end{array}$	$\begin{array}{l} \textbf{2.05} \pm \\ \textbf{0.02}^{\text{b}} \end{array}$
9.5	$\begin{array}{l} \textbf{3.59} \pm \\ \textbf{0.06}^{\text{b}} \end{array}$	$\begin{array}{l} \textbf{4.60} \pm \\ \textbf{0.01}^{bc} \end{array}$	$\begin{array}{l}\textbf{4.15} \pm \\ \textbf{0.01}^{\textbf{b}}\end{array}$	$\begin{array}{l} 1.28 \pm \\ 0.02^a \end{array}$	$\begin{array}{c} \textbf{1.85} \pm \\ \textbf{0.01}^{\text{c}} \end{array}$

Means \pm standard error values followed by different lowercase letters are significantly different (p < 0.05).

Table 2 Amylose content and chain length distribution of RC treated with different concentrations of MA^1 .

Enzyme	Amylose	Chain le	Chain length distribution (%)					
concentrations (10 ⁻³ U/g)	content (%)	DP < 6	DP 6-12	DP 13–24	DP 25-36	DP > 37		
Control	$\begin{array}{l} 28.09 \pm \\ 0.33^{b} \end{array}$	36.20 ± 0.43 ^e	21.40 ± 0.08 ^c	34.83 ± 0.34 ^a	6.37 ± 0.03 ^a	0.70 ± 0.01 ^b		
1.5	$\begin{array}{l} \textbf{27.64} \pm \\ \textbf{0.09}^{d} \end{array}$	40.08 ± 0.51 ^d	19.53 ± 0.15 ^d	34.36 ± 0.28 ^b	5.76 ± 0.01°	0.27 ± 0.05 ^c		
4.0	$\begin{array}{c} \textbf{27.77} \pm \\ \textbf{0.41}^c \end{array}$	$^{44.42}_{\pm}$	21.44 ± 0.04 ^b	29.00 \pm 0.19^{c}	5.20 ± 0.10 ^e	0.16 ± 0.01 ^d		
7.0	$\begin{array}{c} \textbf{27.02} \pm \\ \textbf{0.38}^e \end{array}$	43.58 ± 0.21 ^c	21.96 ± 0.13 ^a	28.63 ± 0.25^{d}	5.65 ± 0.08 ^d	0.17 ± 0.02^{d}		
9.5	$\begin{array}{l} 29.68 \pm \\ 0.12^a \end{array}$	$^{44.89}_{\pm}$ $^{0.26}$	19.47 ± 0.11 ^e	28.32 ± 0.31 ^e	$^{6.23}_{\pm}$ $^{0.11^{b}}$	1.06 ± 0.05 ^a		

 $^{^1}$ Means \pm standard error values followed by different lowercase letters are significantly different (p < 0.05).

Table 3 Gelatinization properties and retrogradation enthalpy of RC treated with MA1.

Enzyme concentrations (10 ⁻³ U/g)	Onset temperature (T _o)/°C	Peak temperature (T _p)/°C	Conclusion temperature (T _c)/°C	7d Enthalpy change (ΔH)/ (J/g)
control	45.45 \pm 0.09°	53.05 ± 0.05^{c}	63.26 ± 0.01^a	4.57 ± 0.08^{a}
1.5	47.25 ± 0.12^{a}	53.65 ± 0.01^a	62.42 ± 0.01^{b}	4.36 ± 0.01^{b}
4.0	45.87 ± 0.17^{b}	53.51 ± 0.07^{b}	60.83 ± 0.01^{c}	2.23 ± 0.02^{c}
7.0	$43.42 \pm 0.22^{\rm e}$	$51.31\pm0.01^{\mathrm{e}}$	60.25 ± 0.03^{d}	$\begin{array}{c} 1.26 \pm \\ 0.03^{\rm d} \end{array}$
9.5	$\begin{array}{l} 40.35 \pm \\ 0.08^d \end{array}$	52.39 ± 0.01^{d}	$60.16\pm0.01^{\mathrm{e}}$	0.84 ± 0.01^{e}

 $^{^1}$ Means \pm standard error values followed by different lowercase letters are significantly different (p < 0.05).

