

INFLUENCE OF RELATIVE HUMIDITY ON DRIED Ca^{++} -ALGINATE FILMS AND COMPOSITES MADE WITH SOY AND PECTIN

S. BARBUT* and A. HARPER

Food Science Department, University of Guelph, Canada, N1G 2W1

*Corresponding author: sbarbut@uoguelph.ca

ABSTRACT

Dried Ca-alginate films were manufactured±pectin or soy protein isolate (SPI) as well as dried un-gelled (no Ca^{2+}) pure alginate films: conditioned at either 57% or 100% relative humidity (RH). With the exception of the un-gelled alginate films, all films conditioned at 100% RH were more transparent than at 57% RH. High RH films resulted in higher % elongation at break, distance and work to puncture values than their corresponding films conditioned at 57% RH. ATR-FTIR scans showed several peak shifts when the film forming solutions were gelled with Ca^{2+} and when the 'wet' films were dried.

Keywords: alginate, film, pectin, relative humidity, soy protein isolate, tensile strength

1. INTRODUCTION

Global production of packaging materials is increasing and was estimated to exceed 180 million tons per year (CUTTER, 2006; NURA, 2018). Food packaging accounts for a large portion of this material and is estimated to attribute to roughly 70% of the \$100 billion packaging market in the U.S. alone (CUTTER, 2006). Much of this packaging material is made from synthetic plastics. However, mounting environmental concerns about plastic use and the rising cost of petroleum has led to an increased interest in natural food packaging material such as polysaccharides, proteins and/or lipids (RHIM, 2004; CUTTER, 2006; DA SILVA *et al.*, 2009; JANJARASSKUL and KROCHTA, 2010). However, edible packaging materials are not normally meant to entirely replace conventional packaging. Rather, the efficiency of food preservation can be improved by using primary edible packing together with less nonedible packaging. A good example is replacing plastic or cellulose casings used for frankfurters with manufactured co-extruded (in line) collagen casings, alginate or their combination (BARBUT, 2015). The frankfurters are then packaged in a strong oxygen barrier plastic pouch, which can be used as a barrier during cooking and/or distribution.

A popular example of a polysaccharide that has been used in edible films is alginate. It is used as food wraps (e.g., spring rolls) and for small diameter sausage casings. One of the more desirable properties of alginate is its ability to very quickly form cold-set gels in the presence of calcium (OUWERX *et al.*, 1998; MØRCH *et al.*, 2006). Alginates are derived from marine brown algae (*Phaeophyceae*) and also produced by soil bacteria (STEPHEN *et al.*, 2006). Chemically, alginate is made up of (1→4) β-D-mannuronic (M) and α-L-guluronic (G) acid. Regions in alginate made up of solely M or G residues are referred to as M or G blocks and these areas are interspersed with MG alternating blocks (FANG *et al.*, 2007; LEE *et al.*, 2018).

Several factors influence the use of edible films in food applications including: availability, cost, functional attributes, mechanical properties, optical quality, barrier properties, structural resistance to water and sensory acceptability (DA SILVA *et al.*, 2009; JANJARASSKUL and KROCHTA, 2010). Typically protein and polysaccharide films are fairly good oxygen barriers at low to medium RH and have good mechanical properties but, due to their hydrophilic nature, are poor water vapour barriers (RHIM and NG, 2007). Today, several large and medium size sausage manufacturers are using co-extrusion technology. Unofficial estimates put this number at 30% of the US market. This allows sausage manufacturers to move from a batch to a continuous process and thus increase production volumes (BARBUT, 2015). Additionally, fewer people handle the product and thus the risk of microbial contamination of fresh sausages is reduced (ANONYMOUS, 2012). Some manufacturers are also using cook in the bag technology, and therefore further reduce the risk of re-contamination (after cooking) with pathogens such as *Listeria*. However, there are several challenges of casings manufactured from solely alginate or collagen. Researchers have reported that alginate casings are prone to calcium migration (e.g., casings dissolve over time), while collagen casings may shrink more during frying (VISSER, 2012). One solution is producing composite co-extruded casings/films, which take advantage of synergistic interactions between individual components. The industry has already adopted this approach by adding ingredients such as starch and cellulose to co-extruded alginate casings, as well as producing co-extruded alginate-collagen hybrid casings. Although these composite alginate casings are used commercially, there is little or no scientific literature regarding the properties of such 'wet' films. In fact, most of the literature reports on 'dry' alginate films for use after they have been cast. For example,

researchers have created composite 'dry' alginate films with proteins such as, whey, soy, and whey (SHIH, 1994; VILLAGOMEZ-ZAVALA *et al.*, 2008; WANG *et al.*, 2010), or polysaccharides such as pectin, kappa-carrageenan, pullulan, sago starch, and carboxymethyl cellulose (XU *et al.*, 2003; TONG *et al.*, 2008; DA SILVA *et al.*, 2009; FAZILAH *et al.*, 2011; GOHIL, 2011; BIERHALZ *et al.*, 2012; PAŞCALĂU *et al.*, 2012; XIAO *et al.*, 2012; GALUS and LENART, 2013). In certain cases, synergism was seen when 'dry' composite films made from 50:50 alginate and low methoxyl pectin showed higher tensile strength and elongation than either pure alginate or pectin films (GALUS and LENART, 2013). HARPER *et al.* (2013) started publishing on the 'wet' film area (90-95% water content), which recently became popular with the use of co-extruded sausage casings. They examined how various protein (whey, soy, and gelatin) and carbohydrate (pectin, cellulose, starch, carrageenan, and gellan gum) additives influence the mechanical and microstructural properties of composite films/casings. However, so far very few comparisons have been made between the physical properties of 'wet' and dried alginate films. Understanding the role water plays in these films is important for future development of this area. Therefore, the objective of this work was to explore how drying 'wet' alginate and alginate composite films affect these physical properties. Additionally, the effect of rehydration of the dried films (i.e., exposing the dried films to high humidity) on their physical properties has been studied. For this work, composite alginate films were manufactured with either soy protein isolate (SPI) or low-methoxylated pectin and compared to pure alginate films.

