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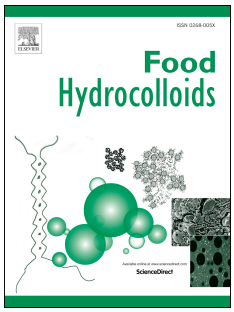

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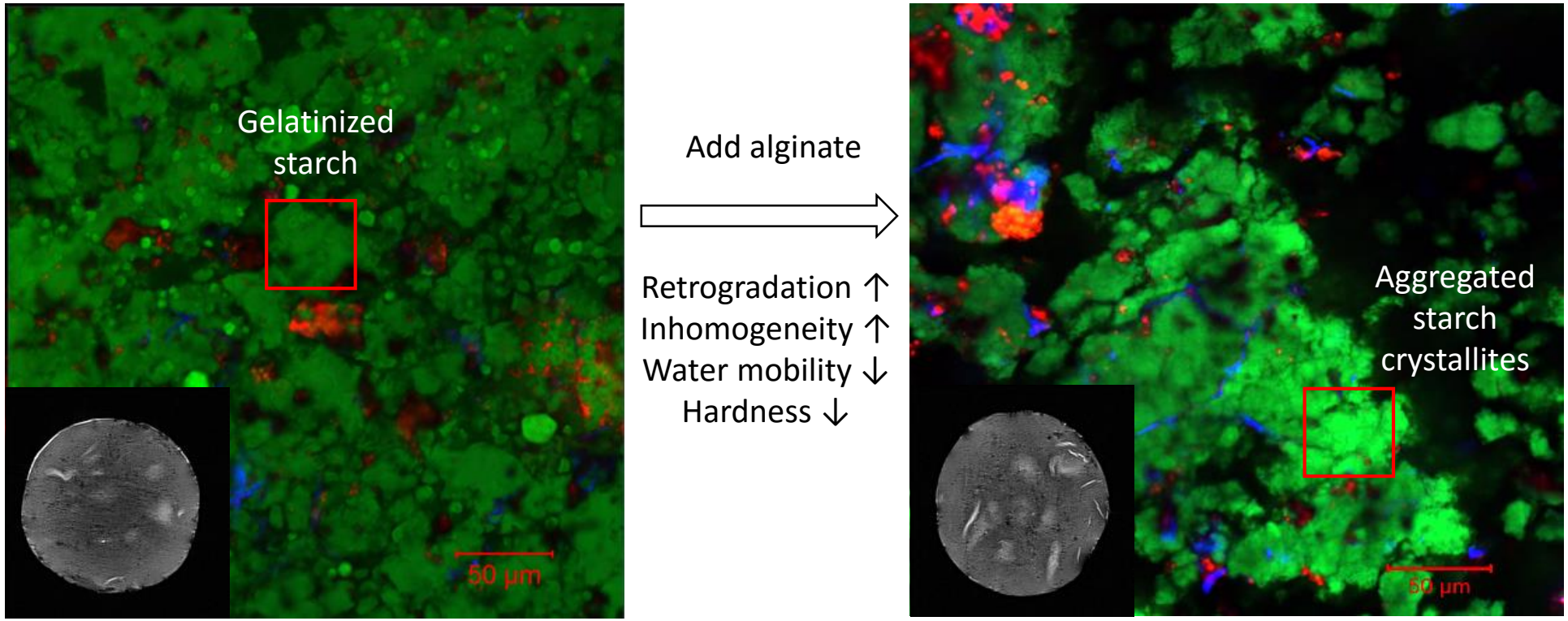
Anti-staling action of alginate in steamed rice cakes



Anti-staling of high-moisture starchy food: effect of hydrocolloids, emulsifiers and enzymes on mechanics of steamed-rice cakes

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Abstract

High-moisture rice snacks, such as steamed rice cakes, develop firmness on storage which decreases shelf life significantly. By analogy with lower moisture bread systems, this staling was hypothesised to be due to a combination of starch retrogradation and moisture re-distribution. Therefore, food additives which are commonly used to retard starch retrogradation during bread staling, including enzymes, hydrocolloids and emulsifiers (alpha-amylase, alginate, xanthan, guar gum, carrageenan, carboxymethyl cellulose, distilled monoglyceride, and sodium stearoyl lactylate) were investigated for their anti-hardening effects in high-moisture rice snacks. The results showed that only alginate significantly reduced the firming rate of rice cakes. However, differential scanning calorimetry measurements surprisingly indicated that rice cakes with alginate had higher levels of starch retrogradation than the control after storage for 7 days. Magnetic resonance imaging results were characterised by a redistribution of signal intensity from the edge to the centre of rice cakes and the formation of high intensity regions. These features were stronger with the addition of alginate. We propose that the alginate forms a continuous phase with water that has high mobility, whereas the partially gelatinized starch granules are an included phase distributed within the continuous phase. The reduced hardness of aged rice cakes with alginate is more dependent on the soft continuous phase than the hard starch granules, therefore leading to a softer texture. This mechanism is different to that proposed to operate for lower water content baked systems, therefore hydrocolloid and other anti-staling agents which are effective in bread systems may not be applicable in higher moisture starchy foods.

Key words: firming; high-moisture; water mobility; additives

1 Introduction

High-moisture starchy foods that contain 35% or greater moisture content or a water activity above 0.9 experience significant staling processes which result in hardened texture and off flavours that limit shelf life. The staling mechanisms that are widely studied in intermediate-moisture starchy foods are also thought to be responsible for the hardening of high-moisture foods, namely moisture loss and redistribution as well as starch retrogradation (Ji, Zhu, Zhou, & Qian, 2010; Lee*, et al.*, 2014).

Various anti-staling agents such as enzymes, hydrocolloids and emulsifiers have been applied successfully in intermediate-moisture foods, particularly breads, to control staling. The enzymes α-amylase and β-amylase have an anti-staling effect on bread by partially degrading starch and generating low-molecular-weight dextrins (De Stefanis, Ponte Jr, Chung, & Ruzza, 1977; Hebeda, Bowles, & Teague, 1991; Katina, Salmenkallio-Marttila, Partanen, Forssell, & Autio, 2006; Nguyen*, et al.*, 2015; Outtrup & Norman, 1984). The dextrins are thought to hinder gluten-starch interactions and interrupt the starch network (Goesaert, Leman, Bijttebier, & Delcour, 2009; Goesaert, Slade, Levine, & Delcour, 2009).

Hydrocolloids enhance water retention and limit its redistribution within starchy baked food structures (Sozer, Bruins, Dietzel, Franke, & Kokini, 2011), which provides an anti-staling effect. The type, source and molecular weight of hydrocolloids are all important factors that influence the anti-staling ability in different food products (Guarda, Rosell, Benedito, & Galotto, 2004; Gujral, Haros, & Rosell, 2004). Guarda*, et al.* (2004) reported that alginate and hydroxypropyl methyl cellulose (0.1% - 0.5%) showed wheat bread crumb hardening retardation and reduction of moisture loss while xanthan and carrageenan only reduced moisture loss.

Effective emulsifiers retard staling of intermediate-moisture bread by influencing the firming rate instead of simply reducing the initial hardness of bread (Knightly, 1977) which shortening is normally capable of. The interactions between emulsifiers and starch and/or protein are thought to be the reason for anti-staling effects (Gray & Bemiller, 2003). Emulsifiers form single helical V-type complexes with amylose, which may reduce starch swelling and amylose leaching from starch granules or prevent migration of water from gluten to facilitate starch crystallisation, further reducing hardness of bread (Dragsdorf, 1980; Zobel, 1973, 1988). This complex-forming ability relies on amylose structure, the type, pH, ion concentration of emulsifiers (Krog, 1973), and their physical state before adding to the dough, with aqueous, liquid-crystalline gels showing best results (Krog & Jensen, 1970). However, this may not be the only reason for the anti-staling effects of emulsifiers, as emulsifiers can complex with amylopectin as well, although at a lower level, to reduce

amylose-amylopectin network formation (Gudmundsson & Eliasson, 1990). Mikus, Hixon, and Rundle (1946) and Tang and Copeland (2007) suggested that monoglycerides form helical complexes of aggregated structure with amylose to decrease hardness caused by amylose retrogradation and amylose complexes with lipids and other components. In contrast to the many reported studies of anti-staling effects in breads and other intermediate moisture foods, there have been few comprehensive studies of anti-staling effects in high moisture foods. We hypothesised that anti-staling agents with proven effectiveness in breads would also have anti-staling properties in high moisture foods.