Table 4 Pasting properties of RC treated with MA¹.

Enzyme concentrations (10 ⁻³ U/g)	Peak viscosity (cP)	Trough viscosity (cP)	Final viscosity (cP)	Breakdown viscosity (cP)	Setback viscosity (cP)
Control	777.33	744.67	1054.67	32.67 \pm	310.00
	$\pm~10.41^{a}$	$\pm~11.59^{a}$	\pm 11.68 $^{\mathrm{a}}$	2.08^{a}	\pm 11.14 $^{\mathrm{a}}$
1.5	541.67	523.33	783.67	18.33 \pm	260.33
	\pm 32.87 $^{\mathrm{b}}$	$\pm~29.69^{ m b}$	\pm 32.87 $^{\mathrm{b}}$	3.21^{b}	$\pm~3.21^{ m b}$
4.0	276.67	257.67	462.00	19.00 \pm	204.33
	$\pm~10.26^{c}$	\pm 13.32 c	\pm 13.11 $^{\rm c}$	4.00 ^b	\pm 9.45 $^{\rm c}$
7.0	199.00	181.00	323.00	18.00 \pm	142.00
	$\pm~14.53^{ m d}$	$\pm~17.00^{ m d}$	\pm 7.55 $^{ m d}$	2.65 ^b	\pm 9.54 $^{ m d}$
9.5	118.00	103.33	185.33	17.00 \pm	82.00 \pm
	$\pm\ 16.52^e$	$\pm~8.50^{\rm e}$	$\pm\ 14.01^e$	1.41 ^b	6.08 ^e

 $^{^1}$ Means \pm standard error values followed by different lowercase letters are significantly different (p < 0.05).

Table 5 Texture properties of RC treated with MA¹.

