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Research Article

Effects of NaCl and $CaCl_2$ on Physical Properties of *Mesona Blumes Gum*/Rice Starch Mixed Gel

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Abstract: The objective of this study was to investigate the effects of Sodium Chloride (NaCl) and Calcium Chloride ($CaCl_2$) on pasting, texture, elastic modulus and structure of the Rice starch (RS)/*Mesona Blumes Gum* (MBG) mixture for the purpose of determine the suitable concentration for the favorable gel properties of RS/MBG mixture. NaCl and $CaCl_2$ increased the pasting temperature with the increasing salt concentration. Compared to NaCl, $CaCl_2$ had a more significant influence on pasting temperature whereas NaCl slightly enhanced the peak viscosity and increased the final and setback viscosities. In addition, $CaCl_2$ significantly decreased the peak, final, breakdown viscosities of the RS/MBG mixture, regardless of the concentration. Texture Profile Analysis (TPA) demonstrated that the hardness of RS/MBG gels increased with the concentration of NaCl in a proper range and springiness and cohesiveness showed no significant trend. Moreover, $CaCl_2$ interfered with the internal structures of RS/MBG mixture, leading to the loss of gel-forming ability. The elastic modulus of the mixture increased by the addition of NaCl and decreased by the addition of $CaCl_2$. The internal structure of the RS/MBG system showed evident changes among the samples, in the occurrences of salts. In conclusion, a proper concentration of NaCl could contribute to the favorable gel properties of RS/MBG mixture. The results in this study facilitate the development of starch-based food products containing MBG and salts.

Keywords: $CaCl_2$, *Mesona Blumes Gum*, nacl, physical properties, rice starch

INTRODUCTION

Since rice starch has many useful attributes, it has been extensively incorporated into a variety of food systems as thickener, stabilizer, gelling agent. However, rice starch displays negative properties for the preparation of certain food products, such as tendency to retrogradation, undergoing syneresis, prolonging heating time and producing weak-bodied, cohesive, rubbery pastes and undesirable gels (Samutsri and Suphantharika, 2012). To overcome these problems, polysaccharides have been widely used to mix with native starch, improving rheological and textural properties and thus enhancing the ability of moisture retention and controlling water mobility. In this way, this approach could ensure and maintain overall products quality during processing and storage phase (Samutsri and Suphantharika, 2012).

Mesona Blumes Gum (MBG) is an ionic polysaccharide gum extracted from *Mesona Blumes*, a plant native to the southern China. MBG could form a low-viscosity solution with a pronounced shearing-thinning characteristic compared with other commercial gums (Feng *et al*., 2007a). The gelatinization, texture and rheological properties of the mixed system consisting of *Mesona Blumes Gum* (MBG) and various starches were reported (Feng *et al*., 2007b; Feng *et al*., 2010a, 2010b; Feng *et al*., 2014; Lai and Liao 2002a; 2002b; Lai *et al*., 2003). In addition, the mixture system has been used as a fat substitute or binder for meats because of its unique and useful properties, such as traditional Chinese sausages (Feng *et al*., 2013).

In numerous food formulations, the starch/polysaccharides mixtures often co-exist with other ingredients, for instance salt. It is well-known that

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the addition of salts would significantly affect the pasting and rheological properties of various starch gels (Ahmad and Williams, 1999; Chiotelli *et al*., 2002; Rumpold and Knorr, 2005; Samutsri and Suphantharika 2012). Ahmad and Williams (1999) found that salts had significant impact on gelatinization temperature, elasticity modulus and gel strength. The effect of sodium chloride on the gelatinization and rheological properties of wheat and potato starches revealed that the concentration of NaCl would significantly influence the gelatinization temperature and elasticity modulus. Chiotelli *et al*. (2002) suggested that the effect of solute on the properties of aqueous solution and direct polymer-solute interactions were highly associated with the mechanism of starch gelatinization in salt solutions and these two reversed effects would lead to complex effect patterns in a concentration dependent manner (Chiotelli *et al*., 2002). Therefore, the effects of salts on these properties mainly depend on the type and concentration of the salts, as well as the type of starches.