2. MATERIALS AND METHODS

2.1. Film preparation

The 'wet' alginate films were produced according to the methods described by Harper *et al.* (2013). Briefly, 5% (w/w) alginate (GRINDSTED® Alginate FD 6965, Danisco USA Inc., Rochester, NY, USA) solution was prepared. Later, 1.5 g portions were rolled onto a plastic covered stainless steel board using a stainless steel roller with a recess of 0.34 mm. The roller, with the film on it, was placed into a 5% (w/w) CaCl₂ (Fisher Scientific, Fair Lawn, NJ, USA) bath for 1 min to gel the film.

Soy protein isolate (SPI; Soy Protein Supro 515, protein 90%, supplied by Hela Spice Canada, Inc., Uxbridge, ON, Canada) was dissolved in 23°C double deionized water to form a 1% wt. protein solution. A 0.25% wt. pectin solution was made by dissolving low methoxyl pectin (LMP; GENU® pectin type LM-104 AS-Z, CP Kelco, Lille Skensved, Denmark) into 70°C double deionized water. 5% wt. alginate was also mixed by hand (total mixing time of 15 min) into both the protein and pectin solutions. Additionally, a control sample of 5% wt. alginate with no protein or pectin was made. To remove some of the air incorporated into the solutions during mixing, the solutions were degassed by using a vacuum packager (Multivac Canada Inc., Woodbridge, ON, Canada) at 7.3 kPa for 25, 50 and 75 sec. The solutions were allowed to hydrate for a minimum of 2 hr at 23°C prior to film formation. The 'wet' films were produced using a stainless-steel roller and then placed into the calcium bath. Alginate films were also produced without calcium as a gelling agent. In this case, alginate solution was rolled between two sheets of plastic on the stainless-steel board. The top layer of plastic was carefully peeled away from the film, leaving the film on the bottom sheet of plastic for drying.

2.2. Drying and conditioning of the films

The 'wet' alginate and alginate composite films were pinned to a plastic covered smooth piece of wood (to prevent curling) and left to dry overnight at room conditions (~23°C and ~52% RH). The alginate films without calcium were simply left on the bottom sheet of plastic to dry. The following day the films were removed from the plastic and placed in jars with either 57% RH (NaBr controlled) or 100% RH, and left to condition overnight. Prior to conditioning, the films that would later be used for tensile testing were cut into 75 mm X 25 mm strips using a razor blade, and their thickness measured. Three thickness measurements (top, centre and bottom) were taken on each film using a digital micrometer (Testing Machines Inc., Islandia, NY, USA) and the average thickness of each film was used for the tensile stress calculations.

2.3. Mechanical properties

The puncture and tensile properties of the films were evaluated using a texture analyzer (TA.XT Icon. Texture Technologies Corp., Hamilton, MA/Stable Micro Systems, Godalming, Surrey, UK). The films were kept in their RH respective jars right until the testing. For the puncture test a 5 mm ball probe was used to puncture the uncut films. The trigger force was set at 5 g and the test speed was 10 mm/s. From the generated force-distance graph, the force (N), distance (mm) and work (N mm) to puncture the films were determined. Tensile testing was conducted according to the ASTM-D882 standard tensile testing procedure (ASTM, 2010). The grippers were spaced 50 mm apart and the test distance was set at 75 mm. The trigger force for testing was 8 g and the test speed was 2 mm/s. The tensile strength (MPa), % elongation at break and the Young's modulus of the films were determined from the generated stress-strain graphs.

2.4. Optical properties

The visible light transmission (400-780 nm) of the films was measured using a single beam spectrophotometer (USB 2000, Ocean Optics Inc., Dunedin, FL, USA). The integration time was 25 ms with 2 scans to average and a boxcar width of 4. In total eighteen films per treatment were scanned (six per trial). The average light transmission of the eighteen films was calculated for each of the treatments.

2.5. ATR-FTIR analysis

Spectra were acquired using an FTIR spectrometer (IRPrestige-21, Shimadzu Corporation, Tokyo, Japan) equipped with a total attenuated reflection press (MIRacle™, Pike Technologies, Madison, WI, USA). Scans were taken of the alginate and alginate-low methoxyl pectin film forming solutions, gelled 'wet' films prior to drying and the gelled dried films conditioned at 57% RH. Additionally, the alginate and low methoxyl pectin powders used to make the films were scanned. All of the powders were conditioned at 57% RH overnight. For consistency, all of the films were scanned with the side of the film that was touching the roller, during gelling in the calcium bath, face up. All samples were tested at 23°C. The data were collected from 600-4000 cm⁻¹ with an average of 30 scans per sample and a resolution of 4 cm⁻¹. The second derivatives of the original spectrums were

taken using Grams-32 spectral analysis software (Galactic Industries Corp., Salem, NH, USA) to determine the wavenumber of the peaks. Sampling was performed in triplicate.

2.6. Statistical analysis

The experimental design was a completely randomized block with three independent trials. For both the puncture and tensile tests six films per treatment were tested in each of the three trials. The average of the six measurements was taken for each treatment in each trial and used for statistical analysis (SAS Version 9.2, SAS Inst., Cary, NC, USA). A General Linear Model was used for analysis of variance (ANOVA) and a Tukey's multiple comparison analysis (P -value ≤ 0.05) was used to detect statistical significance between film type means.