The research example of high-moisture starchy foods in this study, steamed rice cakes, are a traditional snack food in some Asian countries. Steamed rice cakes have a moisture content of 40%-65% and a water activity of around 0.92 (Eunhye Choi & Ko, 2014; Ji, Zhu, Qian, & Zhou, 2007; Sang, Shao, & Jin, 2015). A few studies have shown that additives such as tea polyphenols, oligosaccharides and polysaccharides (β-glucan, β-cyclodextrin, xanthan gum, carrageenan etc.) can reduce the rate and extent of retrogradation in rice starch (Banchathanakij & Suphantharika, 2009; Tang, Hong, Gu, Zhang, & Cai, 2013; Tian, *et al.*, 2009; Wu, Chen, Li, & Li, 2009). However, the mechanisms behind the retardation of starch retrogradation in these cases are not well characterised. The oligo- and poly-saccharides might function as hydrocolloids by holding water to control the amount of water available for inclusion in starch crystallites. Tea polyphenols may act as plasticizers to interact with starch by hydrogen bonding, and reduce self-entanglement of side chains of starch polymers (Wu, *et al.*, 2009). The hydrogen bonding depends on size, number and reactivity of OH groups in the polyphenol additives (Smits, Kruiskamp, Van Soest, & Vliegenthart, 2003). No work has been reported for ready-to-eat high-moisture rice products to investigate starch retrogradation retardation and anti-hardening effect of additives, or to reveal the potential mechanisms.

The aim of this study is to evaluate the anti-staling effect of several enzymes, hydrocolloids and emulsifiers on high-moisture steamed rice cakes. Uniaxial compression testing was used to determine the mechanical properties of rice cakes, particularly hardness, as a function of storage time. Factors thought to be closely related to hardness development were investigated with the goal of defining the anti-staling mechanisms: distribution of water (magnetic resonance imaging) and major components (confocal laser scanning microscopy); starch recrystallization (differential scanning calorimetry); and molecular mobility (magnetic resonance imaging).

2 Materials and Methods

2.1 Materials

Sticky rice flour and rice flour were obtained from Erawan marketing Co., Bangkok, Thailand. The amylose contents of the sticky rice flour and rice flour were 1.87 and 20.84%, respectively, measured using the size exclusion chromatography method of Fitzgerald*, et al.* (2009). The protein content of sticky rice flour and rice flour are 6.61% and 6.98%, respectively (from the manufacturer's nutrition information table). The total fat content of both flours is less than 1%. Sugar was obtained from Sugar Australia Pty Limited, Australia. Enzyme (fungal α-amylase with enzyme activity of min. 1000 units/g), hydrocolloids (guar, carboxymethyl cellulose (CMC), carrageenan, alginate (extracted from brown algae *Laminaria digitate* with M/G ratio of 1.5:1), and xanthan) and emulsifiers (sodium stearoyl lactylate (SSL), distilled monoglyceride (DM)) were provided by Danisco Australia Pty Ltd (Banksmeadow, Australia).

2.2 Preparation of steamed rice cake

Control steamed rice cakes were produced by mixing sticky rice flour, rice flour, sugar and water together in a ratio of 3:2:1:3.6 (w/w) using a bench mixer (GS-6118, Homemaker, Kmart Australia) at low speed for 1 min following a traditional recipe for steamed rice cakes in Asian countries. The dough was then shaped into 50 g balls and cooked in a steamer (RC-4700-A, Homemaker, Kmart Australia) for 15 min. After cooling down, each rice cake was packaged into a 12µm polyethylene terephthalate/120µm linear low density polyethylene stand up pouch (10010001, West's Packaging Services P/L, Australia) and sealed by a food packaging machine (Sous Vide 260, Australia Vacuum Packaging Machines Pty Ltd, Australia). Anti-staling additives including enzymes, hydrocolloids and emulsifiers were mixed with water first in concentrations as shown in Table 1, then the mixture of sticky rice flour, rice flour and sugar was added to the anti-staling additive-water system using the same ratio as control steamed rice cakes. The same mixing and shaping processes were performed. The two sets of doughs with amylase were covered with cling wraps (Cling Wrap, Berry Plastics Pty Ltd, Australia) to prevent moisture loss and left at room temperature for 0.5h and 1h, respectively, allowing time for amylase action on starch. All the samples were then steamed and packaged in the same way as control steamed rice cakes.

2.3 Properties of steamed rice cakes during storage

The packaged steamed rice cakes were stored at room temperature (22±1°C, tested using wireless thermocouples (Hitemp 140-FP-36, MadgeTech Inc., USA)) for 2, 5 and 7 days.

The hardness of all samples was measured on days 0, 2, 5 and 7 to screen for effective anti-staling additives in steamed rice cakes. The rice cakes with effective anti-staling additives and control rice cakes were further used to measure starch retrogradation, microstructure, and moisture content on days 0, 2, 5 and 7. Moisture distributions of rice cakes were measured on days 0 and 7.

2.3.1 Hardness

An Instron universal testing machine (5800, Illinois Tool Works Inc., USA) was used to measure the compression response of rice cakes. Bluehill2 software (Instron Corp., USA) was used to operate the experiment and record data. The centre of the rice cake samples was cut into a cylinder of 20 mm (diameter) × 20 mm (height) for instrumental texture measurement. The height of each cylindrical sample was measured by a 150mm digital Vernier Caliper (Work Zone, Australia) and recorded in the software. Then the sample was placed on an Instron compression cylindrical plate (T1223-1021) with a diameter of 50 mm. Another cylindrical compression plate (T1223-1021 with diameter of 50 mm) was placed directly above the sample and the gauge length was reset as 0mm. A 2000 N load cell was used to compress the sample until reaching the maximum load of 1500 N at a crosshead speed of 2 mm/s. The compressive stress and strain were recorded and graphed. The hardness was reported as the modulus in a linear region of compressive strain between 10% and 20%.

2.3.2 Starch retrogradation by differential scanning calorimetry (DSC)

Analyses were performed in a DSC Q2000 (TA Instruments, United States), using Tzero aluminium hermetic sample pans and lids (901683.901). 10 mg of rice cake was weighed by an ABT 120-5DM analytical balance (Kern & Sohn GmbH, Balingen, Germany) into Tzero aluminium sample pans. All the sample pans were hermetically sealed using a Tzero sample press and hermetic die kit (TA Instruments, United States). An empty sealed pan was used as a reference. Each sample was heated from 30 to 130 °C at a speed of 10 °C/min with nitrogen flow of 50 mL/min. The heat flow curves were recorded and TA Universal Analysis software was used to analyse the data. The temperature values obtained were for the onset (T_o), peak (T_p), and completion (T_c) of the endothermic transition. The enthalpy of transition was estimated from the integrated heat flow over the temperature range of the transition, and is expressed as joule per gram sample (J/g).

2.3.3 Microstructural properties of rice cake by confocal laser scanning microscopy (CLSM)

The micro-structural properties of rice cakes were investigated using confocal microscopy (LSM 700, Carls Zeiss, Germany). A triple-staining technique including the combination of Calcofluor White, FITC (1% w/v, ethanol) and Rhodamine B (0.1% v/w, ethanol) was used.

A very thin slice of rice cake (obtained by hand using a razor blade ~ 10 mg) was incubated with 1 ml of FITC for 48 hours under gentle shaking conditions followed by washing with water until the washed water was observed to be clear. The FITC stained cake was further incubated with 1 ml of Rhodamine B for 4 hours under shaking conditions followed by washing until the supernatant was clear. A small section of double stained cake was placed on a glass slide and infiltrated with Calcofluor white for 5 min. The excess of Calcofluor white was absorbed by a filter paper tip. The sample was observed after excitation at 405, 488 and 555 nm for Calcofluor White, FITC and Rhodamine B respectively. Normally, double-staining of mixed starch and protein systems with a mixture of Rhodamine B and FITC leads to staining of proteins by Rhodamine B and starch by FITC. However, both Rhodamine B and FITC can bind non-covalently with each of starch and protein, depending upon the concentration of the components in the system. In a low protein system, such as rice cake, and a high concentration of FITC (FITC 1% vs Rhodamine B 0.1%) the FITC binds with both starch and protein. In contrast, Rhodamine B binds more specifically with protein allowing the visualisation of both starch and protein simultaneously (Zheng, Stanley, Gidley, & Dhital, 2016). The use of Calcofluor White allows visualisation of the non-starch polymers, mainly the distribution of added hydrocolloid and rice cell wall material.