However, no attempt has been made to study the effect of salts on these properties of MBG/ rice starch mixed gels. Therefore, the main objective of this study was to determine the effect of salts on the pasting, texture, rheological properties and the microscopic structure changes of MBG/ rice starch mixed gels.

MATERIALS AND METHODS

Materials: Rice Starch (RS) was provided by Jiangsu Baobao Group Co., Ltd (China) and the rice starch composition was: moisture 13.5%, ash 0.3%, protein 0.89%, fat 0.3% and carbohydrate 85.01%. *Mesona Blumes* gum (MBG) was prepared following the method described by our previous study (Feng *et al*., 2007b). The salts used in this study were sodium chloride (NaCl) and calcium chloride ($CaCl_2$). All chemical reagents were analytical grade and purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd.(China).

Methods:

Sample preparation: Rice starch and MBG samples were prepared on a dry weight basis. RS/MBG dispersions were prepared by mixing starch (6% w/w) and MBG (0.5% w/w) in a stainless steel cask. Finally, 25, 50, 75 mM of NaCl or $CaCl_2$ (based on MBG/starch dispersion) was added into the dispersion, respectively.

Determination of pasting properties: The pasting properties of the MBG/rice starch mixed gels were determined in the presence of different salts, using Brabender Viscograph (Brabender Instruments, Inc., Duisburg, Germany). The amylographic procedure followed the method described by Cristina et al. (Rosell *et al*., 2001). In brief, the temperature profile employed was as following: heating the dispersion from 30°C to 95°C at a rate of 5°C/min, then holding for 10min at 95°C, cooling to 50°C at a rate of 5°C/min, finally holding at 50°C for 10 min. The values recorded in this study were pasting temperature, peak viscosity (maximum viscosity attained), trough viscosity (minimum viscosity at 95°C), final viscosity at 50°C (at the end of the cycle), breakdown viscosity (peak-trough), setback viscosity(final- trough).All the experiments were carried out in triplicate.

Texture Profile Analysis (TPA) of gels: The starch–MBG dispersions in the presence of NaCl or $CaCl_2$ were prepared as previously. The solution was moderately stirred for 1 h at room temperature and then was heated at 95°C in a water bath with mild agitation till starch gelatinized. The gels were poured into cylindrical plastic tubes (25 mm diameter and 25 mm height), then cooled down to ambient temperature (25°C) and stored at 4°C for 12h. TPA of Gels was evaluated using aTA-XT2i Texture Analyzer (Texture Technologies Corp., NY, USA) equipped with accompanying computer software XTRAD. The gels were compressed with a cylinder probe (10 mm diameter and 40 mm height) at a test speed of 5.0 mm/s. The deformation level was 20% of original sample height and the gels were compressed twice, interval time was 5s. Three replicate samples were tested. Texture parameters, including hardness, springiness and cohesiveness were measured. And, gel strength or hardness is defined as the deformation peak force during the first compression cycle, used as an indicator for the samples structure strength.

Rheological measurements: The MBG/starch gels added with NaCl or $CaCl_2$ (the gels were made as above mentioned method) were sliced and placed on the parallel plate (40 mm diameter and 1.0 mm gap) fixture in AR100 rheometer (Rheometrics Inc., Piscatway, NJ, USA). Trim off the expelled materials and then the exposed sample edge was covered with silicone oil to prevent evaporation during measurements. In order to relax the samples and some recovery motion might occur, all samples were allowed to rest at the initial temperatures for 15 min before measurements. Small deformation oscillatory experiments conducted at a constant deformation (1.0% strain) and frequency sweeps within the linear viscoelastic range from 0.1 to 10 Hz at 25°C. The rheological measurements were conducted in triplicate. The mechanical spectra were obtained recording the experimental data and to calculate storage modulus (G') related to the material response as a solid. The elastic moduli were compared with each other in order to observe the effect of salt on the viscoelastic properties of MBG/rice starch gels.