Enzyme concentrations (10 ⁻³ U/g)	Days	Hardness/N	Springiness/mm	Chewiness/mJ	Resilience	Cohesiveness
control	0	875.060 ± 0.384^a	0.916 ± 0.016^a	568.542 ± 0.031^a	0.342 ± 0.007^a	0.721 ± 0.010^{a}
1.5		636.884 ± 0.601^{b}	0.866 ± 0.006^{b}	401.642 ± 0.354^{b}	0.356 ± 0.005^a	0.733 ± 0.023^a
4.0		$436.825 \pm 0.881^{\circ}$	0.867 ± 0.006^{b}	266.450 ± 0.298^{d}	$0.321 \pm 0.020^{\circ}$	0.703 ± 0.012^{a}
7.5		$475.191 \pm 0.259^{\circ}$	0.876 ± 0.001^{b}	280.737 ± 0.101^{c}	0.299 ± 0.011^{d}	0.675 ± 0.011^{b}
9.5		466.316 ± 0.361^{d}	0.869 ± 0.006^{b}	$255.473 \pm 0.330^{\circ}$	$0.214 \pm 0.004^{\circ}$	0.509 ± 0.010^{b}
control	1	3824.192 ± 0.245^a	0.815 ± 0.002^{b}	1756.622 ± 0.381^a	0.287 ± 0.003^{c}	$0.564 \pm 0.011^{\circ}$
1.5		1769.828 ± 0.477^{b}	0.729 ± 0.004^{d}	814.530 ± 0.217^{b}	0.323 ± 0.008^a	0.636 ± 0.026^{d}
4.0		762.812 ± 0.219^{c}	0.894 ± 0.002^a	457.367 ± 0.285^{c}	0.320 ± 0.001^{ab}	0.667 ± 0.011^{c}
7.0		$592.909 \pm 0.408^{\circ}$	0.788 ± 0.002^{c}	312.633 ± 0.252^{e}	0.314 ± 0.004^{b}	0.787 ± 0.021^{b}
9.5		633.212 ± 0.165^{d}	0.808 ± 0.008^{b}	334.463 ± 0.304^{d}	0.242 ± 0.008^{d}	0.807 ± 0.008^a
control	3	8535.808 ± 0.040^a	0.915 ± 0.012^a	1525.653 ± 0.260^{b}	0.254 ± 0.001^{a}	$0.460 \pm 0.009^{\circ}$
1.5		4708.418 ± 0.157^{b}	0.917 ± 0.002^{a}	2095.589 ± 0.245^{a}	0.233 ± 0.004^{b}	0.484 ± 0.015^{d}
4.0		1479.488 ± 0.129^{c}	0.905 ± 0.052^a	$674.742 \pm 1.066^{\circ}$	0.229 ± 0.007^{b}	0.524 ± 0.013^{c}
7.0		1092.442 ± 0.040^{d}	0.783 ± 0.002^{b}	465.708 ± 0.118^{d}	0.230 ± 0.006^{b}	0.547 ± 0.016^{b}
9.5		$878.472 \pm 0.051^{\circ}$	0.637 ± 0.002^{c}	307.691 ± 0.271^{e}	0.212 ± 0.013^{c}	0.552 ± 0.007^a
control	5	8535.808 ± 0.040^a	0.728 ± 0.012^{d}	1599.721 ± 0.087^{b}	0.103 ± 0.001^{e}	$0.198 \pm 0.021^{\circ}$
1.5		4708.418 ± 0.157^{b}	0.814 ± 0.006^{b}	2621.740 ± 0.235^{a}	0.276 ± 0.003^a	0.536 ± 0.018^{ab}
4.0		1479.488 ± 0.129^{c}	0.845 ± 0.005^{a}	815.446 ± 0.254^{c}	0.237 ± 0.006^{b}	0.557 ± 0.010^a
7.0		1092.442 ± 0.040^{d}	0.793 ± 0.007^{c}	565.552 ± 0.216^{d}	0.221 ± 0.002^{c}	0.545 ± 0.023^{ab}
9.5		$878.472 \pm 0.051^{\circ}$	0.664 ± 0.009^{e}	378.515 ± 0.319^{e}	0.195 ± 0.003^{d}	0.525 ± 0.025^{b}
control	7	10552.454 ± 0.239^a	$0.696 \pm 0.008^{\circ}$	4130.257 ± 0.495^{b}	0.133 ± 0.001^{e}	0.235 ± 0.042^{d}
1.5		6512.708 ± 0.118^{b}	0.943 ± 0.003^a	4209.483 ± 0.334^a	0.366 ± 0.003^a	0.687 ± 0.013^a
4.0		1807.580 ± 0.202^{c}	0.933 ± 0.014^{b}	$1060.515 \pm 0.280^{\circ}$	0.265 ± 0.004^{b}	0.629 ± 0.022^{b}
7.0		1420.622 ± 0.167^{d}	0.814 ± 0.004^{c}	680.656 ± 0.237^{d}	0.232 ± 0.002^{c}	0.592 ± 0.007^{b}
9.5		$1228.286 \pm 0.120^{\circ}$	0.744 ± 0.003^{d}	$499.480 \pm 0.236^{\circ}$	0.202 ± 0.002^{d}	0.549 ± 0.016^{c}

 $^{^{1}}$ Means \pm standard error values followed by different lowercase letters are significantly different (p < 0.05).

Table 6 Rice control sensory assessment standard control table.

Project	Score	Scoring criteria
Color	15	a. The color is white and bright (10 \sim 15); b. The color is white and slightly dark (4 \sim 9); c. The color is yellow and dark (\leq 3)
Flavor	20	a. Moderate sour and sweet, fragrant rice (15 \sim 20); b. Slightly sour or sweet, or lacking in aroma (10 \sim 14); c. Too sour or too sweet, no obvious aroma (\leq 9)
Hardness	30	a. Soft hard moderate (23 \sim 30); b. Slightly stiffer or softer (15 \sim 22); c. Too hard or too soft (\leq 14)
Viscosity	15	a. It is sticky and does not stick to the teeth (9 \sim 15); b. A little sticky (5 \sim 8); c. Viscosity is too large (\leq 4)
Chewiness	20	a. Chew for a moderate amount of time, with a chewy texture (15 \sim 20); b. Chew slightly longer, more chewy (10 \sim 14); c. Chew too long (\leq 9)

Table 7 The sensory evaluation of RC treated with MA.