2.3.4 Moisture content

Moisture was determined by drying the sample to a constant weight in an oven at 105 °C for 24h. The difference in the weight of the sample before and after drying was used to calculate the moisture content (AOAC, 1990).

2.3.5 Water distribution by magnetic resonance imaging (MRI)

Rice cakes were imaged in a preclinical MRI system, comprising a 300mm bore 7T ClinScan (Bruker, Germany), running Siemens software version VB17. A 72 mm ID MRI rf coil was used to acquire the images. The rice cakes were placed on a ruler taped to the bottom of a rat-sized bed. The following images were acquired.

1. Localiser images: Three sets of orthogonal gradient echo images were acquired with the following parameters: repetition time (TR) = 41 msec, echo time (TE) = 4.43 msec, field of view 130 X 130 mm, slice thickness = 1 mm, number of slices in each direction = 5, matrix = 128 X 128, flip angle = 25°, bandwidth = 400 Hz/Px, averages = 1, concatenations = 3, total scan time = 16 sec.

2. T2 TSE images – Coronal T2 weighted spin echo images were obtained with the following parameters: TR = 2800 msec, TE = 49 msec, field of view 60 X 54 mm, matrix = 256 X 256, slice thickness 0.6 mm, number of slices = 19, averages = 1, bandwidth = 130 Hz/Px, turbo factor = 7, total scan time = 3 min 12 sec.

3. T1 parameter images were generated using the 3D_VIBE_t1mapit sequence using the following parameters: TR = 12msec, TE = 1.02 msec, field of view 60 X 55 X 60 mm, image matrix = 160 X 160 X 120, flip angle = 6°, and 33°, bandwidth = 400 Hz/Px, averages = 2, total scan time = 8 min 31 sec. A 3D T1 map was automatically generated following acquisition of the images.

4. T2 parameter images were generated using multi-echo T2 images acquired with the following parameters: TR = 2500 msec, TE = 10-120 ms in 10 msec increments, field of view 60 X 58 mm, matrix = 192 X 192, slice thickness 1.0 mm, number of slices = 20, averages = 2, bandwidth = 196 Hz/Px, total scan time = 15 min 34 sec. T2 parameter images were automatically generated following acquisition of the images.

2.4 Statistical analysis

All measurements were performed with at least 3 replicates. The mean values and standard deviations were reported. Microsoft Excel 2013 and R software (3.2.3) were used for statistical analysis.

3 Results and Discussion

3.1 Hardness

Staling of starchy foods is typically associated with increases in compressive hardness due to starch retrogradation and redistribution of moisture. Thus the hardness of the rice cakes was monitored to represent staling over time and to differentiate between successful and unsuccessful anti-staling interventions. Examples of the full range of compressive stress-strain curves of rice cakes are shown in Figure 1a. The stress-strain curves are divided into 4 regions: a region of partial contact because of the uneven surfaces of rice cakes; a linear region before breaking (Figure 1b) which was used to calculate the modulus to represent the hardness of the rice cakes; the breaking region; and a linear compressive region after breaking.

The compression modulus of all rice cakes, shown in Figure 2, increases significantly after 7 days of storage. All the concentrations of amylases added to rice cakes with both incubation times showed significantly higher hardness than the control (Figure 2a). In contrast to bread (Goesaert, Slade*, et al.*, 2009), amylase appears to be ineffective at preventing staling of high-moisture rice cakes. The mechanism of anti-staling effect of amylase in intermediate-moisture bread is complex and incompletely understood but is likely to involve effects on both starch retrogradation and consequent firming processes (Akers & Hoseney, 1994; León, Durán, & de Barber, 1997). Low-molecular-weight amylase products, such as branched dextrins, maltotriose or maltotetraose, are likely to reduce starch retrogradation as indicated

246 by a lower endotherm enthalpy on re-heating (Lin & Lineback, 1990; Schults, Schoonover,
247 Fisher, & Jackel, 1952). Conversely, amylase products with higher molecular weight might
248 show the opposite effect because the starch recrystallization processes underlying
249 retrogradation is promoted by the more mobile polymers produced by enzyme action (Akers,
250 *et al.*, 1994). In this study, the increased hardness of steamed rice cakes following amylase
251 treatment suggests that the effects on the starch network may have been caused by the
252 presence of staling-accelerating hydrolysis products in rice cakes.

253 Most concentrations of DM and SSL show significantly higher hardness than control across
254 the whole storage time (Figure 2a). Similar to amylase, staling retardation due to emulsifiers
255 is not observed in high-moisture rice cakes, which is opposite to the effect normally reported
256 in intermediate-moisture bread (Gomes-Ruffi, Cunha, Almeida, Chang, & Steel, 2012).

257 Most of the rice cakes with suitable amounts of hydrocolloid have a lower hardness
258 compared to the control after storage for 5 and 7 days (Figure 2b), presumably due to the
259 general water holding capacity of hydrocolloids. This is in line with Davidou, Le Meste,
260 Debever, and Bekaert (1996) where addition of 0.6% w/w of locust bean gum, 0.3% alginate
261 or xanthan in wheat bread, and Sim, Noor Aziah, and Cheng (2011) and Sim, Noor Aziah,
262 and Cheng (2015) where addition of 0.2% w/w flour of alginate or 0.8% w/w flour of konjac
263 glucomannan in Chinese steamed bread reduced hardness by reducing the dehydration rate
264 of samples, or influencing protein-starch interaction or macromolecular entanglement. The
265 statistical analysis shows that only the rice cakes with an addition of 0.3% w/w alginate show
266 significantly lower hardening rate ($p<0.05$), while the rest are not significantly different from
267 the control rice cakes. This suggests that alginate is a potential anti-staling agent for
268 application in high-moisture starchy foods such as steamed rice cakes, although the
269 mechanism needs to be uncovered for predictable application. One potential mechanism is
270 that, in high-moisture starchy foods, hydrocolloids might absorb more water which reduces
271 the amount of water available to be included in starch crystallites. Xanthan is reported to
272 have a greater water holding ability (Sánchez, Bartholomai, & Pilosof, 1995), but its anti-
273 staling effect on steamed rice cakes is less than alginate. However, water holding ability as
274 an isolated hydrocolloid may not be a good indicator of water holding in a high starch and
275 sugar environment as found in rice cakes. One possibility is that xanthan binds more to
276 (neutral) starch than the more highly charged alginate does, and therefore contributes less to
277 water-holding. Further work (e.g. using labelled xanthan to identify its location within rice
278 cakes) would be needed to test this possibility. In order to further investigate the
279 mechanism(s) by which alginate exerts its anti-staling effect, the enthalpy required for
280 melting retrograded amylopectin, and the rice cake moisture content were measured.

3.2 Starch retrogradation

Starch retrogradation has been found to be an important factor influencing the hardness of starchy food systems such as bread and cake (Katz, 1928; Maga & Ponte, 1975; Zhu, 2016). As the main component on a dry basis of steamed rice cakes, starch experiences structural changes both in the steaming process (gelatinization) and on storage (retrogradation) which greatly influence the texture. Therefore, the retrograded starch may play a major role in the staling mechanism. DSC tests were applied to control and 0.3% alginate-added rice cake samples to capture the heat flow curves and compare the levels of starch retrogradation.

A typical peak representing the melting of retrograded amylopectin was detected and the onset temperature, peak temperature, conclusion temperature, and enthalpy were recorded, as shown in Table 2. With increasing storage time, the width of the transition (T_c – T_o) increased and the peak shifted to a lower temperature in the control group, while the alginate group did not show this shift. This suggests that after long term storage, amylopectin in rice starch forms a broad range of imperfect and (for control) slightly more labile crystalline structures, consistent with the ageing process involving more of the starch in crystallisation rather than an annealing of the same crystallites into more perfect crystals. Rice cakes with 0.3% alginate show lower onset and peak temperatures than the control, suggesting that alginate influences the formation of crystalline structures by interacting with starch molecules and/or interacting with water to control the availability of water within starch networks.