Scanning Electron Microscopy (SEM): The MBG/rice starch gels with different concentration of NaCl or $CaCl_2$ were cut into sections of 1 μm of thickness and then frozen in liquid nitrogen instantly. Frozen samples were then dried using a freeze drier (Dynavac Freeze Drier, Dynavac Engineering Australia). The samples were cross linked by immersion into 6% glutaraldehyde in 0.1 M phosphate buffer (pH 7) followed by dipping into 2% osmium tetraoxide in 0.1 M imidazole solution overnight. Then gradually dehydrated in 50, 70, 90%, respectively and absolute ethanol at room temperature for 24 h at each concentration before being critical point dried through CO_2 using a critical point dryer. (CPD-030, Boeckeler Instruments, Inc., Arizona, USA). Finally, the samples were mounted on aluminum stubs and coated with gold-palladium, then the images were recorded using a scanning electron microscope (SEM) (QUANTA-200, FEI Company, NE, USA) at an accelerating voltage of 10 kV and at magnification 600×(Han, 2000).

Statistical analysis: Results are expressed as the mean value with a SD of triplicate analyses for each sample. The analysis of variance (ANOVA) and Duncan's test were used to establish the significance of differences among the mean values at the 0.05 significance level. Statistical analyses were performed using SAS program (version 9.2) (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Pasting properties: The pasting characteristics of RS/MBG mixtures in the presence of different concentrations of salts determined by Brabender Viscograph were depicted in Fig. 1. It was illustrated that the pasting curve of rice starch/MBG system was affected by NaCl (Fig. 1a) and $CaCl_2$ (Fig. 1b). All pasting parameters are summarized in Table 1. The addition of salts increased the pasting temperature of the RS/MBG system with the increasing salt concentration. Of note, this effect was significantly increased as the concentrations of $CaCl_2$ increased. The increase of pasting temperature could be originated from the inhibition of starch swelling, resulting in the failure to gelatinize on account of the decreased amount and mobility of water molecules (Gil and Yoo, 2015). Considering the effect of the NaCl concentration on RS/MBG mixtures, the addition of NaCl caused a slight increased peak viscosity of the composed system and significant increases in the final and setback viscosities. The increase of the final viscosity values in combination with the interaction between NaCl and starch could be due to the starch exchanged cations from the solution for hydrogen ions, resulting in the increase of the swelling volume and the decrease of the mobility of starch granules, causing the increased paste viscosities of RS/MBG mixture systems in the presence of NaCl (Gil and Yoo, 2015; Sudhakar *et al*., 1995, 1996), as previously discussed. On the contrary, it was indicated that the peak, final, break viscosities of the RS/MBG composed system significantly decreased in the present of $CaCl_2$ and the viscosities have little difference between different concentrations of $CaCl_2$. That might be that Ca^{2+} addition would decline the ionization degree of MBG molecules, along with the reduction of electrostatic interactions among them, which promoted the gathering of numerous MBG molecules and damaged the formation of gel structures between starch and MBG molecules (Sudhakar *et al*., 1995).

Texture profile analysis: The effects of RS/MBG gels with different concentrations of salts on gel texture properties, including hardness, springiness, cohesiveness, were summarized in Table 2. In general, with addition of NaCl, gels hardness significantly increased ($p<0.05$) to a maximum at the concentration of 25 mM and then evidently decreased with the increase of NaCl concentration. It was illustrated that there was a critical level of NaCl concentration for hardness of the mixed gels. When NaCl concentration is below the critical value, the hardness value increases. In contrast, when NaCl concentration is above that certain concentration, the hardness value decreases. Thus, the presence of a proper concentration of NaCl could improve the hardness of RS/MBG gels. Moreover, springiness and cohesiveness of the gels showed no significant trends of consistent change. They slightly decreased with the addition of NaCl. Whereas, the hardness, springiness and cohesiveness of RS/MBG/$CaCl_2$ mixture system could not be detected by the texture analyzer, because the addition of $CaCl_2$ destroyed the structure and could not form a gel, accompanied by the precipitation.

