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Physical and mechanical properties of sago starch – alginate films incorporated with calcium chloride

*Fazilah, A., Maizura, M., Abd Karim, A., Bhupinder, K., Rajeev Bhat,
Uthumporn, U. and Chew, S. H.

*Food Technology Division, School of Industrial Technology, University Sains Malaysia,
11800 Penang, Malaysia*

Abstract: Edible films were prepared from sago starch: alginate mixtures with ratios of 1:0, 4:1, 3:1, 2:1, 1:1 and 0:1. The physical and mechanical properties of films were modified with the addition of calcium chloride ($CaCl_2$) (2 x 10^4 mg/kg) in the film solutions and by immersion of the films into a $CaCl_2$ (2% w/w) solution. The films were characterized for tensile strength (TS), percentage of elongation at break (% E), calcium content and water solubility (WS). The calcium content in 100% alginate films was significantly higher compared to those after immersion for 1 min in a $CaCl_2$ solution. Immersion of films into a $CaCl_2$ solution for 1 min increased the TS, except for 100% sago starch film. Prolonging the immersion time for 10 minutes did not significantly affect the TS of the films. Moreover, increasing the immersion time did not significantly decrease the WS of the films. Addition of $CaCl_2$ in film solutions with higher ratios of alginate significantly increased their TS and % E. Films treated with $CaCl_2$ by the immersion process led to markedly increased TS compared to those treated by $CaCl_2$ addition in the film solutions.

Keywords: Alginate, calcium chloride, mechanical properties, physical properties, sago starch

Introduction

The production of edible films from natural polymers has received much attention due to the excellent biodegradability, biocompatibility and edibility of the films. Preparing these films involves the use of at least one film-forming agent (macromolecules), a solvent and a plasticizer. The most-used macromolecules are polysaccharides, proteins and lipids (Krochta and Mulder-Johnston, 1997; Cuq *et al*., 1998; Krochta, 2002). There are many polysaccharide sources for the production of edible films, such as root and cereal starches, plant cell pectin, alginate and carrageenan from seaweed and cellulose (Kester and Fennema, 1986). However, starches are commonly used as a raw material because they are renewable resources, widely available and inexpensive (Mali and Grossmann, 2003; Famá *et al*., 2005).

Starch is a major food component and a complex biodegradable carbohydrate made up of thousands of glucose units (Zhou *et al*., 2009). It consists primarily of linear and branched chains of glucose molecules, namely amylose and amylopectin. Amylose, which is the linear fraction of starch, is responsible for the formation of coherent, relatively strong and free-standing films (Rindlav-Westling *et al*., 1998). Physical crosslinkages in the macromolecular network of starch are formed mainly by microcrystalline amylose and affect the mechanical properties of films (Rindlav-Westling *et al*., 1998). In contrast, the branched structure amylopectin generally leads to films that are brittle and reduced tensile strength.

Alginate is the water-soluble salt of alginic acid. It is a naturally occurring, non-toxic polysaccharide found in brown algae (Rubio and Ghaly, 1994; Al-Musa *et al*., 1999) and consists of β-(1-4)-linked D-mannuronic acid (M) and α-(1-4) linked L-guluronic acid (G). Three types of glycosidic linkages exist in the block structures: diequatorial M-M, diaxial G-G and dequaltorial-axial MG (Yotsuyanagi *et al*., 1991; Donati *et al*., 2005; Dong *et al*., 2006). Alginate has potential use in films or as a coating component because of its unique colloidal and gel-forming properties (King, 1982; Rhim, 2004). However, the most useful and unique property of alginate is its ability to react with polyvalent metal cations, such as calcium ions in aqueous media, to generate strong gels and insoluble polymers (Grant *et al*., 1973; Braccini and Pérez, 2001; Fang *et al*., 2008; Olivas and Barbosa-Cánovas, 2008). As such, several studies have analyzed the properties of alginate-calcium films (Pavlath *et al*., 1999; Rhim, 2004; Olivas and Barbosa-Cánovas, 2008; Silva *et al*., 2009).