3. RESULTS AND DISCUSSION

3.1. Mechanical properties of formed films

The purpose of this work was to compare the physical properties of dried alginate and alginate composite films to their 'wet' film counterparts. In our previous work, when low methoxyl pectin (LMP) or soy protein isolate (SPI) were added to 'wet' alginate films, the composite films exhibited different mechanical properties from the pure 'wet' alginate films (HARPER *et al.*, 2013; HARPER *et al.*, 2015). For this reason, pectin and SPI were chosen as ingredients for the alginate composite films manufactured in this study. In order to be able to directly compare the dried films in this study to the 'wet' films produced in earlier work, the films were manufactured in the same way. In the present study, the films were dried and then conditioned at 57% and 100% relative humidity in order to also evaluate how rehydration influences the films' physical properties. Overall, all of the dried films (conditioned at 57% or 100% RH) showed higher puncture force and tensile strength but lower puncture distance and elongation values compared to their corresponding 'wet' films. For example, the percent elongation at break was 140% for the 'wet' alginate-pectin films (HARPER *et al.*, 2015) and 4.6% and 12.3% for the dried alginate-pectin films conditioned at 57% and 100% RH, respectively (Table 1). The control alginate film gelled with Ca had an elongation value of 88% while after drying and conditioned it to 57% or 100% RH the values were only 5.8% and 12.0%, respectively. Therefore, it is important to emphasize that the rehydrated dried films (i.e., 100% RH films) did not have the same mechanical properties as their original 'wet' films, thus demonstrating that drying caused irreversible changes in the alginate film structure.

All the dried and reconditioned films could be tested after conditioning at 57% and 100% RH, with the exception of the alginate films that were not gelled with calcium. After conditioning at 100% RH, the un-gelled alginate films were very sticky and unable to hold their shape for testing. For this reason, no mechanical or optical tests were performed on these films. As expected, differences in puncture force were seen between the films conditioned at 57% and 100% RH (Fig. 1). Interestingly, the trend was not consistent for all treatments. In the case of the gelled alginate and alginate-pectin films, the films conditioned at 100% RH required less force to puncture than those conditioned at 57% RH, while the opposite was true for the alginate-SPI films. REMUNAN-LOPEZ and BODMEIER (1997) also explored the differences in puncture strength between 'dry' and 'wet' alginate films. They found that 'wet' films had lower puncture strength than 'dry'

films, which they attributed to the plasticizing effect of water. A similar trend was expected in this work. It should be noted that the 'wet' films in their study were immersed in water as opposed to exposed to humid air, which may have influenced the uptake of water by the films and thus their resulting mechanical properties.

Table 1. Mechanical properties of dried calcium-alginate (ALG) films with and without low methoxyl pectin (LMP) or soy protein isolate (SPI) conditioned at 57% and 100% relative humidity.

Treatment	Distance to Puncture (mm)	Work to Puncture (N mm)	Tensile Strength (MPa)	Elongation at Break (%)	Young's Modulus (MPa)	Thickness (um)
ALG (no Ca) 57	3.2±0.3 ^c	7.3±0.4 ^c	90.9±32.8 ^a	3.7±1.3 ^b	2729.2±298.4 ^a	0.007±0.002 ^c
ALG 57	6.6±0.5 ^b	29.4±1.9 ^b	30.0±5.2 ^b	5.8±1.3 ^b	867.2±294.0 ^b	0.018±0.002 ^b
ALG 100	11.3±0.5 ^a	39.5±4.3 ^a	9.9±0.3 ^b	12.0±2.9 ^a	179.7±15.5 ^c	0.020±0.001 ^b
ALG LMP 57	5.7±0.3 ^b	27.8±3.5 ^b	38.8±5.7 ^b	4.6±0.6 ^b	1190.9±228.1 ^b	0.018±0.002 ^b
ALG LMP 100	11.9±0.1 ^a	41.0±1.8 ^a	9.9±1.5 ^b	12.3±2.4 ^a	178.7±45.2 ^c	0.018±0.002 ^b
ALG SPI 57	2.7±0.4 ^c	4.8±1.1 ^c	24.5±3.5 ^b	3.2±0.3 ^b	989.6±265.6 ^b	0.035±0.002 ^a
ALG SPI 100	11.6±0.3 ^a	31.6±2.4 ^b	4.4±0.1 ^b	16.1±3.2 ^a	64.5±4.4 ^c	0.034±0.001 ^a

Means±standard deviation, * show significant differences (P<0.05) between means (n=18).

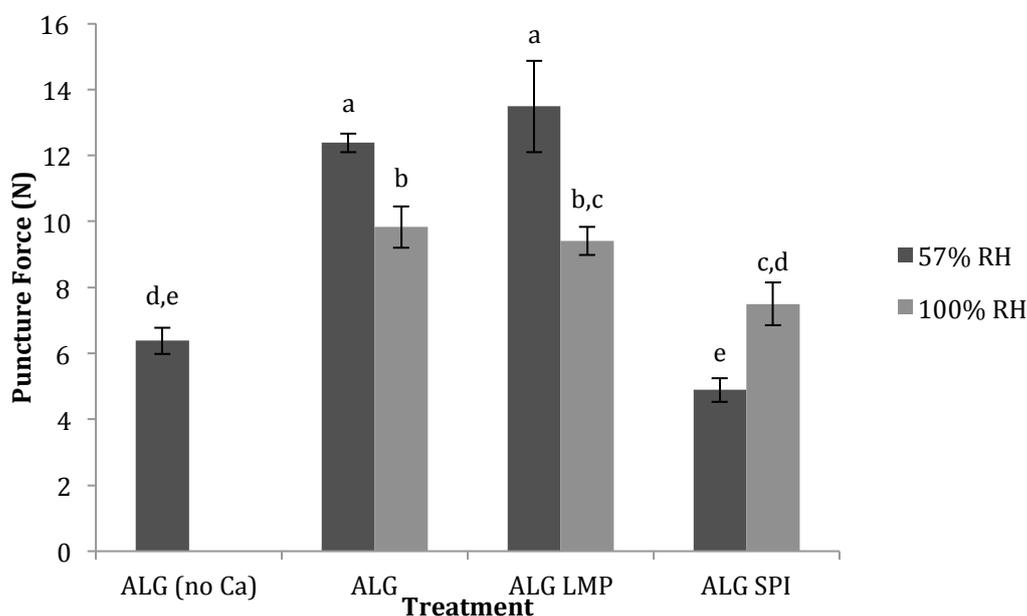


Figure 1. Puncture force of dried calcium-alginate (ALG) films with and without low methoxyl pectin (LMP) or soy protein isolate (SPI) conditioned at 57% and 100% relative humidity (RH). Note: ALG with no Ca does not form a film and hence could not be evaluated.