Table 1: Effects of various concentrations of NaCl or $CaCl_2$ on the parameters of Brabender curves of rice starch and MBG mixture

Samples	Salt concentration (mM)	Pasting temperature (°C)	Peak viscosity (BU)	Trough viscosity (BU)	Final viscosity (BU)	Breakdown viscosity (BU)	Setback viscosity (BU)
RS/MBG	0	64.7±0.461^{a}	89.2±0.82^{bc}	59.4±0.6^{c}	156.7±2.0^{d}	29.7±1.3^{a}	97.2±1.2^{c}
RS/MBG/NaCl	25	65.3±0.36^{b}	92.3±1.40^{a}	65.8±0.4^{b}	340.2±1.3^{c}	26.7±0.9^{ab}	274.6±0.7^{b}
RS/MBG/NaCl	50	65.9±0.26^{bc}	88.7±1.08^{c}	66.2±0.8^{b}	377.0±3.1^{a}	22.4±0.8^{bc}	311.0±2.0^{a}
RS/MBG/NaCl	75	66.2±0.15^{c}	91.4±0.60^{ab}	86.5±1.2^{a}	360.5±1.0^{b}	5.0±0.5^{d}	274.1±0.5^{b}
RS/MBG/$CaCl_2$	25	87.6±0.30^{d}	67.6±0.80^{d}	45.3±0.3^{d}	100.2±1.5^{e}	22.3±0.3^{bc}	55.1±1.4^{d}
RS/MBG/ $CaCl_2$	50	88.2±0.20^{de}	63.5±1.60^{e}	44.7±0.5^{d}	94.9±0.8^{f}	18.9±0.6^{c}	50.3±0.2^{e}
RS/MBG/ $CaCl_2$	75	88.4±0.42^{e}	63.7±1.20^{e}	46.4±0.7^{d}	96.3±0.7^{ef}	17.5±0.2^{c}	50.0±0.9^{e}

RS: Rice Starch; MBG: Mesona Blumes Gum; The values are expressed as mean±SD. Different letters in each column indicated that there were significant differences according to Duncan's Multiple Range Test ($p\leq0.05$)

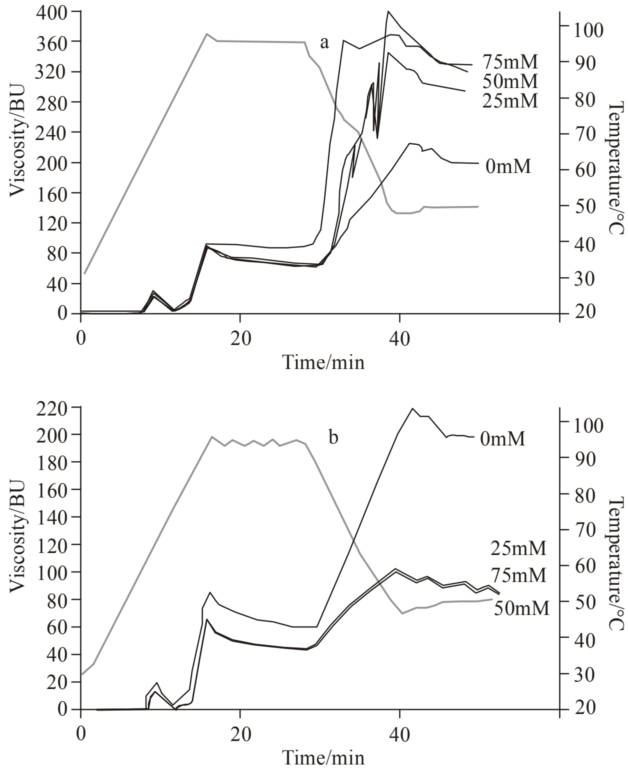

Fig. 1: Pasting curves of rice starch/MBG with sodium chloride (a) and calcium chloride (b)