The enthalpy values increase with storage time, consistent with increasing amounts of retrograded amylopectin. Control rice cakes have less retrograded amylopectin (smaller enthalpies) compared to rice cakes with 0.3% alginate at the same storage time (Table 2). Alginate is a macromolecule and is unlikely to be able to penetrate either intact starch granules as only molecules smaller than 1000 g/mol are able to penetrate starch granules (Lathe & Ruthven, 1956) or swollen granules (Appelqvist & Debet, 1997). In addition, alginate absorbs water first as it was pre-dissolved prior to mixing with dry flour. Due to competition with the alginate, less water would be expected to be available in this concentrated system for the starch granule swelling (Gonera & Cornillon, 2002; Ramírez*, et al.*, 2015). The more intact granules and ordered structure of starch in alginate-added rice cakes are proposed to result in larger DSC enthalpies than the control. However, from the compression test results (Figure 2), the rice cakes with addition of 0.3% alginate have a lower hardness than controls. This suggests that amylopectin retrogradation is not responsible for the firming of high-moisture rice cakes and that the anti-firming action of alginate has another origin. From phase separation theory (Appelqvist*, et al.*, 1997), alginate might form a continuous soft phase with water and leached amylose, which separates from a second phase made of stiffened starch granules. The balance of these two phases may

affect the hardness of steamed rice cakes. Possibly the soft alginate-water phase is dominant and yields the softer rice cake texture found in this study. To test this hypothesis, direct observation of the microstructure of rice cakes using microscopy can be used to examine if there are two phases. In addition, the softness of the continuous phase is likely related to the water behaviour for which MRI can be used to derive information on molecular mobility.

3.3 Microstructure

Since amylopectin retrogradation was not correlated with hardness development of high-moisture steamed rice cakes, the microstructure is of interest to reveal any difference between samples with and without 0.3% alginate. Figure 3 shows CLSM images of stained samples from control and alginate-containing rice cakes. Both groups showed similarities in changes during storage for 7 days. In fresh rice cake samples (day 0, Figure 3 (a1), (b1)), most of the starch molecules, stained green, formed a continuous phase as they were gelatinised. Bright green areas show that some starch granules are kept intact and aggregate with limited swelling (Figure 3c). With increasing storage time, starch granule aggregates are clearly observed and their size increases by day 7. This suggests that some phase separation is occurring, perhaps driven by starch molecules crystallizing and forming a more ordered structure compared to the fresh samples (as evidenced by DSC – Table 2). Proteins (stained red) are dispersed between starch granules apparently uniformly. Even though the two groups of samples show a similar overall behaviour, the control group appears to have less and smaller aggregates of residual partially-swollen granule structures compared to the alginate group. In addition, they tend to be more continuous and have a looser structure. This observation is consistent with the DSC results that the control group has a smaller enthalpy for retrograded amylopectin than the alginate group.

Calcofluor stained non-starch polymers blue, including cell walls and alginate in this study. The blue parts observed in Figure 3 (a1-3) and (b1-3) are believed to be the cell walls, as cell wall polysaccharides account for 2% of the dry weight of polished rice grains (Palmer*, et al.*, 2015), while the alginate content is only 0.3% of rice flour basis, which is less than cell wall polysaccharides. Alginate appears to be distributed uniformly in the inter-granular spaces of the rice cakes (in the background of Figure 3 (a1-3) and (b1-3)). This reduces the inter-particle contact, interrupting the continuity of the network of starch granules and weakening the composite network structure (Biliaderis, Arvanitoyannis, Izydorczyk, & Prokopowich, 1997). From these results, it is hypothesised that aged rice cakes with addition of alginate have a lower overall hardness because of a softer continuous alginate/water continuous phase despite containing harder swollen starch granules as an included phase.

3.4 Moisture content

The results of the present study (Figure 2) show that only 0.3% alginate addition in the rice cakes exhibited less firming than the control. As starch retrogradation is not positively correlated with the firming of aged rice cakes, it is possible that water content and/or distribution play an important role in the firming process. Therefore, the moisture contents of the control group and the alginate 0.3% group were tested, but the results (Figure 4) show no significant difference of moisture content between the two groups before or after different storage periods. Moisture loss can be an important factor influencing staling of starchy food products, however, in this study, moisture loss was prevented by sealed polyethylene packaging and was not responsible for rice cake staling. Even though the total moisture content of the rice cakes did not change significantly during storage, there may be a change of water mobility and moisture distribution. In studies related to bread staling, the moisture redistribution between components, particularly starch and protein, changes the physical structure of the molecules and their properties, and influences the staling process (Schiraldi & Fessas, 2000).

3.5 Moisture distribution

MRI was used to capture the moisture distribution and water mobility of rice cakes on day 0 (fresh) and day 7 (stale) to better understand the effect of water on staling. Three central images of the proton-density, T1 (longitudinal relaxation time) and T2 (spin-spin relaxation time)-weighted images of fresh (day 0) and stale (day 7) control and 0.3% alginate-added steamed rice cakes are shown in Figure 5. The MRI images (Figure 5) consist of dark areas representing air bubbles in the rice cake samples and an intermediate-intensity background representing the rice cake base. In the stale samples, high image intensity (bright) regions develop, consistent with areas of higher water density and/or mobility. Steamed rice cakes have a foam-like structure with an extensive number of fine pores, while the void areas might be filled with air and/or liquid. The porous structure of steamed rice cakes makes it hard to convert proton density to water content in local areas and calculate the precise T1 and T2 values for analysing the difference in water mobility between samples quantitatively. However, a difference between fresh and aged samples is observed in the proton density, T1 and T2-weighted MRI images with the appearance of bright areas in aged samples. The pixel intensity distribution in the proton density, T1 and T2 images are shown in Figure 6.

The proton density images of fresh control and fresh alginate added samples are very similar in that they both have higher signal intensity near the edges than in the centre, which suggests that the water distribution in fresh samples is not uniform. The porous structure of rice cakes leads to two possibilities. Firstly, the pores may not be evenly distributed, with an

outer layer containing a lower density of pores and/or smaller pore sizes below the image resolution compared to the central region. Secondly, the outer layer of pores may be filled with water to a greater extent than the inner pores. Water vapour transfers to the pores near the edges during the steaming process, but the short time (15min) and the hydrophilic nature of starch may limit diffusion of water from the surface into the centre. With increasing storage time, water tends to move to the centre as the whole system moves towards an equilibrium state.

This is different from water movement in intermediate-moisture bread which has a higher initial moisture content in the internal crumb (25%-35%) than the external crust (15%-20%) (Czuchajowska & Pomeranz, 1989; Mandala, Karabela, & Kostaropoulos, 2007) due to water evaporation during baking. During equilibration, water moves from the crumb to the crust and evaporates from the crust to the surrounding air (Maga*, et al.*, 1975), i.e. the opposite direction to steamed rice cakes. The cling wrap covering of the rice cakes in this study would minimize evaporation, and enhance water re-distribution in the rice cakes. This is likely to be one of the main reasons for the apparently different mechanisms of staling in intermediate- and high-moisture starchy foods.

Both fresh and aged alginate-added samples have brighter centres than the control and water movement is more pronounced in control samples, which may be due to the water holding ability of alginate. Fresh and aged samples can be differentiated as a few areas with high proton density are observed in both aged samples (Figure 5a). These areas might be composed of gel structures with limited porosity. Generally, the pixel intensity in the centre of the stale rice cakes displayed higher maximum intensity and greater variation in the pixel intensity, than the fresh samples after 7 days' storage (Figure 6a). These bright areas are consistent with regions of higher water content and reduced air bubbles. This may occur as air bubbles are filled with water expelled from starch structure in the rice cakes. However, the alginate added samples have more bright areas than the control, consistent with comparatively more water binding with the alginate. Morris (1990) suggested that water can be lost from a starch-rich phase due to the molecular associations between starch chains as occurs during retrogradation. The DSC results show that rice cakes with alginate added have more retrograded starch than the control, which may expel more water and result in brighter areas (more inhomogeneity) in the alginate group. There is also experimental evidence that some types of hydrocolloids work as a coating agent to reduce interaction of starch molecules and retard syneresis (Charoenrein, Tatirat, Rengsutthi, & Thongngam, 2011; Ferrero, Martino, & Zaritzky, 1994; Hahm & Kuei, 2015; Lee, Baek, Cha, Park, & Lim, 2002). Shi and BeMiller (2002) suggested that this effect was likely due to interactions between certain leached molecules, primarily between amylose and certain gums. In this

study, alginate neither reduced starch retrogradation, nor inhibited syneresis, so this mechanism does not seem to apply.

