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Influence of surfactants and proteins on the properties of wet edible calcium alginate meat coatings

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Abbreviations

a_w: water activity

DSC: differential scanning calorimeter

Fmax: maximum force

FTIR: Fourier transform infrared

OTR: Oxygen transfer rate

TEM: Transmission electron microscopy

WVTR Water vapour transfer rate

Abstract

Calcium alginate structures are of interest as replacers for natural casings due to their high availability, biodegradability and low price. The aim of this paper is to study the effect of oil, surfactants and proteins (pea and collagen) on the water transfer, mechanical and microstructural properties of the wet calcium alginate films. The addition of oil and surfactants tended to reduce the water permeance and the weight loss rate, reaching values between those shown by natural and collagen artificial casings. The addition of proteins did not improve the adherence of the films and it decreased the maximum force of the film at puncture test, which was even lower with the presence of the surfactant E475. The TEM micrographs showed that the differences in mechanical properties are mainly related to the differences in the compaction of the microstructure. Wet alginate films with E475 are envisaged as a substitute of natural and collagen artificial casings in the stuffed meat products industry.

Keywords: Alginate film, edible coating, water transfer, film strength, adherence, drying,

1. Introduction

Calcium alginate structures are of interest to the meat industry as replacers for natural casings due to their high availability, biodegradability and low price compared to natural casings (Frye, 1996). Alginate films are impermeable to fats and oils, but are poor moisture barriers. However, hydrophobic substances have been used to form highly water impermeable films (Carulo & Kieckbusch, 2005; Cottrell & Kovacs, 1980). Therefore, composite polysaccharide-lipid films, in emulsion or laminated forms, combine structural integrity and oxygen-barrier characteristics of polysaccharide films with moisture-barrier properties of lipid films (Hambleton, Debeaufort, Bonnotte, & Voilley, 2009; Karbowiak, Debeaufort, & Voilley, 2007; Wu, Weller, Hamouz, Cuppett, & Schnepf, 2001). Films formed with solid lipids, might become thicker and more brittle with low mechanical strength (Carulo & Kieckbusch, 2005; Li Liu, Kerry, & Kerry, 2006; Phan The, Debeaufort, Voilley, & Luu, 2009). Proteins form poor moisture barriers because of their hydrophilic nature (Hambleton et al., 2009; Li Liu et al., 2006), but they may improve particular aspects (adhesion, oil absorption …) when added to complex batters (Varela & Fiszman, 2011). The adhesiveness of a coating on food product surface mainly depends on the nature and on the number of interactions or bondings between film and support (Debeaufort, Quezada-Gallo, & Voilley, 1998). Several studies reported improved adhesion properties with protein addition to coatings and batters (Mukprasirt, Herald, Boyle, & Rausch, 2000; Suderman, Wiker, & Cunningham, 1981). Other authors reported that when pea protein interacts with polysaccharides may contribute to new functions, regarding particularly solubility and surfactant properties (S. Liu, Elmer, Low, & Nickerson, 2010) and collagen protein is expecting to exert a reinforcement effect with improved mechanical properties (Wolf, Sobral, & Telis, 2009). Thus, composite films and casings can be formulated to combine the advantages of hydrocolloid, lipid and protein components to acquire the physicochemical and mechanical properties similar to standard natural and artificial casing used in the elaboration of salami type meat products. Co-extruded alginate casings consist of a thin layer of alginate solution extruded onto the meat batter as it is being extruded from the stuffer. The coated sausage then enters a brine bath of calcium chloride to form the cross-linked wet calcium alginate casing (Harper, Barbut, Lim, & Marcone, 2013). However, this wet casing have the drawback of a limited mechanical

strength to hold the manipulation and drying process of the salamis (Arnau, Comaposada, & Grebol, 2009).

Barrier and mechanical properties depend on film microstructure, which in turn is influenced by film composition, formation and method of product containment (Li Liu et al., 2006). In order to understand the changes in the coatings, several techniques can be used to determine structural changes in the films. Among them the Fourier transform infrared (FTIR) spectroscopic techniques (Li Liu et al., 2006), the differential scanning calorimeter (DSC) (García, Martino, & Zaritzky, 2000; Kumarnaidu, Sairam, Raju, & Aminabhavi, 2005) analysis and the transmission electron microscopy (Brun et al., 2011; Wright et al., 2009).

The objective was to study the effect of surfactants and proteins on mass transfer and mechanical properties of wet alginate films used as substitute of natural and artificial casings in the stuffed meat products industry.