Table 2: Effect of NaCl or $CaCl_2$ on textural parameters of rice starch and MBG gels

Samples	Salt concentration (mM)	Hardness (g)	Elasticity	Cohesiveness
RS/MBG	0	148.5±8.336[b]	0.972±0.004[a]	0.562±0.072[a]
RS/MBG/NaCl	25	164.5±5.170[a]	0.963±0.016[a]	0.501±0.023[a]
RS/MBG/NaCl	50	150.5±9.648[b]	0.972±0.001[a]	0.490±0.046[a]
RS/MBG/NaCl	75	78.3±3.096[c]	0.942±0.002[b]	0.505±0.010[a]
RS/MBG/CaCl2	25	/	/	/
RS/MBG/ CaCl2	50	/	/	/
RS/MBG/ CaCl2	75	/	/	/

RS: Rice Starch; MBG: Mesona Blumes Gum; The values are expressed as mean ±SD. Different letters in each column indicated that there were significant differences according to Duncan's Multiple Range Test ($p \leq 0.05$)

Hardness is characteristic related to the strength of the gel structure under compression, expressed by the peak force during the first compression cycle. The change of hardness of RS/MBG/NaCl gels could be attributed to the fact that a large number of uronic acid groups in MBG molecules might combine with cations hydrolyzed from NaCl solution in RS/MBG gel system. The combination would inhibit the ionization degree of MBG molecule and then affect its configuration, the formation amount of hydrogen-bond and the strength of electrostatic attraction needed for the interaction between MBG and starch molecules, conducive to the polymerization of these two molecules (Samutsri and Suphantharika, 2012). However, excess cations might occupy anionic sites of MBG molecules, thus preventing the formation of linkages between adjacent polymer chains, obstructing the formation of network structure of the mixture system and decreasing the gel strength.

Given the effect of molecule structure and interaction of RS/MBG should take into consideration, RS/MBG/$CaCl_2$ mixture could not form gels. In addition, calcium ions might cause the formation of an inordinate number of ordered nuclei, resulting in MBG molecule gathered together and structure tightening, which would hamper the interaction of MBG and starch molecules and finally weaken the gel network structures (Lau *et al*., 2000).

As shown in Table 2, the addition of NaCl had no remarkable effect on the springiness of the gel system. Springiness (sometimes is referred to as elasticity) represented the extent and speed of deformation recovery of gels when external force removed, counted by the ratio of waviness width after a double compression cycle. Since all the gelled foodstuffs were viscoelastic materials, elasticity tested by texture profile analysis should be comprehensive performance of Elastic instant caused by elastic materials and deferred recovery originated from the viscous materials. Additionally, the structure of RS/MBG gel system might be destroyed during the first compression, which could influence the accuracy of elasticity, resulted in