Starch-based films exhibit physical characteristics similar to synthetic polymers, such as being transparent (Lourdin *et al*., 1997; Myllärinen *et al*., 2002; Parra *et al*., 2004), odorless, tasteless (Wolff *et al*., 1951), semi-permeable to carbon dioxide and resistant to oxygen passage (Nisperos-Carriedo, 1994). In addition, they have good barrier and mechanical

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*Corresponding author.
Email: *fazilah@usm.my*
Fax: +604 6573678

properties (Myllärinen *et al*., 2002; Larotonda *et al*., 2005). Several researchers have tried to develop more sophisticated starch-based edible films with enhanced barrier and mechanical properties by blending them with other biopolymers, such as alginate (Wu *et al*., 2001), potato starch with agar (Wu *et al*., 2009), cassava starch with arabinoxylan (Phan The *et al*., 2009), and tapioca starch with hsian-tsao leaf gum (Chen and Lai, 2008).

Wu *et al*. (2001) and Maizura *et al*. (2007) studied on starch-alginate mixtures to form edible films. However, sago starch-alginate mixtures in the presence of $CaCl_2$ have not been reported for use in the formation of edible films. Starch-alginate mixtures crosslinked with calcium ions are desirable to produce films with enhanced tensile strength and decreased solubility. The objective of this research was to investigate the effect of $CaCl_2$ on the physical and mechanical properties of sago starch-alginate edible films.

Materials and Methods

Sago starch (*Metroxylon sagu*) was obtained from Nitsei Sago Industries, Sdn. Bhd. Penang, Malaysia. Alginate (Acros Organic, Fairlawn, N.J., U.S.A.), glycerol (Qrec, New Zealand) and calcium chloride (Systerm, Shah Alam, Selangor, Malaysia) were also used in this study.

Preparation of sago starch-alginate film

Film-forming solutions were prepared from mixtures of sago starch and sodium alginate containing different ratios (1:0, 4:1, 3:1, 2:1, 1:1, 0:1) based on the total mass of dry solid (3.5 g) and glycerol (0.5 g) in 200 ml of distilled water. Films were treated with $CaCl_2$ in two different ways. In the first treatment, 2 x 10^4 mg/kg of $CaCl_2$ was mixed into the film solutions. The mixture was heated to 85°C with continuous stirring for 45 min, followed by homogenization at 1000 ^{1}/s using a homogenizer (IKA-Werke GmbH, Staufen, Germany) for 5 min. The solution (90 g) was cast onto a 16 x 16 cm polyacrylic plate, cooled down to room temperature, and oven-dried at 40°C for 24 hr. For the second treatment, sago starch-alginate films were immersed in a 2 % w/w $CaCl_2$ solution for 1, 5 and 10 min and dried at 40°C for 6 hr.

Film thickness

The film thickness was measured using a micrometer (Dial thickness gauge 7301, Mitutoyo Co. Tokyo, Japan) with accuracy to the nearest 0.01 mm at five locations along the film strips; the average value was used in tensile strength calculations.

Mechanical properties

The tensile strength and elongation at break percentage of films were tested using a TA-TX2 texture analyzer (Stable Micro System, Goldaming, Surrey, UK) in accordance with ASTM (ASTM, 1981). Five sample strips (14.0 x 2.0 cm) of each formulation were cut and clamped between tensile grips. Films were previously equilibrated in 52% relative humidity (RH) at 30°C for 7 days. Each film strip was placed in pneumatic grips (30 psi) and stretched at 1.5 mm s^{-1}. The initial distance between the grips was 100 mm. The tensile stress (Mpa) and percentage of elongation at break (%E) were determined. The tensile strength of the films is a measure of the maximal force per original cross-sectional area that the film could sustain before breaking, and the elongation at break percentage measures the capacity of the film to extend before breaking (Silva *et al*., 2009). At least five strips were used per treatment, with five replicates.

Calcium content

The concentration of calcium in the crosslinked films was determined with an atomic absorption spectrophotometer (Perkin Elmer, Waltham, Massachusett, U.S.A.). The film samples (1 g) were dissolved in 6 mL of a concentrated nitric acid (HNO_3) solution and 1 mL of 30% (v/v) hydrogen peroxide (H_2O_2). The mixture was digested using a microwave digester for 20 min. The clear solutions were then mixed with 5 mL of lanthanium oxide (La_2O_3), and distilled deionized water was added until the solution volume was 25 mL. Each determination was done in triplicate.

Staining microscopy

Dried film samples (1.5 x 2 cm) were placed onto a glass slide. Three drops of an iodine solution were added on the films. The films were left for a few seconds to allow the iodine solution to completely disperse on the films before covering with a cover slip. Then, the film structure was examined using light microscopy.