2. Materials and methods

Two experiments were developed. Experiment 1 studied the mass transfer of alginate films with several surfactants (E472a, E472c, E322 $_{\text{high grade}}$, E475), considering its addition into the sodium alginate solution or as a double coating on the calcium alginate film. Experiment 2 studied the combined effect of proteins (pea and collagen) and the surfactant selected in experiment 1 (E475), added into the sodium alginate solution, on the calcium alginate film. Calcium alginate films were compared with natural and artificial casings.

2.1 Experiment 1

2.1.1 Materials

Two commercial sodium alginates Protanal GP 3350 and Protanal RF 6650 from FMC BioPolymer (Drammen, Norway) were used. Technical information on these alginates was reported in Comaposada et al. (2015). E471 (mono and diglycerides of fatty acids – Verol N-20), E472a (mono-diglyceride acetylated 70% grade - Veracet 70), E472c (mono and diglycerides citric esters of fatty acids - Coris I), E475 (poliglyceride ester of fatty acids - Verol P/PH) and E322 (high grade hydrolysis lecitine - Giralec HE-60) from Lasenor Emul, S.L. (Olesa de Montserrat, Spain) , food grade anhydrous calcium chloride from Cargill Inc. (Minneapolis, MN, USA) and sunflower oil (Borgesol) from Borges Mediterranean Group, S.L.U. (Reus, Spain) were used.

2.1.2 Preparation of sodium alginate solutions and calcium alginate films

2.1.2.1 Emulsions

Solutions of 2% (w/w) of sodium alginate were prepared in deionised water using a Thermomix blender (Vorwerk, Wuppertal, Germany). The control alginate solutions were stirred at 12 °C for 2 minutes at 1500 rpm and 3 minutes at 5000 rpm. The surfactant E471 was added in melted state to the sodium alginate solution at 37 °C after 2 min of stirring. The rest of surfactants were previously stirred with hot water (37 °C for E472a and E472c and 50 °C for E322 $_{\text{high grade}}$ and E475) for 2 minutes at 1500 rpm. After cooling down to 25 ºC, the sodium alginate was incorporated together with sunflower oil, when this was required, and stirred for 3 more minutes at 5000 rpm. The alginate solutions were stored for 24 h at 12 °C in order to stabilize the temperature and to facilitate deaerating.

Alginate films were obtained using a hand-operated Thin Layer Chromatography Plate Coater (CAMAG, Muttenz, Switzerland). The gate for layer thickness was adjusted to 0.5 mm. Sodium alginate solutions were crosslinked by immersion in a 10% (w/w) $CaCl_2$ solution in water (pH 6.7) for 30 seconds at 12 °C. The calcium alginate films were covered with a high density polyethylene film to prevent dehydration until analysis.

2.1.2.2 Double coating

The calcium alginate films obtained from control solutions were covered with surfactants (E472a, E472c, E322 $_{\text{high grade}}$, E475) and/or sunflower oil by brushing (double coating).

2.1.3 Water transfer properties

2.1.3.1 Permeance

A modified method of the international standard ISO 2528:1995(E) was used to determine the water permeance of the calcium alginate films. Petri dishes (86 mm diameter and 12 mm high) sealed to their corresponding lids and containing distilled water were used. The lid had a central opening of 29 mm diameter (water transfer area) covered with a metallic mesh of 6 mm gaps. Alginate films were placed upon the metallic mesh, which were in contact with the distilled water. The petri dish was placed into a climatic chamber with air flow at 3.8 ±0.2 m/s, 13.9 ±1.1 °C and 65.4 ±4.1 % RH.

Weight loss of the petri dish was recorded for 24 h and the drying curve was used to calculate permeance according to the Equation (1).

$$P = \frac{m}{A \cdot t \cdot \Delta p} \quad (1)$$

Where m is the weight loss (g), A is the water transfer area (m^2), t is time (s) and Δp is partial vapour pressure difference (Pa) between distilled water and drying air.
Three independent films per treatment were analysed. The measurement on each film was done in triplicate.

2.1.3.2 Weight loss of minced meat mixture coated with calcium alginate films

Minced meat mixture was elaborated with lean meat ground into a 3 mm plate and mixed for 3 minutes with other ingredients and additives (salt, 20 g/kg meat; sodium nitrite, 0.15 g/kg; sodium nitrate, 0.15 g/kg; black pepper, 3 g/kg; dextrose, 2 g/kg; lactose, 20 g/kg; sodium ascorbate, 0.5 g/kg). The minced meat mixture was stuffed into 50 mm diameter plastic casings. Then sausages were frozen at -18 °C.