* show significant differences (P<0.05) between means (n=18).

The puncture force trends between treatments are similar to what we have previously reported for the 'wet' alginate films (HARPER *et al.*, 2015). In both the dried (57 & 100% RH) and 'wet' alginate films, it took significantly less force to puncture the alginate-SPI films (3.8N) than the pure alginate films (6.3N). This may be a result of the soy proteins interrupting the alginate film network. No differences in puncture force existed between the alginate-pectin (5.8N) and pure alginate films (5.3N) for the 'wet' or dried films (HARPER *et al.*, 2015). As expected, the un-gelled pure alginate films had significantly lower puncture force than the gelled pure alginate films. Similarly, it has been reported that 'dry' alginate films gelled with calcium have increased tensile strength over their un-gelled counterparts (FAZILAH *et al.*, 2011).

Differences in the puncture distance were also observed between the dried films conditioned at 57% and 100% RH (Table 1). In all cases, the films conditioned at 100% RH showed a greater distance to puncture than the films conditioned at 57% RH. Again, this is likely due to the plasticizing effect of water in the 100% RH conditioned films. Although no differences were observed between the three 100% RH treatments, the alginate and alginate-pectin films conditioned at 57% RH showed significantly higher puncture distance values than the alginate-SPI and un-gelled alginate films conditioned at 57% RH. Similar trends were observed for 'wet' alginate films where the pure 'wet' alginate films had significantly higher puncture distance values (17mm) than 'wet' alginate-SPI films (13mm) (HARPER *et al.*, 2013), but not significantly different values from the 'wet' alginate-pectin films (17mm) (HARPER *et al.*, 2015). The work to puncture results followed a similar pattern as the puncture distance, with the films conditioned at 100% RH having significantly higher work values than those conditioned at 57% RH (Table 1). Both the alginate-SPI and un-gelled alginate films conditioned at 57% RH required less work to puncture than the pure alginate films conditioned at 57% RH. No difference in the work to puncture existed between the alginate-pectin and pure alginate films conditioned at either relative humidity.

Tensile testing is another method used to measure the strength and elasticity of biopolymer films. Fewer differences were seen in the tensile strengths of the dried films gelled with calcium and then reconditioned at either 57% or 100% RH (Table 1). Our previous work indicated that, 'wet' alginate pectin films had significantly higher tensile strength than the pure 'wet' alginate films. This was also the case here when the films conditioned at 57% RH were compared. In general, it was expected that films conditioned at 57% RH would have higher tensile strength than those conditioned at 100% RH. Other work has shown that increasing the relative humidity of 'dry' calcium-alginate films decreases their tensile strength (OLIVAS and BARBOSA-CÁNOVAS, 2008). However, the authors also reported that this effect was less pronounced when plasticizers such as glycerol, sorbitol, fructose or PEG-8000 were absent from the film formulations. They argued that the non-plasticized films had a reduced capacity to absorb water and thus their mechanical properties were less influenced by changes in relative humidity. In the current work, plasticizers were not used. This may explain why more differences between the 57% and 100% RH films tensile strengths were not observed.

The tensile strengths of the Ca-alginate films in the present study were lower than some of the values reported in the literature for 'dry' alginate films. Tensile strengths of 85.9, 64.7, and 24.1 MPa were reported for dried glycerol-plasticized calcium-alginate films conditioned at 50%, 56%, and 98% RH, respectively (RHIM, 2004; OLIVAS and BARBOSA-CÁNOVAS, 2008). In each of these cases, the alginate films were dried prior to being immersed into CaCl₂. Alternatively, the films in the present study were gelled with CaCl₂ prior to drying, which may account for some of the differences observed between the

studies. The percent elongation at break (% EAB) values in the present study (Table 1) were similar to those reported for other 'dry' alginate and alginate composite films (RHIM, 2004; OLIVAS and BARBOSA-CÁNOVAS, 2008; FAZILAH *et al.*, 2011; GOHIL, 2011; GALUS and LENART, 2013).

Similar to the puncture distance, the % EAB was significantly higher for films conditioned at 100% RH than films conditioned at 57% RH (Table 1). Unlike the puncture distance, no significant differences were found between the various films conditioned at 57% RH. Contrarily, GALUS and LENART (2013) reported higher elasticity values for blended 'dry' alginate-pectin films than pure alginate or pure pectin 'dry' films. GOHIL (2011) also reported that adding up to 60% low methoxyl pectin improved the elasticity of 'dry' alginate films. Additionally our previous work on 'wet' alginate films showed that alginate-pectin films had significantly higher % EAB than pure alginate films (HARPER *et al.*, 2015). Therefore, it appears that any benefit low methoxyl pectin imparted on the elasticity of 'wet' alginate films was lost when the films were dried. On the other hand, adding soy protein isolate to the alginate films did not influence the % EAB of either the 'wet' (HARPER *et al.*, 2013) or dried (57% 100% RH) films.

The Young's modulus is a measurement of the film's stiffness. Similar to the tensile strength, the un-gelled alginate film had the highest Young's modulus of all of the films (Table 1). No differences in modulus were seen between the gelled alginate, alginate-pectin and alginate-SPI films conditioned at the same relative humidity, but in all three cases the films conditioned at 57% RH had higher stiffness values than those conditioned at 100% RH. The alginate-SPI films were significantly thicker than the alginate-pectin and gelled alginate films while the un-gelled alginate films were the thinnest (Table 1). Since the un-gelled films were rolled between two sheets of plastic (with a roller), and not submerged in a CaCl₂ bath, these films were thinner than the gelled films prior to drying and thus, it is no surprise that the dried un-gelled films were thinner than the dried gelled films.