Water solubility

The water solubility of the films was determined according to the method described by Romero-Bestida *et al*. (Romero-Bastida *et al*., 2005). Pieces of film (2 x 3 cm) were cut from each film formulation and stored in a dessicator with silica gel (0% RH) for 7 days. The samples were weighed to the nearest 0.0001 g and placed in beakers with 80 ml of deionized water. The samples were maintained under constant agitation for 1 hr at room temperature (approximately 25°C). After soaking, the remaining pieces of film were filtered through filter paper (Whatman No. 1), followed by oven drying at 60°C to constant weight. The samples were measured in triplicate and the percentage of total soluble matter (% solubility) was calculated according to Eq. (1):

$$\%\ \text{solubility} = \frac{(\text{initial dry weight} - \text{final dry weight}) \times 100}{\text{initial dry weight}} \qquad (1)$$

Statistical analysis

The mean data was analyzed by two-way and one-way analysis of variance (ANOVA) to evaluate the effects of $CaCl_2$ and the starch alginate ratios on the physical and mechanical properties of the films using the SPSS 15.0 system software. Duncan's multiple-range test ($P<0.05$) was used to determine the significant differences between means.

Results and Discussions

Whereas the starch films appeared translucent, stiff, slightly brittle, odorless and colorless, the alginate films were transparent and pliable. Mixtures of these two polymers formed films that were almost transparent, had good flexibility and were easily removed from the casting plate. The viscosity of the film-forming solution with higher amounts of alginate increased after the addition of $CaCl_2$, likely due to the development of crosslinks between the carboxyl groups in the film molecules and the calcium ions. After the immersion of films into the $CaCl_2$ solution, the surface was not as smooth as that of the control films. A similar occurrence was also observed by Rhim (2004). These results were attributed to disruption of the film matrix during the immersion and an uneven rate of water removal from the films during the drying process.

The film thickness with different treatments of $CaCl_2$ and different starch alginate ratios is presented in Table 1. The thickness of films with sago starch: alginate ratios of 1:0 and 4:1 decreased significantly ($P<0.05$) after immersion in the $CaCl_2$ solution for 5 min and 10 min respectively compared to films without treatment with $CaCl_2$. These results suggest that the starch component in the starch-dominated films solubilized into the solution, thus reducing the thickness of the film. However, no significant difference ($P>0.05$) in thickness was observed in films with higher ratios of alginate (3:1, 2:1, 1:1 and 0:1) after immersion in a $CaCl_2$ solution for 10 min compared to films without $CaCl_2$ (control).

The mechanical properties of edible films are of great importance due to their influence on product performance. The effect of $CaCl_2$ on the tensile strength of the films at different sago starch: alginate ratios are shown in Figure 1. The results show that films without the addition of alginate had lower tensile strengths compared to those with alginate. Many studies (Romero-Bastida *et al*., 2005; Turhan and Sahbaz 2004; Laohakunjit and Noomhorm 2004; Fama *et al*., 2005; Mali *et al*., 2005) was done on the tensile strength analysis of starch based films. The tensile strength of sago starch based films obtained in this study was similar as found by Romero-Bastida *et al*. (2005) for banana starch film which was 25 Mpa. However, the presence of alginate at sago starch: alginate ratios 4:1 had significantly increased the tensile strength due to the linear structure of the polymer, which is capable of producing tough and flexible films. In addition, films with sago starch: alginate ratios 2:1, 1:1 and 0:1 exhibited increased tensile strength by more than 40% compared to films without alginate.

The results in Figure 1 also clearly indicate that the tensile strength of films with $CaCl_2$ significantly increased ($P<0.05$) compared to those films without $CaCl_2$ at sago starch: alginate ratios of 2:1, 1:1 and 0:1. These results could be attributed to crosslinking between carboxyl groups in alginate with calcium ions. According to Glickmans (1982), crosslinks could be formed by simple ionic bridging of two carboxyl groups on adjacent polymer chains with calcium ions. However, calcium ions could also form connections with either polymannuronic acid or polyguluronic acid segments.