T1-weighted images (Figure 5b) of both fresh control and fresh alginate-added samples have greater intensity towards the surface than in the centre, with water distribution moving towards a more homogeneous state after 1 week's storage, similar to that observed in proton density images. The T1 images also showed higher pixel intensity and greater variation in the pixel intensity following 7 days of storage. The reduced variation in the profiles in the stale samples is consistent with the high intensity regions not being as prominent in the T1 images. This suggests similar T1 times for the water distributed throughout the rice cake and in the regions of high intensity. Figure 6b shows that the intensity of fresh alginate added samples are slightly higher than that of the fresh control, which may indicate a general shortening of T1 or different porosity caused by the alginate. In this study, alginate was first added to water, followed by the mixture of rice flours and sugar, thus less water is available for binding with starch in alginate-added samples. This is consistent with a reduced overall water mobility / porosity after addition of alginate. The intensity difference between the surface and the centre is more pronounced in the control, suggesting that an alginate-water phase facilitates moisture diffusion into the rice cake. This is consistent with the presence of alginate increasing the phase volume of the extra-granular phase by reducing the amount of water associated with swollen starch granules. The slower hardness development in alginate-added samples might therefore result from a continuous phase with alginate which remains soft with less reduction of water mobility.

Bread staling has been studied extensively, however, the movement of water between components at the molecular level is still not clear. In bread staling research, water transportation from starch to gluten and from gluten to starch have both been hypothesized based on instrumental measurements (Bachrach & Briggs, 1947; Cluskey, Taylor, & Senti, 1959; Eliasson, 1983; Ribotta & Le Bail, 2007; Senti & Dimler, 1960). However, steamed rice cake has a very different composition to wheat bread, particularly more water, less protein and fat, and no gluten, which makes the mechanisms of staling behaviour different. With no gluten and less protein content, water might be more mobilized and involved in the starch network to reduce crystal formation and starch retrogradation, as a smaller DSC enthalpy (1.35 J/g) was observed in the control samples after 7 days' storage than was reported for bread (more than 3 J/g) (Baik & Chinachoti, 2000; Rogers, Zeleznak, Lai, & Hoseney, 1988).

T2-weighted images (Figure 5c) of both control and alginate-added samples have a bright layer on the surface, indicating that during steaming free water diffuses into the pores in this layer or there is a thin layer of water between the packaging and the rice cake which

provides the high image intensity. The contrast between the background rice cake and the regions of high intensity in the stale cakes was greatest in the T2 images, in particular the alginate stale sample (blue dashed line, Figure 6c). After 1 week's storage, there was a general increase in the image intensity at the centre of both samples (Figure 6c). This is consistent with water diffusing from the surface to the centre resulting in a more homogeneous water distribution. In addition, the aged samples have distinct bright areas, more obvious in alginate-added samples (Figure 6c), similar to what was found in the proton density images. The bright areas are consistent with longer T2 values and/or lower porosity. The higher intensity regions may result from areas with lower porosity, pores filled with water or free water filling cracks in the rice cakes. The aggregated bright areas possibly represent a liquid phase with high water mobility, similar to the hypothesis of a continuous aqueous phase leading to soft texture of fresh rice cakes. For wheat bread samples, T2 measurements suggest three distinct ranges: 8-14µs, 280-360µs, and 2000-3000µs (Chen, Long, Ruan, & Labuza, 1997). These ranges are proposed to represent water (8-14µs) strongly associated with other molecules by hydrogen bonding, particularly macromolecules such as starch and gluten in bread; water (280-360µs) associated with macromolecules to a certain degree; and mobile water (2000-3000µs) which was closely correlated with the firming process. Some studies related two ranges of water (microsecond and millisecond range) to two mobility fractions of water (Ruan*, et al.*, 1996). Two fractions are observed in T2 images of rice cakes as well. One is the portion of water with low mobility shown as the intermediate intensity background of rice cakes, while the other is the portion with high mobility shown as high intensity separated areas in Figure 5c. The changes of water mobility might be caused by macromolecules such as starch including water to form crystals during retrogradation or expelling water as the physical structure of the macromolecules change due to e.g. re-crystallisation. Overall, the MR imaging results are characterised by movement of signal intensity from the edge to centre of the rice cakes and formation of high intensity regions in aged cakes.

4 Conclusions

Among three categories of additives tested (hydrocolloids, enzymes and emulsifiers), alginate was the only one that showed a significant reduction of hardening rate for steamed rice cakes during storage. The additives were chosen based on their anti-staling effect on low-moisture foods such as breads, but the results suggest most are not effective in higher moisture systems. Amylase treatment and emulsifier addition to rice cakes led to higher hardness compared to the control, while other hydrocolloids had no significant effect on hardness. The staling of rice cakes is accompanied by starch retrogradation, but addition of

alginate increased the DSC enthalpy for melting retrograded amylopectin, whilst decreasing hardness. The microstructure of rice cakes with and without alginate is similar, however after staling, the alginate samples had a smaller reduction in water mobility and more inhomogeneity than in the control, which might be related to the reduced hardness. We hypothesise and provide evidence to suggest that the properties of high-moisture steamed rice cakes are due to a phase-separated system containing a low water mobility included phase of stiffened starch granules and a continuous soft aqueous phase containing added alginate with high water mobility. The continuous phase apparently plays a key role in determining hardness and storage ability of steamed rice cakes. More generally, for high moisture starchy foods, anti-staling agents function by different mechanisms in high moisture starchy foods than for the well-studied but lower moisture bread systems.

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Figures

Figure 1 Compressive stress-strain curves: (a) full strain range, regions from left to right are region for partial contact, linear region before breaking, breaking region, and linear region after breaking; (b) linear region before breaking for modulus calculation

Figure 2 Firmness of rice cakes with added anti-staling agents: (a) enzyme and emulsifiers; (b) hydrocolloids. DM: distilled monoglyceride; SSL: sodium stearoyl lactylate; CMC: carboxymethyl cellulose.

Figure 3 Confocal laser scanning microscopy images of control (a1-3), 0.3% alginate (b1-3) added rice cake samples on storage time of day 0, 2 and 7. c: polarised light microscopy image of alginate added sample on day 0. Starch were stained by FITC to green, proteins were stained by Rhodamine B to red, and the non-starch polymers, mainly alginate and rice cell wall material were stained by Calcofluor White to blue..

Figure 4 Moisture contents of rice cakes of control and alginate 0.3% addition groups

Figure 5 The proton-density (a), T1 (b) and T2-weighted (c) magnetic resonance images of control and 0.3% alginate added steamed rice cakes.

Figure 6 Pixel intensity distribution of proton density (a), T1 (b) and T2 (c) images as a function of distance from rice cake centre.

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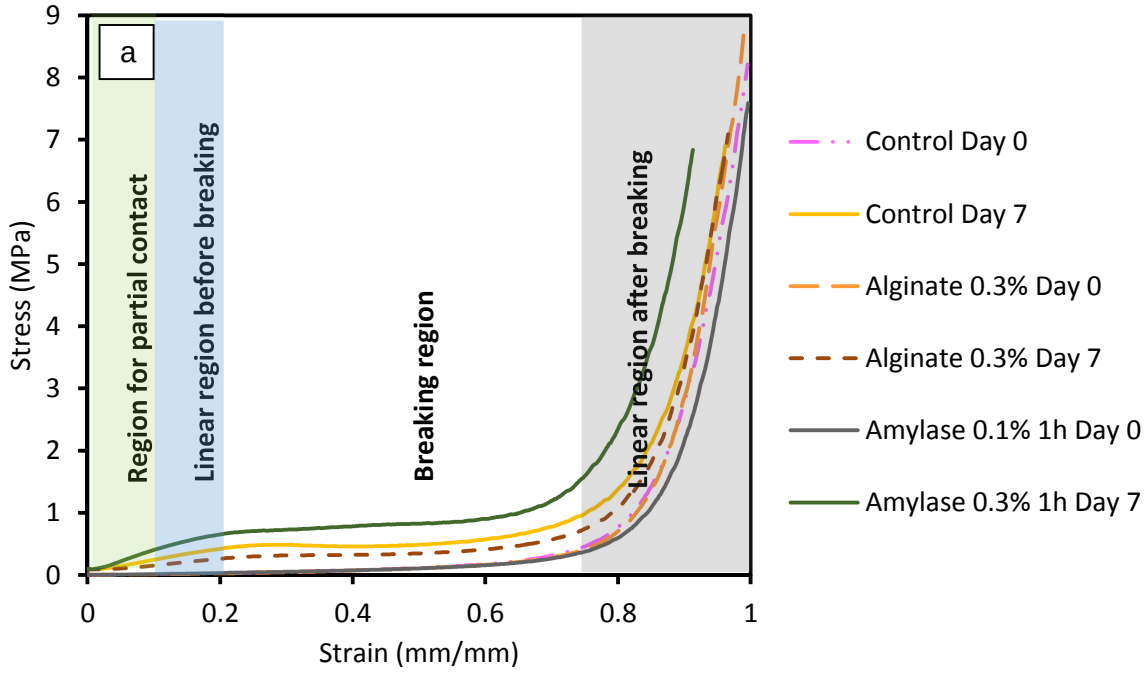