Three mm thick slices from the sausages were defrosted and placed individually on the bottom part of a petri dish and covered with alginate films cut at 84 mm diameter. Depending on the test, the alginate films were covered with surfactants and/or oil by brushing (double coating). The samples were located into a climatic chamber at 13.6 ±1.7 °C and 74.0 ±4.2 % RH with air flow of 3.8 ±0.2 m/s. Weight loss of the test dish was recorded for 8 h. Three independent films per treatment/casing were analysed. The measurement on each film was done in triplicate.

2.2 Experiment 2

2.2.1 Materials

The commercial sodium alginate Algogel 6021 was supplied by Cargill Inc. (Minneapolis, MN, USA). Technical information on this alginate was reported in Comaposada et al. (2015). E475 (poliglyceride ester of fatty acids - Verol P/PH) from Lasenor Emul, S.L. (Olesa de Montserrat, Spain), hydrolysed collagen from Juncà Gelatines (Banyoles, Spain), green pea protein from Provital Group (Barcelona, Spain), food grade anhydrous calcium chloride from Cargill Inc. (Minneapolis, MN, USA) and sunflower oil (Borgesol) from Borges Mediterranean Group, S.L.U. (Reus, Spain) were used.

Salted pork natural casings from Collelldevall S.L. (Banyoles, Spain) and artificial collagen casings from Fibran S.A. (Sant Joan de les Abadesses, Spain) of 50 mm diameter were used for properties comparison with respect the alginate films. Previous to the casings use, the pork natural casings were partially desalted by rinsing them with water and collagen casings were hydrated by maintaining them in a salted bath (2.5 g NaCl/ 100 g water solution) for 20 minutes.

2.2.2 Preparation of sodium alginate solutions and calcium alginate films

The alginate solutions were prepared according 2.1.2. The hydrolysed collagen and green pea protein (with or without E475) were mixed in water for 2 minutes at 1500 rpm, added to the alginate solutions and mixed for 3 minutes at 5000 rpm.
The alginate solutions were stored for 24 h at 12 °C.
Calcium alginate films were obtained according 2.1.2.1.

2.2.3 Water transfer properties

2.2.3.1 Water vapour transfer rate (WVTR)

The water vapour permeability test was performed in a LabThink Model W3-060 equipment (Labthink, 2017) at 23 ºC and 90% Relative Humidity (WVTR measurements every 5 minutes and proportional mode 10%). A multilayer system had to be performed to develop the test. The calcium alginate film sample was located between two cellulose films. Total thickness was 460 μm. A plastic washer (D.ext.73mm, D.int.30mm) was used to reduce the area of water vapour exchange. At least two independent films per treatment/casing were analysed. The measurement on each film was done in triplicate.

2.2.3.2 Water activity and sorption isotherms

Natural casings were rinsed with water to remove the salt and artificial casings were hydrated with a 15 % aprox. salt solution before water activity (a_w) determination. The a_w of alginate films was measured immediately after formation. Measurements were done at 12 °C in duplicate with a Novasina AWSPRINT-TH 500 (Axair Ltd., Pfäffikon, Switzerland) in two independent films per alginate solution/casing.
Sorption isotherms were determined gravimetrically in two independent films per treatment by exposing them to atmospheres of relative humidity controlled by different saturated salts according to the COST90 method (Wolf, Spiess, & Jung, 1985). The

measurements on each film were done in triplicate. Three saturated salts were prepared by mixing salt and distilled water in hermetic containers and stirring them once a day for 7 days. The salts used (Merck, Darmstadt, Germany) were "extra pure" quality for $MgCl_2$ $6H_2O$, $Mg(NO_3)_2$ $6H_2O$, and "for analysis" for NaCl. A_w of saturated salts at 12 °C are 0.334, 0.568 and 0.756 respectively (Greenspan, 1977). Plastic trays were used to place the films into the sorption containers. Finally, the containers were placed in incubators. The equilibrium process ended when the samples achieved constant weight. The moisture content of the wet films used for a_w and sorption isotherms measurement was immediately determined in duplicate after film formation by drying at 103 ±2 °C until constant weight (AOAC, 1980).

2.2.3.3 Weight loss of salami coated with calcium alginate films

The salami matrix was elaborated with pork shoulders and bellies (60:40). They were ground into a 5 mm plate and then mixed for 3 minutes with other ingredients and additives (salt, 20 g/kg meat; sodium nitrite, 0.15 g/kg; sodium nitrate, 0.15 g/kg; black pepper, 3 g/kg; dextrose, 2 g/kg; lactose, 20 g/kg; sodium ascorbate, 0.5 g/kg). The salami matrix was stuffed in 50 mm or 80 mm diameter plastic casings (for weight loss or adhesivity measurement respectively) and sausages were frozen at -18 °C.

The weight loss of salami was determined following the methodology described in section 2.1.3.2.