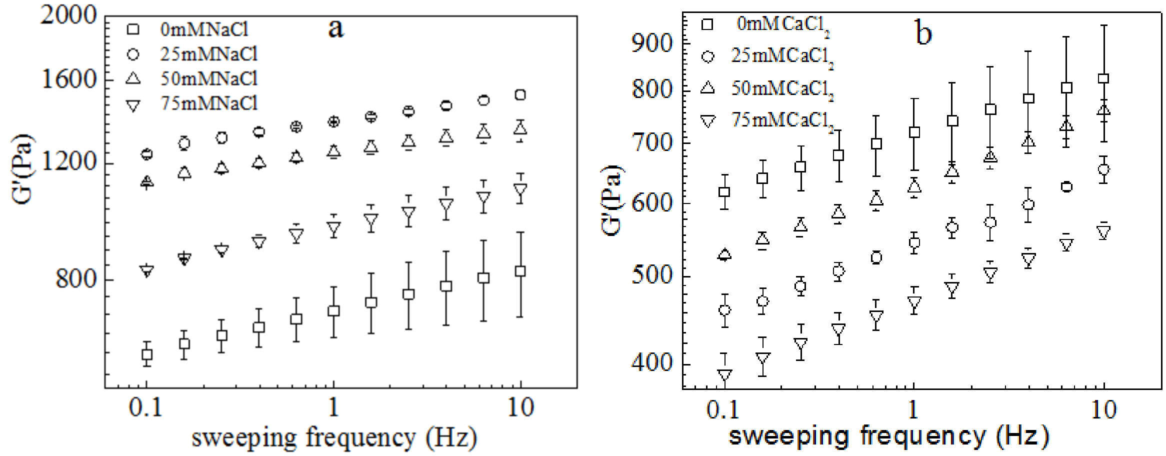

Fig. 2: Dynamic mechanical spectrum of rice starch/MBG with (a) NaCl or (b) $CaCl_2$ addition

the inconspicuous differences of these samples. Furthermore, RS/MBG Gels with various concentrations of NaCl had no significant differences in cohesiveness, which reflects the degree of difficulty in breaking down the gel's internal structure, counted by the ratio of the area under the first and second compression (A2/A1). The occurrence of the strong linkage inside the gel system could generate high resistance to the change caused by the external force, resulting in low A_2/A_1 at the same time, these observation are comparable to locust been gum/κ-carrageenan mixed gels reported by Gonçalves *et al.* (1997).

Elastic moduli assay: Effects of NaCl concentration on storage modulus (G′) of RS/MBG gel are shown in Fig. 2a. It was demonstrated that the addition of NaCl greatly affected the elastic properties of RS/MBG mixed system. G′ value of RS/MBG mixed gels significantly increased with the increase of NaCl concentration to a certain level and reached the maximum with the concentration at 25 mM. Subsequently, G′ value decreased gradually with the increase of NaCl concentration, higher than that of the control. Based on the previous results obtained from this study, such higher dynamic elastic moduli of RS/MBG mixtures in the presence of NaCl may be attributed to the synergistic effect of the addition of NaCl. These results are consistent with the hardness measured by texture analyzer. The addition of salts enhanced the intermolecular interaction of MBG and starch molecules due to the charge screening effect, thereby promoting a network formation and causing an increase in the elastic properties of RS/MBG/NaCl mixtures (Gil and Yoo, 2015). Meanwhile, when the concentration of NaCl excessed 25 mM, electrostatic repulsion between MBG molecules would be extremely weakened, which restrained the network formation between MBG molecules and gelatinized starch, resulting in the decline of G′.

The G′ of RS/MBG gel system with $CaCl_2$ in the various concentrations (Fig. 2b) indicated that the magnitudes of G′ decreased with the concentration of $CaCl_2$ since the addition of $CaCl_2$ would destroy the gel structure and appeared unconsolidated state, along with the precipitation of water. Compared to the change of G′ of RS/MBG gel system, it was illustrated that the impact of $CaCl_2$ was much stronger than that of NaCl, probably due to the factthat Ca^{2+} was a bivalent cation and its capacity of reducing the electrostatic repulsion between MBG molecules was higher than that of Na^+.

Microscopy structure: RS/MBG gel is composed of swollen granules, fragments and MBG molecules, interpenetrating and forming a continuous network. As shown in Fig. 3 and 4, Scanning Electronic Micrographs (SEM) of RS/MBG gels revealed that the gel structures were changed by the addition of salts. Figure 3a and 4a showed that the appearance of RS/MBG displayed a honeycomb-like structure in the absence of salts. When 25 mM of NaCl was introduced to the gel system, gels still could maintain honeycomb-like structures while the partial aperture enlarged, along with the hardness of gels. The apertures and holes on the wall diminished and hardness of the gel system consequentially decreased when the concentration increased to 50 mM and 75 mM. Meanwhile, the gel immediately showed a messy microstructure of MBG/starch gels with the addition of $CaCl_2$ at 25 mM. In addition, the extent of disorder increased as the increasing concentration of $CaCl_2$, illustrating to the damage of the gel structure. This is in good agreement with the previous experiments in this study. Moreover, the gel properties, hardness and elasticity modulus of gels with neatly organized microstructure were higher than those of gels with mixed and disorderl