All the dried films (conditioned at both RH) in the present study had higher puncture force and tensile strength values than their corresponding 'wet' films produced in earlier work. For instance, the 'wet' alginate-SPI films had a tensile strength of 1.6 MPa (HARPER *et al.*, 2013), while the dried alginate-SPI films had tensile strengths of 24.5 and 4.4 MPa for the films conditioned at 57 and 100% RH, respectively. On the other hand, all the 'wet' films had higher puncture distance and % EAB than their corresponding dried (57 & 100% RH) films. The % EAB values in the present study ranged from ~3 to 16% whereas those previously reported for 'wet' alginate films were ~80 to 140%. Therefore, it can be concluded that drying the films improved their puncture and tensile strength but decreased their elasticity. As mentioned before, even after rehydration the films did not have the same properties as the original 'wet' films.

3.2. Films optical properties

With the exception of the un-gelled alginate films, all of the films conditioned at 100% RH were more transparent than the films conditioned at 57% RH (Fig. 2). Visually, the alginate-pectin, alginate-SPI and pure alginate films conditioned at 57% RH were a whitish colour. On the other hand, the pure alginate and alginate-pectin films conditioned at 100% RH were clear and transparent (upper lines in Fig. 2), while the alginate-SPI films conditioned at 100% RH had a slight yellowish-brown tint to them. It is believed that the whitish colour of the films conditioned at 57% RH was a result of salt crystals on the surface of the film. This hypothesis was supported by SEM images which showed very

small salt crystals on the surface of the alginate-SPI, alginate-pectin and pure alginate films conditioned at 57% RH (images not shown). It would also explain why the un-gelled alginate films conditioned at 57% RH were much more transparent than all of the other films conditioned at 57% RH. At both relative humidity conditions, the alginate-SPI films appeared to be least transparent of the films, although a greater difference was seen for the films conditioned at 100% RH. The alginate-pectin films conditioned at 57% RH also appeared to be slightly less transparent than the pure alginate films. This is in agreement with other researchers who have reported 'dry' alginate films to be more transparent than 'dry' pectin films (PARRIS *et al.*, 1995; DA SILVA *et al.*, 2009; GALUS and LENART, 2013).

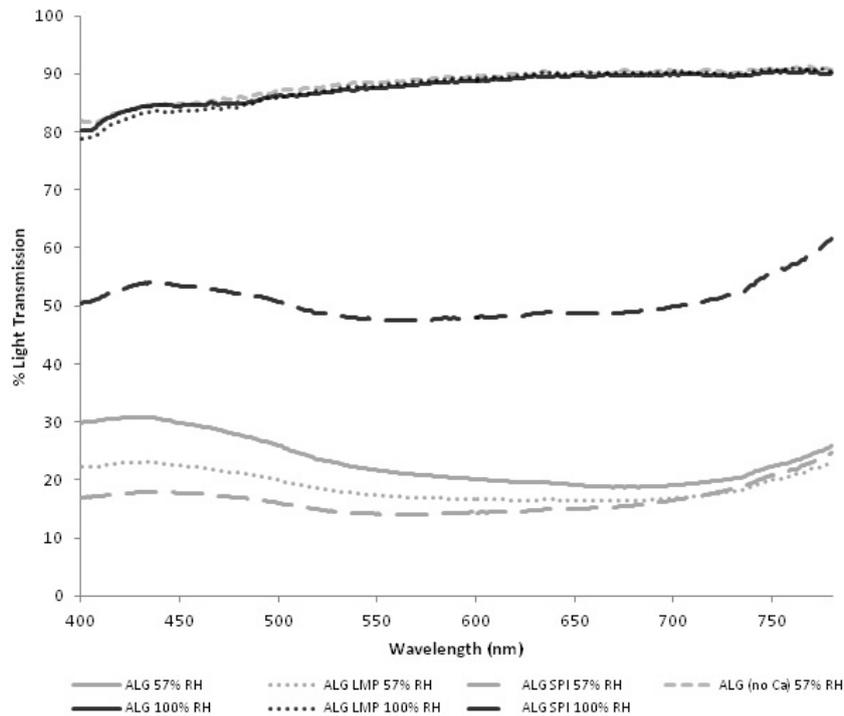


Figure 2. Visible light transmission (n=18) of dried calcium-alginate (ALG) films with and without low methoxyl pectin (LMP) or soy protein isolate (SPI) conditioned at 57% and 100% relative humidity.

3.3. FTIR

The ATR-FTIR spectra of the alginate, and alginate-pectin film forming solutions as well as the 'wet' and dried (57% RH) films are shown in Fig. 3. In addition, the alginate and pectin dry powders that were used to make the films were scanned (Fig. 3).

Table 2 summarizes the main vibrational peaks of the various spectra in the fingerprint region (1750-800 cm^{-1}). Assignments of the peaks was based on literature (SARTORI *et al.*, 1997; KACURAKOVA *et al.*, 2000; PEREIRA *et al.*, 2009; PAPAGEORGIOU *et al.*, 2010). Calcium is known to interact with the carboxylate groups in the alginate. The two absorbance bands associated with the symmetric and asymmetric stretching vibration of the COO^- groups of alginate occurred at 1426-1414 cm^{-1} and 1597-1591 cm^{-1} for the films and film forming solutions. This is in agreement with other studies that reported these two

peaks to occur in the 1422-1404 cm^{-1} and 1622-1596 cm^{-1} ranges (SARMENTO *et al.*, 2006; MOHAMADNIA *et al.* 2007; JAYA *et al.*, 2009; PAPAGEORGIU *et al.*, 2010; PAŞCALĂU *et al.*, 2012). It should be noted that there were actually two peaks detected in the 1600 cm^{-1} region in the present study; however, the peak at 1635-1631 cm^{-1} has been attributed to the water in the films as it was not present in the alginate powder spectra (Fig. 3).