Table 1. Effect of calcium chloride and starch:alginate ratios on film thickness

Treatment	Thickness (mm) x 10^{-2}					
	Sago starch: alginate ratio					
	(1 : 0)	(4 : 1)	(3 : 1)	(2 : 1)	(1 : 1)	(0 : 1)
Control (films without $CaCl_2$)	12.8 ± 0.4^{hijk}	12.4 ± 0.5^{fghij}	11.6 ± 0.5^{cdefg}	12.0 ± 0.7^{defghi}	11.4 ± 0.5^{cdef}	13.4 ± 0.5^{jkl}
Films incorporated with $CaCl_2$	12.4 ± 0.5^{fghij}	12.6 ± 0.5^{ghij}	12.4 ± 0.5^{fghij}	12.2 ± 0.4^{efghi}	11.6 ± 0.5^{cdefg}	12.6 ± 0.5^{ghij}
Films immersed in $CaCl_2$						
1 min	11.8 ± 0.4^{defgh}	11.6 ± 0.9^{cdefg}	11.4 ± 0.9^{cdef}	10.6 ± 0.9^{abc}	11.2 ± 0.8^{bcde}	13.0 ± 1.4^{ijkl}
5 min	11.4 ± 1.3^{cdef}	11.4 ± 0.5^{cdef}	11.0 ± 1.0^{bcd}	11.4 ± 0.9^{cdef}	11.0 ± 1.2^{bcd}	14.0 ± 0.7^{l}
10 min	9.8 ± 0.4^{a}	10.2 ± 0.4^{ab}	11.8 ± 0.4^{defgh}	11.8 ± 0.4^{defgh}	12.2 ± 1.1^{efghi}	13.8 ± 0.4^{kl}

Mean ± standard deviation (n= 5); different superscript letters in each row and column indicate significant differences at ($P < 0.05$).

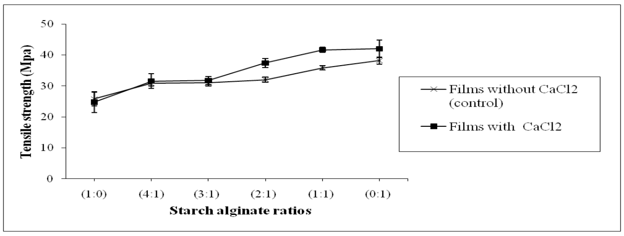

Figure 1. Effect of starch:alginate ratios on tensile strength of films with and without $CaCl_2$. The vertical bars are standard deviations of the means (n=5).

The tensile strength of films with various ratios of starch and alginate after immersion in the $CaCl_2$ solution for different periods of time is presented in Figure 2. The tensile strength of the films drastically increased after immersion for 1 min in the $CaCl_2$ solution. After immersion in the $CaCl_2$ solution for 1 min, films with a sago starch: alginate ratio of 4:1 had higher tensile strengths (39.9 ± 3.0 Mpa) compared to films (30.8 ± 0.8 Mpa) with similar sago starch: alginate ratios incorporated with $CaCl_2$. These results suggest that the effect of increased tensile strength is more pronounced in the immersion process. The calcium ions present in the solution can crosslink with alginate in the films matrix better than calcium ions and alginate in the edible film solutions at similar concentrations of 2% w/w $CaCl_2$. However, there was no significant (P>0.05) increase in the film tensile strength after immersion in the $CaCl_2$ solution for10 min.

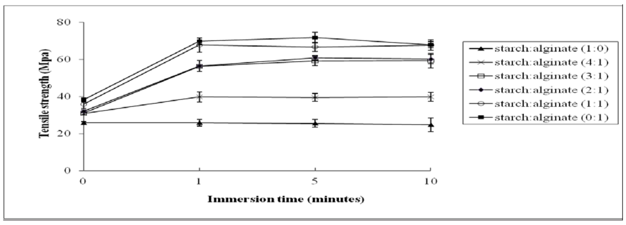

Figure 2. Effect of starch:alginate ratios on tensile strength of films after immersion in $CaCl_2$ for different periods of time.

The effects of $CaCl_2$ on the percentage of elongation at break of the films at different sago starch: alginate ratios are shown in Figure 3. The elongation at break sharply increased in films with a 0:1 ratio (sago starch: alginate), both with and without $CaCl_2$. Generally, increased tensile strength led to a decreased percentage of elongation at break (Rhim, 2004; Olivas and Barbosa-Cánovas, 2008). However, we observed that increased tensile strength of films did not necessarily decrease the percentage of elongation at break. These results are similar to those obtained by Pavlath *et al.* (1999).

Figure 4 shows the percentage of elongation at break of films with various ratios of sago starch and alginate after immersion in $CaCl_2$ solutions for different periods of time. Longer immersion times did not significantly (P>0.05) increase the elongation at break percentage of all the films. The elongation of alginate films was substantially higher compared to other films. A study by Rhim (2004) found that, the elongation at break for alginate films was 14.0% and after immersion in 2% w/w of $CaCl_2$ for 5 mins, the percentage of elongation had decreased significantly to 3.4%. In this study, it was similarly found that immersion of alginate film for 5 mins also had decreased the elongation at break significantly from 8.90% to 7.46%. The films dominated with alginate exhibited higher tensile strength and good flexibility.