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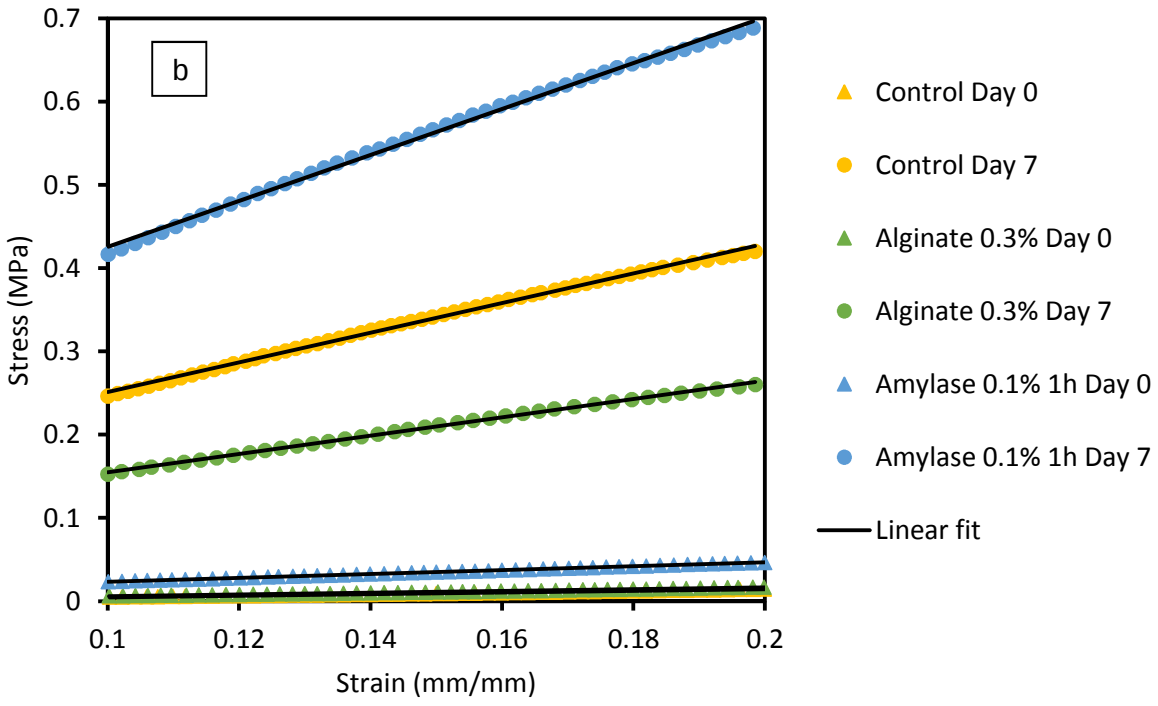

662

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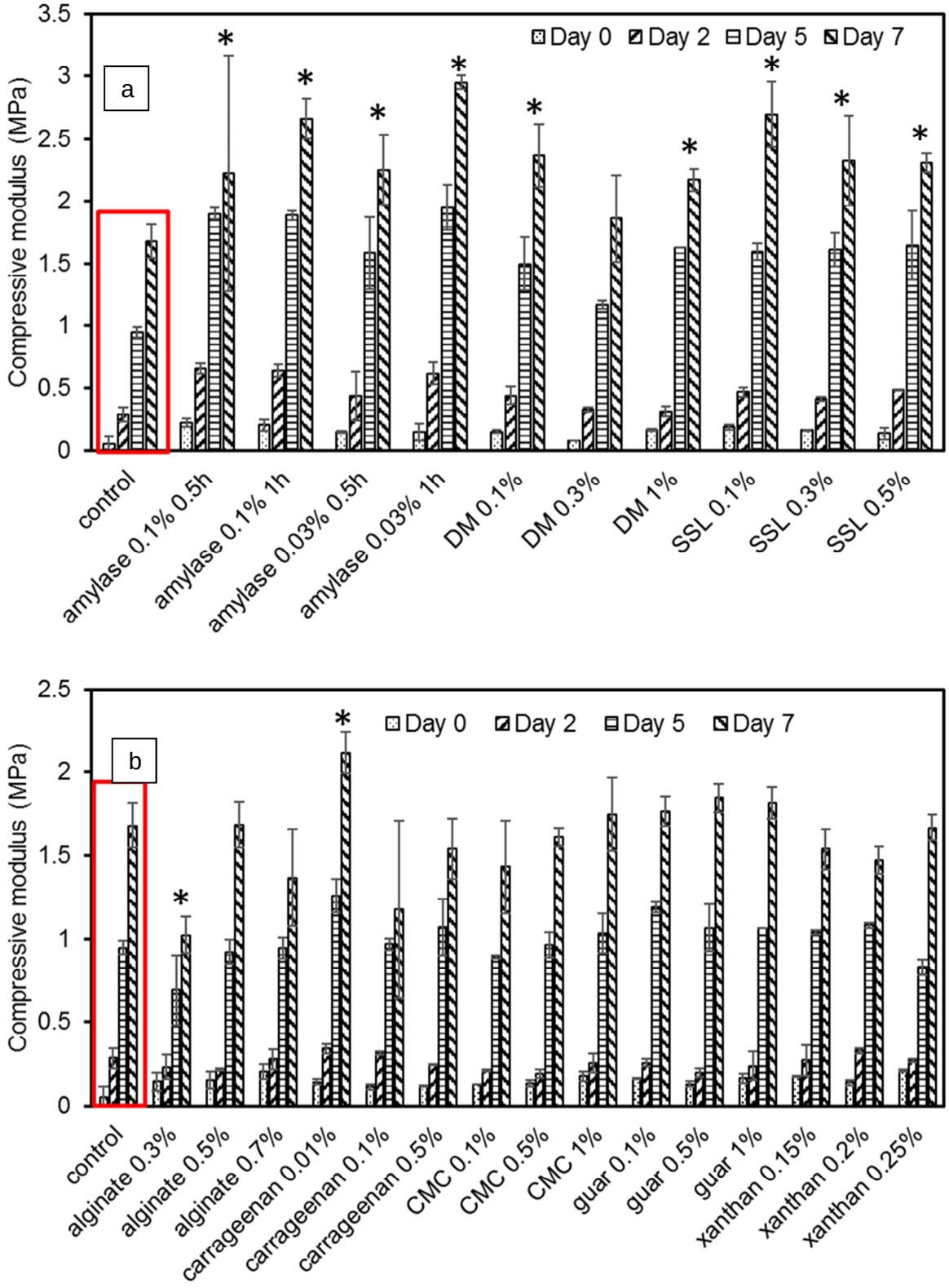
664 *contact, linear region before breaking, breaking region, and linear region after breaking; (b) linear region before*
665 *breaking for modulus calculation*