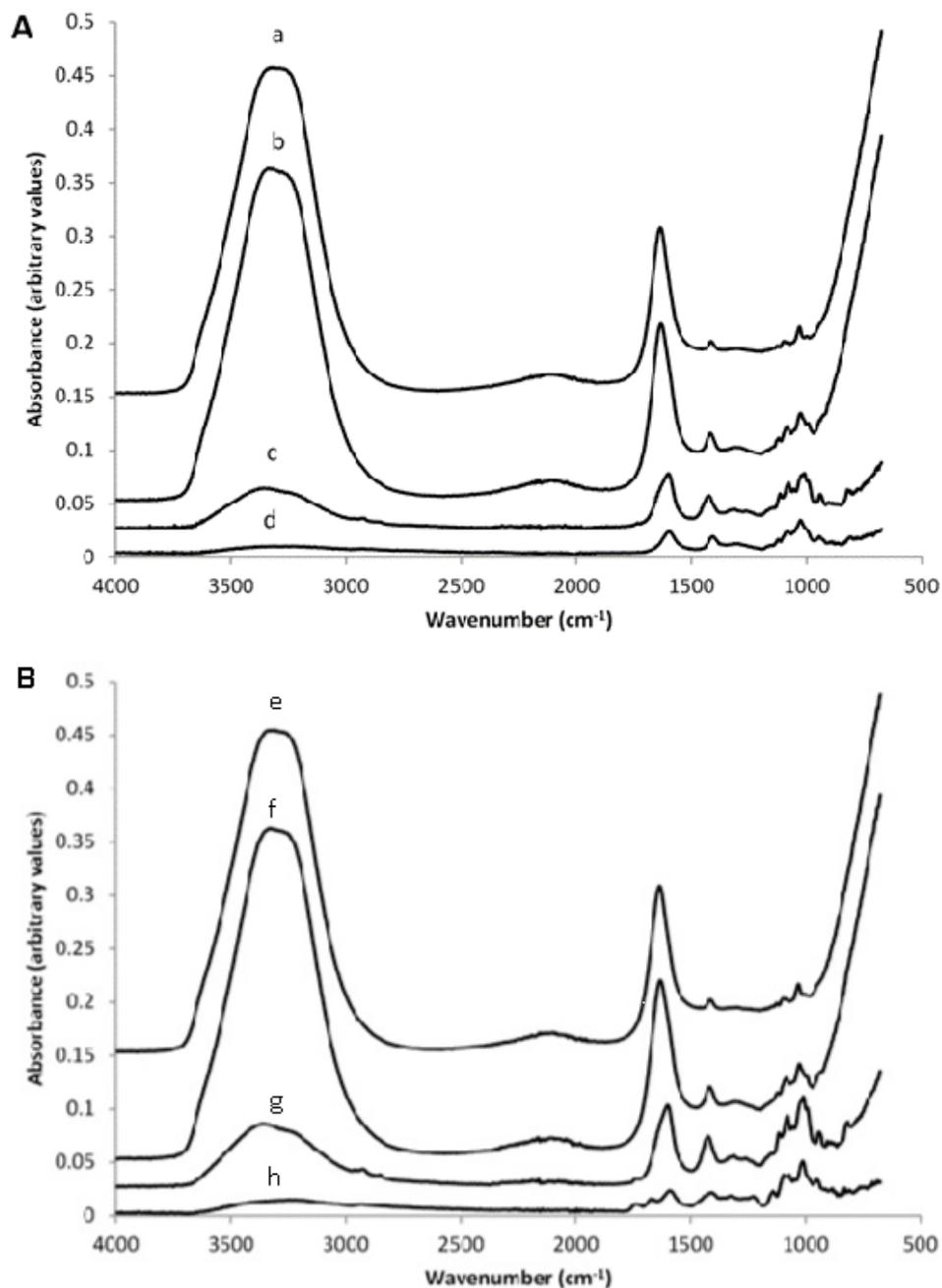


Figure 3. ATR-FTIR spectra in the 4000-675 cm^{-1} range of alginate (A), and alginate-low methoxyl pectin (B). Film forming solutions (a, e); ‘wet’ films (b, f); ‘dry’ films (c, g); and alginate (d), and low methoxyl pectin (h) powders.

Table 2. Assignment of the main vibrational peaks for the ATR-FTIR spectra in the 1750-800 cm^{-1} range.

Vibration ^a	Alginate				Alginate-Low Methoxyl Pectin			
	Film Forming Solution	'Wet' Film	'Dry' Film	Alginate Powder	Film Forming Solution	'Wet' Film	'Dry' Film	Pectin Powder
C=O stretching								1743
-								1676
-	1635	1632	1633s		1633	1632	1631s	
Asymmetric COO ⁻ stretching	1597s	1591s	1596	1594	1596s	1594s	1595	1589
Symmetric COO ⁻ stretching	1414	1418	1426	1410	1414	1418	1422	1441s 1408
C-O stretching			1312	1318			1312	1326
S=O stretching								1224
C-O, C-C & C-O-C stretching	1126	1125	1117	1124	1126	1124	1117	1141
	1102	1085	1080	1088	1101	1086	1081	1100
	1032	1028	1028	1027	1032	1028	1028	1072
			1010				1010	1047
-	999	992	989	995s	1000	993	989	1013
C-O stretching	950	945	941	944	949	944	939	947
C-O of 3,6-anhydrogalactose								886
C-O-SO ₃ on C4 of galactose								830
Mannuronic acid residues?			824	811			822	

^aSARTORI *et al.*, 1997; KACURAKOVA *et al.*, 2000; PEREIRA *et al.*, 2009; PAPAGEORGIU *et al.*, 2010.
s - shoulder peak.

For both treatments, the peak associated with the symmetric stretching vibration of the COO⁻ shifted to a higher wavenumber (1414-1415 cm⁻¹ to 1418-1420 cm⁻¹) when the film forming solution was gelled with calcium. PAŞCALĂU *et al.* (2012) reported a similar shift when 'dry' alginate-kappa-carrageenan films were gelled with calcium. SARTORI *et al.* (1997) also observed an increase in wavenumber of the symmetric stretching vibration of the COO⁻ when sodium alginate was gelled with calcium. They stated that a peak shift should be expected as the environment around the carboxyl group changes when Na⁺ ions are replaced by Ca²⁺ ions, since the two ions have different charge densities, radii and atomic weights. A second shift of the peak associated with the symmetric stretching vibration of COO⁻ to higher energy (wavenumber) was observed when the gelled 'wet' films were dried. In this case, the peak shifted from 1418-1420 cm⁻¹ to 1422-1426 cm⁻¹. Shifts in the peak associated with the asymmetric stretching vibration of the COO⁻ on the alginate were less defined.