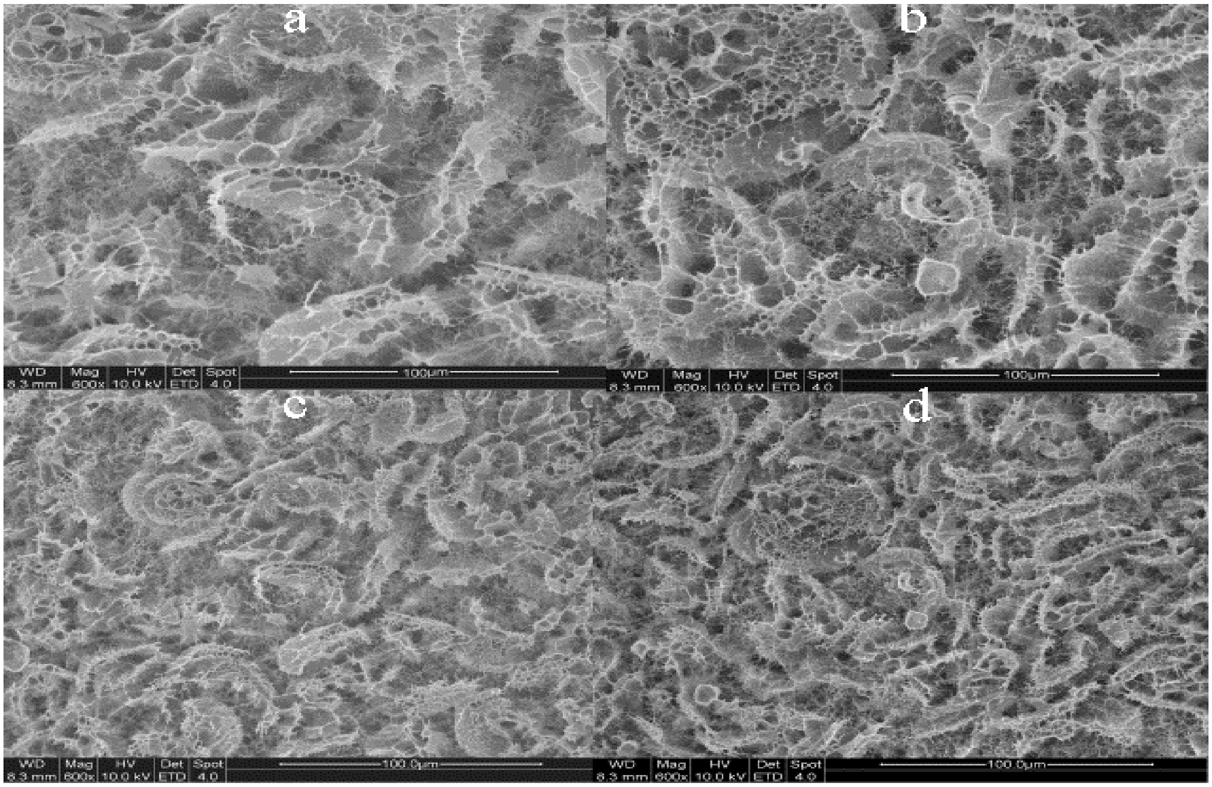

Fig. 3: Microscopic images of rice starch/MBG gels with different sodium chloride concentration; (a): 0; (b): 25; (c): 50; (d): 75 mM