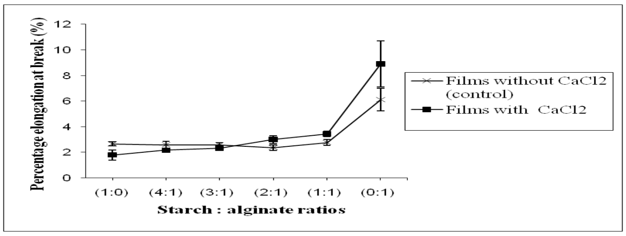

Figure 3. Effect of starch:alginate ratios on percentage of elongation at break of films with and without $CaCl_2$. The vertical bars are the standard deviations of the means (n=5).

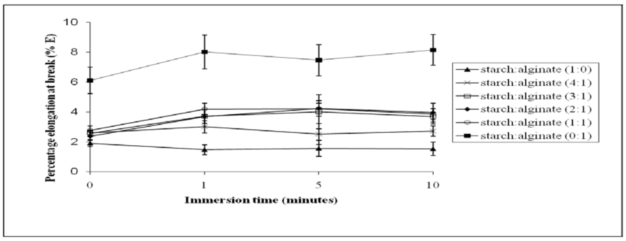

Figure 4. Effect of starch:alginate ratios on percentage of elongation at break of films after immersion in $CaCl_2$ for different periods of time. The vertical bars are the standard deviations of the means (n=5).

Staining microscopy analysis was conducted to determine the homogeneity of the produced films. Figure 5 shows films with different treatment of $CaCl_2$. The mixture of sago starch and alginate produced films with a smooth surface. However, incorporation of $CaCl_2$ into the sago starch and alginate film solutions led to the formation of films that were slightly rough and had a gritty surface, which could be related to the rapid rate of alginate gelation. In the absence of a sequestering agent for calcium, the gelation of the alginate component in a starch-alginate mixture would occur very fast (uncontrolled), thus preventing uniform formation of the film. In the immersion process, calcium ions crosslinked with alginate in the film matrix. We assumed that the presence of calcium ions in the film matrix did not interrupt the sago starch-alginate polymer chain crosslinking, thereby producing films with slightly smooth surfaces. After immersion in the $CaCl_2$ solution, these films dried much faster than the control films, and a similar finding was also reported by Rhim (2004). However, the obtained films were unable to revert back to their smooth original surfaces after the immersion process, which may be due to the variable drying process between different parts of the film surface.

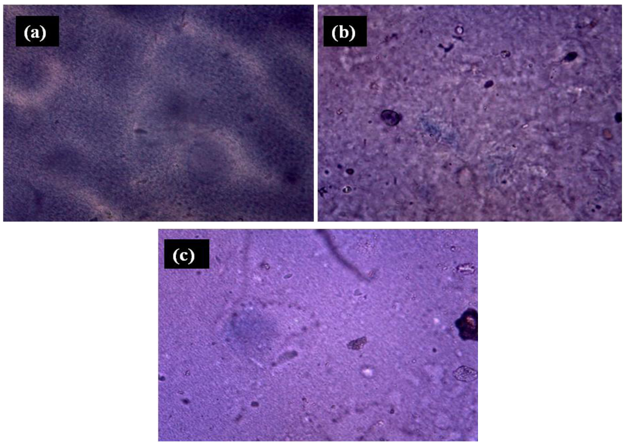

Figure 5. Representative pictures of sago starch-alginate (1:1) films with and without $CaCl_2$ treatment: (a) Control (without $CaCl_2$), (b) Film with addition of 2% w/w $CaCl_2$, (c) Film immersed in 2% w/w $CaCl_2$ for 1 min.

The calcium content of films with different sago starch: alginate ratios after immersion in $CaCl_2$ solution for different periods of time is presented in Figure 6. Increasing the immersion time did not significantly ($P>0.05$) increase the calcium content in the films. The calcium ions probably crosslinked with almost all of the alginate polymers in the film matrix after 1 min of immersion time. Films with higher ratios of alginate have been predicted to have higher calcium content because of the mechanical properties of films (Olivas and Barbosa-Cánovas, 2008). Films with higher calcium content have more crosslinkages between calcium ions and alginate polymers, thus producing films with higher tensile strength. This relationship is supported by the significantly positive correlation between the tensile strength and calcium content values of films after 1 min of immersion in the $CaCl_2$ solution. The correlation value was 0.681, which is significant at the $P<0.01$ level.