Several other peak shifts occurred when the alginate and composite solutions were gelled with calcium into 'wet' films. These included shifts from 1102-1101 cm⁻¹ to 1086-1085 cm⁻¹, 1032 cm⁻¹ to 1028 cm⁻¹, and 1000-999 cm⁻¹ to 993-992 cm⁻¹. SARTORI *et al.* (1997) also reported shifts towards lower wavenumbers for several peaks in the 1150-1000 cm⁻¹ region when sodium alginate was gelled with calcium. They suggested that the shift to lower frequencies was caused by weakening in the C-C and C-O bonds, likely due to these

bonds being shared with calcium ions. They also found a new peak at 1010 cm^{-1} when sodium alginate was gelled with calcium. While this peak was not observed in the 'wet' films in the current work, it was present in the spectra of the dried films. Other peaks that were only observed in the dried film spectrum include the peaks at 1312 cm^{-1} and 824-820 cm^{-1} . It is suspected that these peaks were masked by the large percentage of water (~95%) in the 'wet' films. Drying the 'wet' films also caused shifts towards lower frequencies of several peaks. These included shifts from 1125-1124 cm^{-1} to 1117 cm^{-1} , 1086-1085 cm^{-1} to 1081-1080 cm^{-1} , and 993-992 cm^{-1} to 989 cm^{-1} .

Overall, there were no differences observed between the alginate, and alginate-pectin treatments in either the film or the film forming solutions' spectrum. It was expected that a peak around 1760-1740 cm^{-1} would be observed in the alginate-pectin treatments due to the esterified carboxyl groups of the pectin (ISMAIL *et al.*, 2012). It is thought that because the amount of pectin added to the alginate was low (0.25%), the intensity of these peaks was too low to be detected in the analysis of the composite film and film forming solution spectra.

4. CONCLUSIONS

The results demonstrate that water plays a critical role in determining the mechanical properties of alginate films. Dried alginate films conditioned at 57% RH had different mechanical and optical properties than their corresponding dried alginate films conditioned at 100% RH. While drying gelled 'wet' alginate films appeared to increase their puncture and tensile strength, it also decreased their elasticity. Rehydrated dried films did not have the same properties as the original 'wet' films, suggesting that drying caused irreversible changes in the alginate film structure.

Dried alginate-pectin films did not show significantly different puncture or tensile properties compared to pure Ca-alginate films at either 57% or 100% RH. This was contrary to some of our earlier work on 'wet' films which showed 'wet' alginate-pectin films to have higher tensile stress, % EAB, and puncture work than pure 'wet' Ca-alginate films (HARPER *et al.*, 2015). Therefore, any benefits low methoxyl pectin imparted on 'wet' alginate films, appeared to be lost once the films were dried. On the other hand, adding SPI to both the dried and reconditioned films (57% & 100% RH), as well to 'wet' alginate films, decreased their puncture force and work but did not affect the films' tensile properties. Adding low methoxyl pectin did not cause any detectable differences in the FTIR spectra of the films or film forming solutions in the 1750-800 cm^{-1} region. However, for both treatments, peak shifts were detected when the film forming solution was gelled with Ca^{2+} . These results suggest that inferences on the behaviour of dried alginate composite films cannot necessarily be drawn from the results of corresponding 'wet' alginate composite film, and this is important for further development of such composite films.

ACKNOWLEDGEMENTS

The authors would like to thank the Ontario Ministry of Agriculture, Food and Rural Affairs for providing the funding, as well as Drs. Marcone and Lim for being on the supervisory committee.