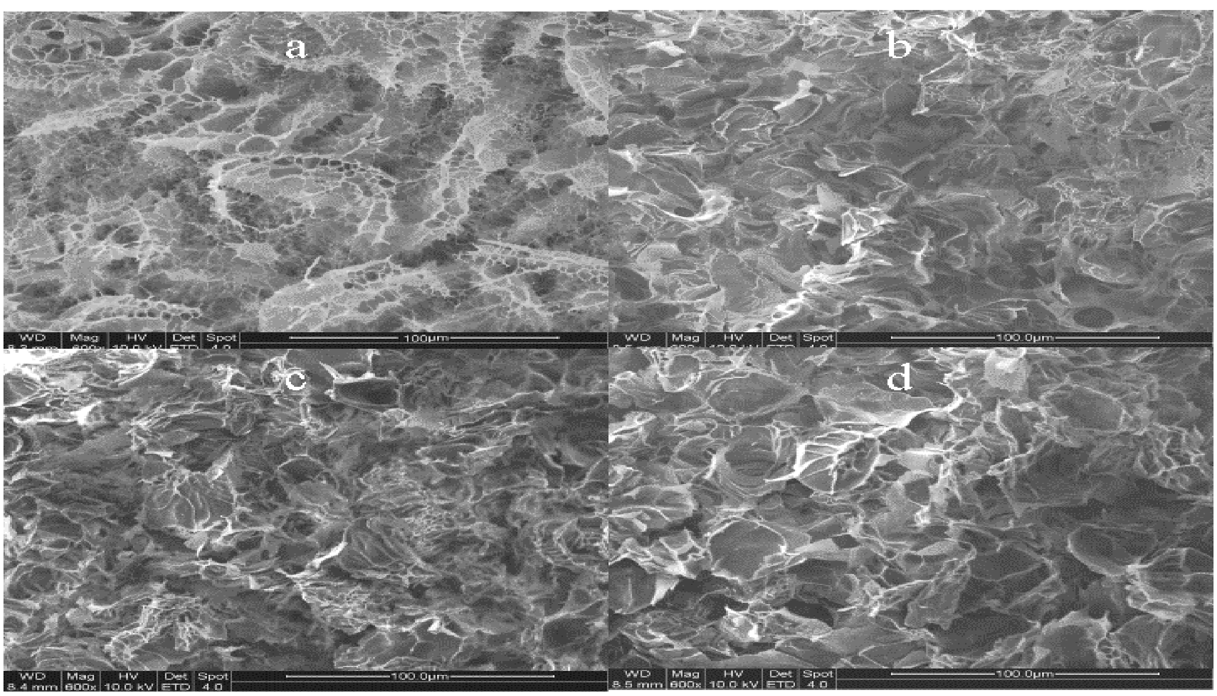

Fig. 4: Microscopic images of rice starch/MBG gels with different calcium chloride concentration; (a): 0; (b): 25; (c): 50; (d): 75 mM

microstructure. Microstructures of gels indicated that the orderly degree of the gel structure is associated with the hardness of the gel and reflected the interaction between these components. The hardness of gel would increase with the degree of its uniformity, as well as the interaction strength of MBG and starch molecules.

CONCLUSION

This study demonstrated that the addition of salts have a pronounced effect on the pasting, texture, elastic modulus and microscopic structure of the rice starch/MBG combined system and the presence of NaCl and $CaCl_2$ with different concentrations influenced these physical properties differently. In general, the RS/MBG mixture in the occurrence of a proper concentration of NaCl contributed to the favorable gel properties enhanced the hardness and strength of gels, whereas the addition of $CaCl_2$ would damage the gel formation. In addition, the changing trends of storage modulus of gel system with salts were basically consistent with those of hardness detected by the texture analyzer. This study contributes to the development of starch-based food products containing MBG and salts.

ABBREVIATIONS

RS : Rice Starch
MBG : *Mesona Blumes Gum*
TPA : Texture Profile Analysis
NaCl : Sodium Chloride
$CaCl_2$: Calcium Chloride
SEM : Scanning Electron Microscopy

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