Sensory	Score	MA concentration (10 ⁻³ U/g)						
indicators		control	1.5	4.0	7.0	9.5		
Color	15	$10.63 \pm \\ 0.35^{e}$	$\begin{array}{l} 9.88 \pm \\ 0.10^{\rm f} \end{array}$	9.75 ± 0.13^{e}	10.00 ± 0.21^{e}	9.13 ± 0.14^{e}		
Flavor	20	$\begin{array}{c} \textbf{14.00} \pm \\ \textbf{0.41}^c \end{array}$	$14.88 \\ \pm 0.21^{\rm c}$	14.63 ± 0.04^{c}	$13.13 \\ \pm 0.13^{c}$	$12.13 \\ \pm 0.16^{\rm c}$		
Hardness	30	$\begin{array}{l}\textbf{20.50} \pm \\ \textbf{0.24}^{b}\end{array}$	$\begin{matrix}22.13\\ \pm\ 0.27^b\end{matrix}$	$25.00 \pm 0.26^{\mathrm{b}}$	$\begin{matrix}23.25\\ \pm\ 0.17^{b}\end{matrix}$	$\begin{matrix}21.25\\ \pm \ 0.11^{b}\end{matrix}$		
stickiness	15	$\begin{array}{l} \textbf{9.25} \pm \\ \textbf{0.28}^{\text{f}} \end{array}$	$10.75 \\ \pm 0.29^e$	$\begin{array}{l} \textbf{7.63} \pm \\ \textbf{0.09}^{\text{f}} \end{array}$	$\begin{array}{l} \textbf{7.75} \pm \\ \textbf{0.06}^{\text{f}} \end{array}$	$\begin{array}{l} \textbf{5.25} \pm \\ \textbf{0.03}^{\text{f}} \end{array}$		
Chewiness	20	$\begin{array}{c} 12.50 \pm \\ 0.31^d \end{array}$	$\begin{array}{l} 14.63 \\ \pm \ 0.15^d \end{array}$	$\begin{array}{l} 12.25 \\ \pm \ 0.11^d \end{array}$	$\begin{array}{l} 12.13 \\ \pm \ 0.12^d \end{array}$	$\begin{array}{l} 10.50 \\ \pm \ 0.12^d \end{array}$		
Total score	100	66.88 ± 0.12^{a}	$72.25 \\ \pm 0.45^a$	$69.25 \\ \pm 0.29^a$	$66.25 \\ \pm 0.33^a$	$\begin{array}{l} 58.25 \\ \pm \ 0.43^a \end{array}$		

 $^{^{1}}$ Means \pm standard error values followed by different lowercase letters are significantly different (p < 0.05).

Table 8 Coefficient of determination (R²) of retrogradation kinetic analysis using Avrami and exponential rise to maximum equations.

		Coefficient of Determination (R ²)						
		Control	RGLY1	RGLY5	RGLY10	RES0.1	RSE0.3	RSE0.5
	Hardness	0.81	0.83	0.94	0.85	0.81	0.89	0.90
	XRD	0.94	0.93	0.96	0.99	0.94	0.98	0.94
Avrami	T _g '	0.83	0.83	0.81	0.94	0.85	0.87	0.82
Avidilli	ΔH_{i}	0.96	0.81	0.79	0.84	0.91	0.93	0.94
	ΔH_r	0.95	0.99	0.95	0.95	0.96	0.97	0.98
	T ₂	0.78	0.71	0.48	0.79	0.82	0.69	N.D.
	Hardness	0.99	0.93	0.95	0.88	0.95	0.91	0.92
Exponential rise to maximum	T ₂	0.96	0.95	0.85	0.90	0.92	0.86	0.92
	T _g '	0.89	0.95	0.94	0.95	0.92	0.94	0.92
	$\Delta H_{\rm i}$	0.98	0.96	0.94	0.97	0.96	0.98	0.96

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