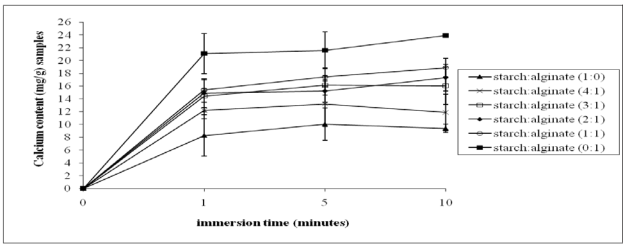

Figure 6. Effect of starch:alginate ratios on calcium content of films after immersion in a $CaCl_2$ solution for different periods of time. The vertical bars are the standard deviations of the means (n=3).

Water solubility is an important property of edible films. Some applications may require water insolubility to enhance product integrity and water resistance. The results of the percentage of water solubility of films at different ratios of sago starch and alginate with and without $CaCl_2$ are shown in Figure 7. Films with $CaCl_2$ had higher water solubility compared to those without $CaCl_2$ at all ratios. The absence of a sequestering agent for calcium produced non-uniform films due to the rapid rate of alginate gelation, thus producing non-homogeneous film structures that disintegrated rapidly during immersion in water. In addition, films with $CaCl_2$ exhibited sharply increased water solubility as the alginate ratio increased. Alginate films with and without $CaCl_2$ completely disintegrated in water, making it impossible to measure the water solubility, as similarly noted by Rhim (2004).

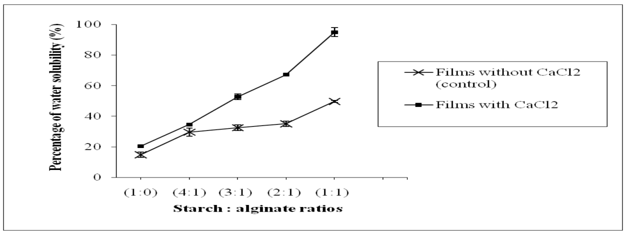

Figure 7. Effect of starch:alginate ratios on water solubility of films with and without $CaCl_2$. The vertical bars are the standard deviations of the means (n=3).

The water solubility of films with various sago starch:alginate ratios at different periods of immersion times is presented in Figure 8. Increasing the immersion time in $CaCl_2$ solution did not significantly ($P>0.05$) enhance the film solubility in water. According to Pavlath *et al.* (1999), alginate films were not moisture-resistant and readily dissolved in distilled water within a minute. However, increases in water resistance of the films are dependent on the immersion time and concentration of calcium chloride (Pavlath *et al.*, 1999, Rhim, 2004). In this study, 100% alginate film, after immersion for 1, 5 and 10 min in a $CaCl_2$ solution (2%w/w), dissolved completely in water. Our results clearly showed that films with higher ratios of sago starch exhibited lower water solubility, indicating higher water resistance.

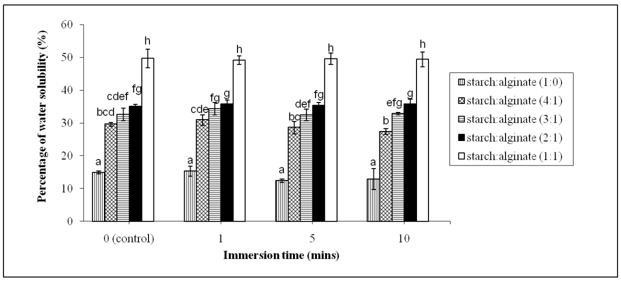

Figure 8. Water solubility of films with various starch:alginate ratios for different immersion times. The vertical bars are the standard deviations of the means (n=3). Different superscript letters within and across the group are significantly different at $P < 0.05$.

In conclusion, our results demonstrated that mixtures of sago starch and alginate produced films with better tensile strength compared to sago starch films. However, immersion of sago starch-alginate films in $CaCl_2$ solution exhibited higher tensile strength than those with added $CaCl_2$. After immersion in $CaCl_2$ solutions, films with different ratios of sago starch and alginate had better flexibility sago starch film. The films with higher ratios of alginate had higher calcium contents. Alginate film had a lower water resistance compared to sago starch film. Sago starch-alginate films had moderate water solubility.

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