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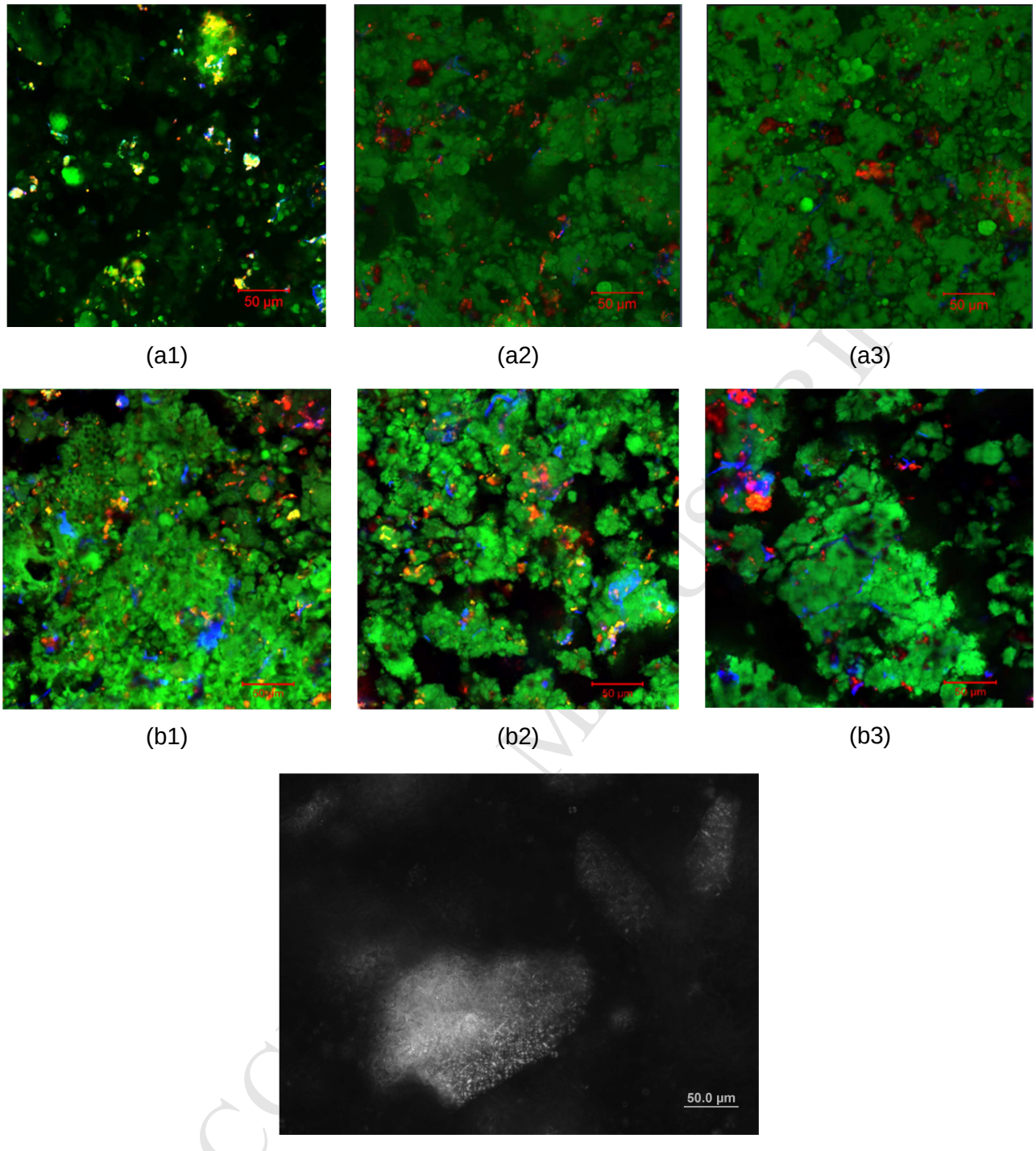

671 *Figure 3 Confocal laser scanning microscopy images of control (a1-3), 0.3% alginate (b1-3) added rice cake*
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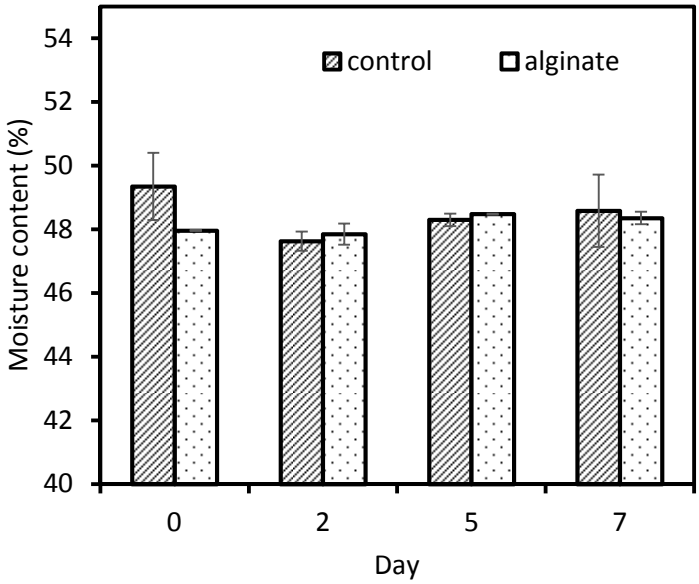
673 *0. Starch were stained by FITC to green, proteins were stained by Rhodamine B to red, and the non-starch*
674 *polymers, mainly alginate and rice cell wall material were stained by Calcofluor White to blue..*

675



676

677 *Figure 4 Moisture contents of rice cakes of control and alginate 0.3% addition groups*

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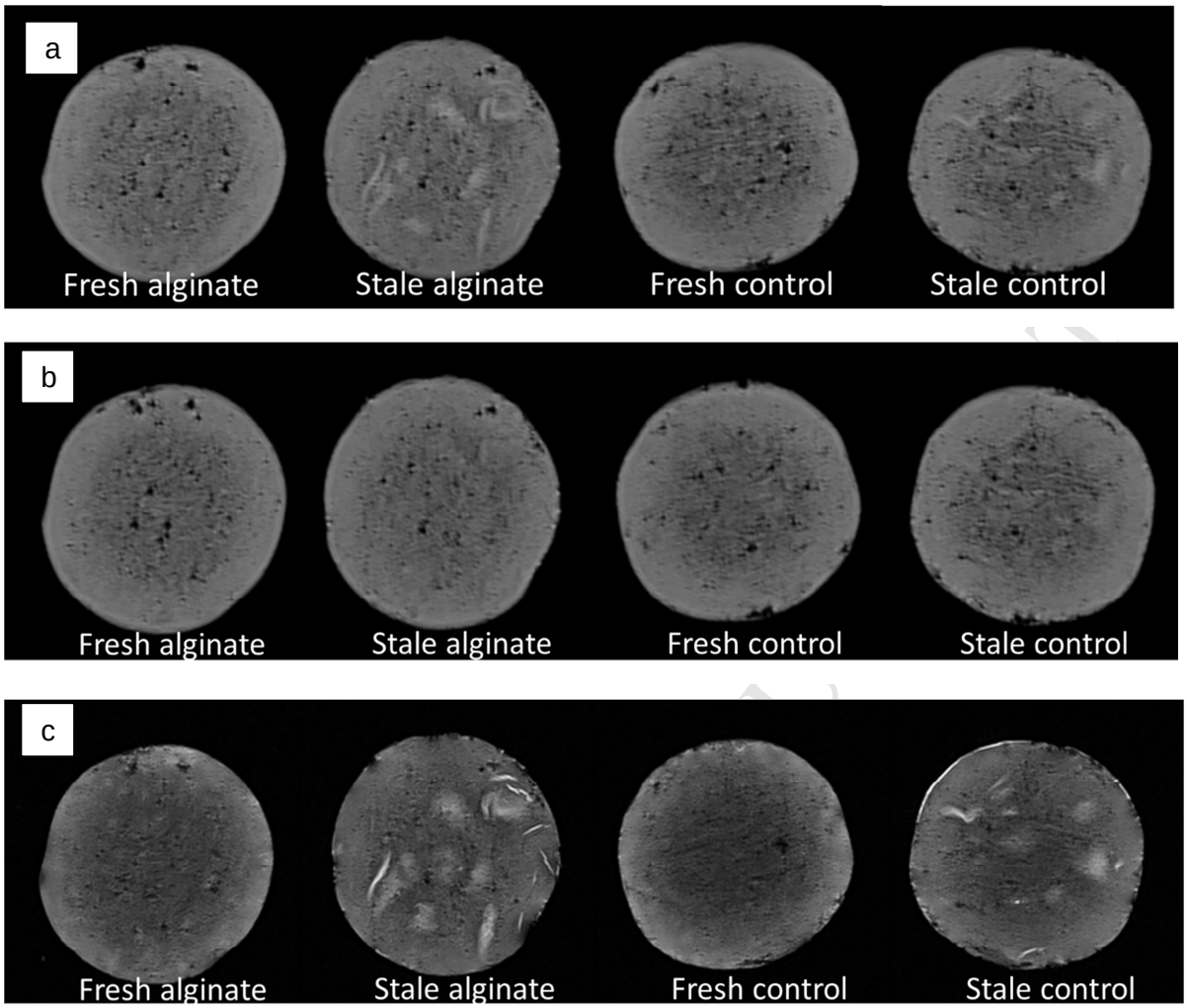