REFERENCES

- Anonymous. 2012. Townsend further processing - QX quality coextrusion. www.townsend.nl/aspx/products/qx.aspx?pcid=6
- ASTM. 2010. Standard test method for tensile properties of thin plastic sheeting, method D882-10. ASTM, Philadelphia, PA.
- Barbut S. 2015. Principles of meat processing. In "The Science of Poultry and Meat Processing". S. Barbut (Ed.), p. 13-1. www.poultryandmeatprocessing.com
- Bierhalz A.C.K., Da Silva M.A. and Kieckbusch T.G. 2012. Natamycin release from alginate/pectin films for food packaging applications. *J. Food Eng.* 110(1):18-25.
- Cutter C.N. 2006. Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods. *Meat Sci.* 74(1):13-142.
- Da Silva M.A., Bierhalz A.C.K. and Kieckbusch T.G. 2009. Alginate and pectin composite films crosslinked with Ca²⁺ ions: effect of the plasticizer concentration. *Carbohydr. Polym.* 77(4):736-742.
- Fang, Y. Al-Assaf S., Phillips G.O., Nishinari K., Funami T., Williams P.A. and Li L. 2007. Multiple steps and critical behaviors of the binding of calcium to alginate. *J. Phys. Chem. B*, 111(10):2456-2462.
- Fazilah A., Maizura M., Abd Karim A., Bhupinder K., Rajeev B., Uthumporn U. and Chew S.H. 2011. Physical and mechanical properties of sago starch--alginate films incorporated with calcium chloride. *Int. Food Res. J.* 18(3):985-991.
- Galus S. and Lenart A. 2013. Development and characterization of composite edible films based on sodium alginate and pectin. *J. Food Eng.* 115(4):459-465.
- Gohil R.M. 2011. Synergistic blends of natural polymers, pectin and sodium alginate. *J. Appl. Polym. Sci.* 120:2324-2336.
- Harper B.A., Barbut S., Lim L.T. and Marcone M.F. 2013. Characterization of 'wet' alginate and composite films containing gelatin, whey or soy protein. *Food Res. Int.* 52(2):452-459.
- Harper B.A., Barbut S., Smith A.K. and Marcone M.F. 2015. Mechanical and microstructural properties of 'wet' alginate and composite films containing various carbohydrates. *J. Food Sci.* 80:E84-E92.
- Ismail N.S.M., Ramli N., Hani N.M. and Meon Z. 2012. Extraction and characterization of pectin from dragon fruit (*Hylocereus polyrhizus*) using various extraction conditions. *Sains Malays.* 41(1):41-45.
- Janjarasskul T. and Krochta J.M. 2010. Edible packaging materials. *Annu. Rev. Food Sci. Technol.* 1:415-448.
- Jaya S., Durance T.D. and Wang R. 2009. Effect of alginate-pectin composition on drug release characteristics of microcapsules. *J. Microencapsul.* 26(2):143-153.
- Kacurakova M., Capek P., Sasinkova V., Wellner N. and Ebringerova A. 2000. FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses. *Carbohydr. Polym.* 43(2):195-203.
- Lee B.B., Bhandari B.R., and Howes T. 2018. Gelation of an alginate film via spraying of calcium chloride droplets. *Chem. Eng. Sci.* 183:1-12.
- Mohamadnia Z., Zohuriaan-Mehr M.J., Kabiri K., Jamshidi A. and Mobedi H. 2007. pH-sensitive IPN hydrogel beads of carrageenan-alginate for controlled drug delivery. *J. Bioact. Compat. Pol.* 22(3):342-356.
- Mørch Ý.A., Donati I., Strand B.L. and Skjåk-Bræk G. 2006. Effect of Ca²⁺, Ba²⁺, and Sr²⁺ on alginate microbeads. *Biomacromolecules* 7(5):1471-1480.
- Nura A. 2018. Advances in food packaging technology- A review. *J. Postharvest Techno.* 6(4):55-64.
- Olivas G.I. and Barbosa-Cánovas G.V. 2008. Alginate-calcium films: water vapor permeability and mechanical properties as affected by plasticizer and relative humidity. *LWT-Food Sci. Technol.* 41(2):359-366.
- Ouwerx C., Velings N., Mestdagh M.M. and Axelos M.A.V. 1998. Physico-chemical properties and rheology of alginate gel beads formed with various divalent cations. *Polym. Gels Networks* 6(5):393-408.

- Papageorgiou S.K., Kouvelos E.P., Favvas E.P., Sapalidis A.A., Romanos G.E. and Katsaros F.K. 2010. Metal-carboxylate interactions in metal-alginate complexes studied with FTIR spectroscopy. *Carbohydr. Res.* 345(4):469-473.
- Parris N., Coffin D.R., Joubran R.F. and Pessen H. 1995. Composition factors affecting the water vapor permeability and tensile properties of hydrophilic films. *J. Agric. Food Chem.* 43(6):1432-1435.
- Paşcalău V., Popescu V., Popescu G.L., Dudescu M.C., Borodi G., Dinescu A., Perhaița I. and Paul M. 2012. The alginate/k-carrageenan ratio's influence on the properties of the cross-linked composite films. *J. Alloys Compd.* 536:S418-S423.
- Pereira L., Amado A.M., Critchley A.T., Van de Velde F. and Ribeiro-Claro P.J. 2009. Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman). *Food Hydrocoll.* 23(7):1903-1909.
- Remunan-Lopez C. and Bodmeier R. 1997. Mechanical, water uptake and permeability properties of crosslinked chitosan glutamate and alginate films. *J. Controlled Release* 44(2-3):215-225.
- Rhim J.W. 2004. Physical and mechanical properties of water resistant sodium alginate films. *LWT-Food Sci. Technol.* 37(3):323-330.
- Rhim J.W. and Ng P.K. 2007. Natural biopolymer-based nanocomposite films for packaging applications. *Crit. Rev. Food Sci.* 47(4):411-433.
- Sarmiento B., Ferreira D., Veiga F. and Ribeiro A. 2006. Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies. *Carbohydr. Polym.* 66(1):1-7.
- Sartori C., Finch D.S., Ralph B. and Gilding K. 1997. Determination of the cation content of alginate thin films by FTIR spectroscopy. *Polym. J.* 38(1):43-51.
- Shih F.F. 1994. Interaction of soy isolate with polysaccharide and its effect on film properties. *J. Am. Oil Chem. Soc.* 71(11):1281-1285.
- Stephen A.M., Phillips G.O. and Williams P.A. 2006. "Food Polysaccharides and their Applications" (2nd ed). CRC Press, New York, NY.
- Tong Q., Xiao Q. and Lim L.T. 2008. Preparation and properties of pullulan-alginate-carboxymethylcellulose blend films. *Food Res. Int.* 41(10):1007-1014.
- Villagómez-Zavala D.L., Gómez-Corona C., Martínez E.S.M., Pérez-Orozco J.P., Vernon-Carter E. J. and Pedroza-Islas R. 2008. Comparative study of the mechanical properties of edible films made from single and blended hydrophilic biopolymer matrices. *Rev. Mex. Ing. Quím.* 7(3):263-273.
- Visser P.R. 2012. Casings for foodstuffs U.S. patent application US 2012/0114807 A1.
- Wang L., Auty M.A.E. and Kerry J.P. 2010. Physical assessment of composite biodegradable films manufactured using whey protein isolate, gelatin and sodium alginate. *J. Food Eng.* 96(2):199-207.
- Xiao Q., Lim L.T. and Tong Q. 2012. Properties of pullulan-based blend films as affected by alginate content and relative humidity. *Carbohydr. Polym.* 87(1):227-234.
- Xu J.B., Bartley J.P. and Johnson R.A. 2003. Preparation and characterization of alginate-carrageenan hydrogel films crosslinked using a water-soluble carbodiimide (WSC). *J. Membrane Sci.* 218(1-2):131-146.

Paper Received July 26, 2019 Accepted October 12, 2019