679 *Figure 5 The proton-density (a), T1 (b) and T2-weighted (c) magnetic resonance images of control and 0.3%*
680 *alginate added steamed rice cakes.*

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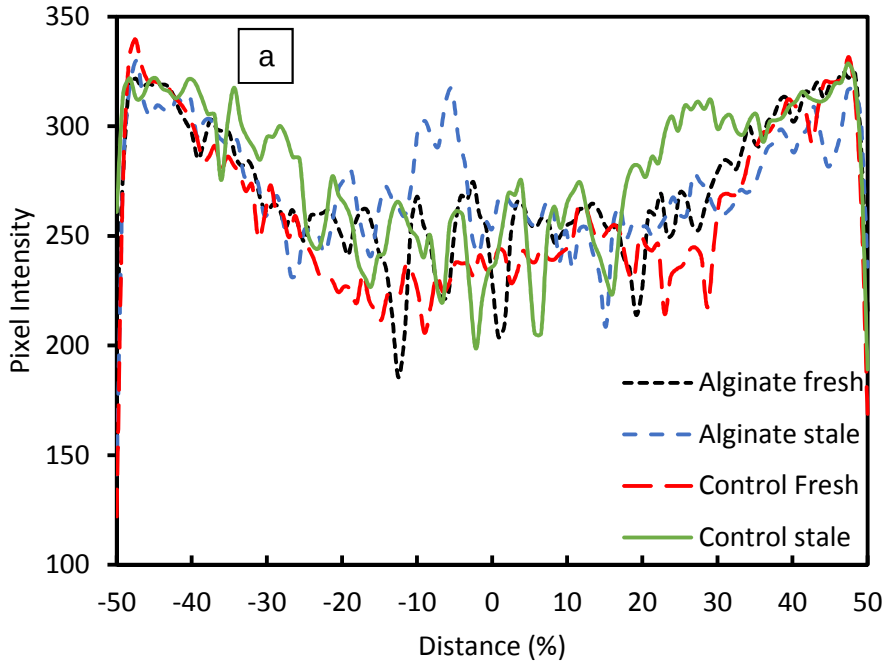
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Pixel Intensity
Distance (%)
Alginate fresh
Alginate stale
Control Fresh
Control stale

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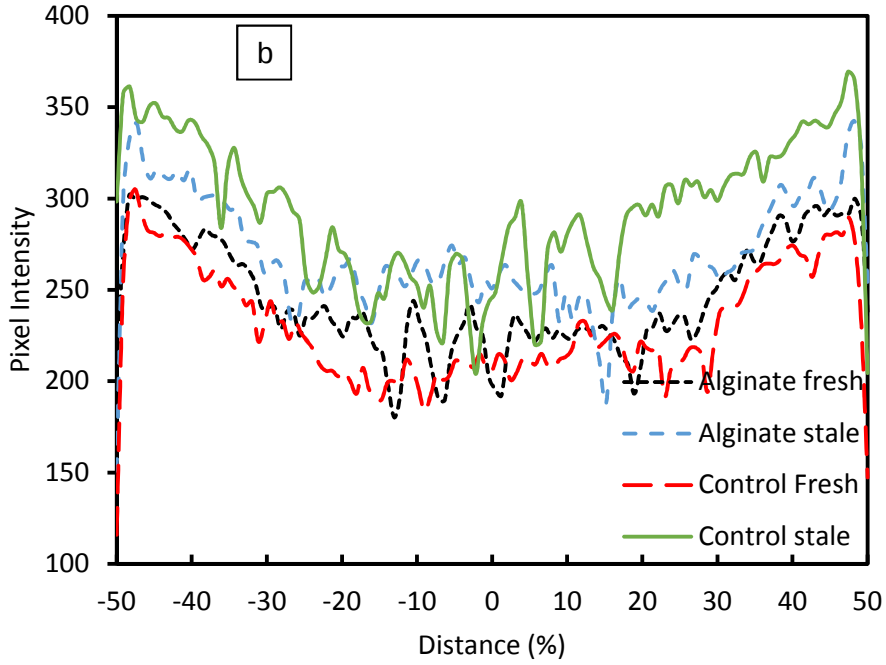
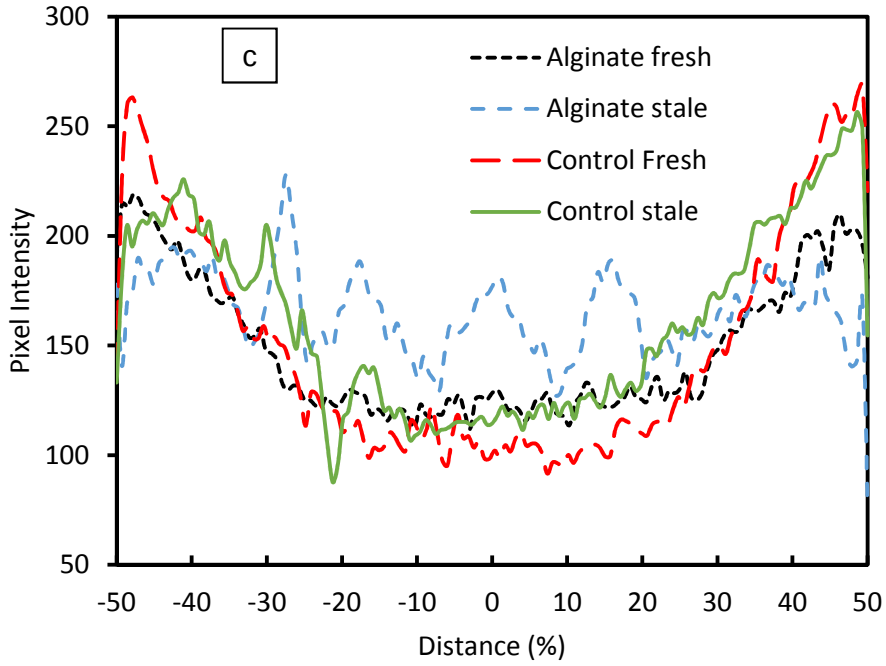

b
Pixel Intensity
Distance (%)
Alginate fresh
Alginate stale
Control Fresh
Control stale

683



684

685 *Figure 6 Pixel intensity distribution of proton density (a), T1 (b) and T2 (c) images as a function of distance from*
686 *rice cake centre.*

Tables

691 *Table 1 Additives and their concentrations used in this study*

Additives	Concentrations tested (w/w rice flour)		
Xanthan	0.15%	0.2%	0.25%
Guar gum	0.1%	0.5%	1%
Carrageenan	0.01%	0.1%	0.5%
CMC	0.1%	0.5%	1%
Alginate	0.3%	0.5%	0.7%
Fungal α-amylase	0.03%	0.1%	Incubation time 0.5h, 1h
SSL	0.1%	0.3%	0.5%
DM	0.1%	0.3%	1%

692

693 *Table 2 DSC characteristics of rice cakes during storage for a week*

Storage time (day)		Retrograded amylopectin			
		T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
Alginate 0.3%	0	-	-	-	-
	2	52.5±1.1	62.2±1.7	75.1±0.6	0.37±0.09
	5	46.9±0.1	58.5±1.1	73.4±0.4	2.00±0.27
	7	48.2±0.7	61.5±0.2	78.3±1.1	2.94±0.51
Control	0	-	-	-	-
	2	58.7±5.0	68.3±1.7	77.5±0.8	0.19±0.17
	5	56.0±2.3	66.2±1.2	76.7±1.0	0.47±0.39
	7	52.9±1.2	64.2±0.8	77.2±0.5	1.35±0.23

694 "-" indicates no peak detected.

Highlights

- Effects of anti-staling treatments used in baking evaluated for steamed rice cakes
- Alginate showed the most effective anti-firming effect but promoted starch retrogradation
- Anti-firming mechanism proposed to be due to structuring of the soft continuous phase
- Hydrocolloid anti-staling mechanisms are different for high and low moisture foods