2.2.4 Oxygen transfer rate

The oxygen permeability test was performed on a LabThink Model VAC-V1. First the film sample was placed between the two chambers, fixed and sealed. Next, the vacuum was performed throughout the system and then oxygen was introduced into the upper chamber. Therefore a constant differential pressure was created and the gas penetrated from the upper to the lower chamber, through the film. From the pressure measurement in the lower chamber the oxygen transfer properties of the sample were obtained. The equipment is exclusively designed for the analysis of plastic films with higher strength and lower permeability than the films studied. Because of that, it was necessary to carry out a study to adapt the methodology of analysis. The tests at 25 ºC was performed in a multilayer system (plastic film/sample/plastic film) with 420 μm total thickness and 97 mm test area. Eight hours of vacuum previous to the beginning or the test was applied. Gas pressure 1.01 kgf/cm^2 and proportional mode was used.

A minimum of two independent films per treatment/casing were analysed. The measurement on each film was done in duplicate.

2.2.5 Film adhesivity

Three meat matrices were used: salami, described in section 2.2.3.3, pork loin and pork back fat. All of them were kept frozen at -18 °C until its use.

The adhesion of the alginate film on loin and salami was evaluated before and after drying (drying process developed following the methodology described in section 2.1.3.2), while the adhesion on pork back fat was evaluated only before drying.

The meat matrices were cut in slices (8 mm thickness, 80 mm diameter) and placed on methacrylate plate. A 30x20 mm PVC strip was placed on top of the food matrix to prevent adhesion and a gauze was placed on top (Figure 1). Alginate solution (2.5 g) was poured on top of the gauze using a 40x55x5 mm plastic mould. Alginate coatings were crosslinked using a 25 % $CaCl_2$ solution for 3 min at 12 °C. The PVC strip and the plastic mould were removed before analysis. The measurement area was kept constant by cutting the edges of the gauze at constant width.

Adhesion of alginate coatings to the different matrices was measured as the average force value needed to separate the alginate coating from the food matrix. A TA.HD Plus Texture Analyser (Stable Microsystems Ltd, Godalming, United Kingdom) with test speed set at 5 mm/s was used for analysis. The methacrylate plate with the matrix-coating system was placed vertically on the texture analyser (Figure 1). A minimum of three independent films per treatment/casing were used for the adhesivity measurement. A minimum of 6 measurements per film were performed.

2.2.6 Puncture test

The puncture test method was performed to evaluate maximum force (Fmax) and elongation (E) of calcium alginate films in its transversal direction according to the methodology described by Marcos, Gou, Arnau, and Comaposada (2016). Alginate films were cut into 7 x 3 cm strips. A TA.HD Plus Texture Analyzer (Stable Micro Systems Ltd, Godalming, UK) with test speed set at 20 mm/s was used for analysis. Puncture test was performed using a 3 mm cylinder probe and a platform with a 10 mm central opening used to place the film support.

2.2.7 Colour measurement

The colour of the alginate films was determined with a Minolta Chroma Meter CR-400 (Minolta Camera Co., Osaka, Japan) set at C illuminant and 2° standard observer. The chromameter was calibrated before each series of measurements using a white ceramic plate. Alginate films were placed on a bigger white plate that was used as a background for colour measurement. Parameters obtained were L*(lightness), a* (redness) and b* (yellowness), according to the CIE Lab (CIELab, 1976).

Three independent films per treatment/casing were analysed. The measurement on each film was done in triplicate.

2.2.8 Fourier transform infrared spectroscopy (FTIR)

Spectra of the films were obtained using a Fourier transform infrared spectrometer Nicolet™ 6700. Film samples were placed onto a diamond crystal (Smart Orbit reflexion) and spectra were taken with 32 scans recorded at 4 cm^{-1} resolution in a wavenumber range of 4000 to 400 cm^{-1}. Prior to recording the film spectra, samples were dried 24-48 h at room temperature (23 ±2 °C; 50 ±5% RH).
Two independent films per treatment/casing were analysed. The measurement on each film was done in duplicate.

2.2.9 Differential Scanning Calorimetry (DSC) analysis

The DSC analysis was done using a Diamond DSC (PerkinElmer Inc., USA). The temperature and heat calibration was done using indium, tin and zinc. Nitrogen was chosen as a purge gas at 50 mL × min-1 according to manufacturer recommendations. Sample (8 - 15 mg) was placed into sealed vented aluminum pans. Samples were heated (20 °C × min-1) from 20 °C to 400 °C.
Two independent films per treatment/casing were analysed. The measurement on each film was done in duplicate.

2.2.10 Microscope analysis

Alginate–carbohydrate films were fixed in a 2.5% glutaraldehyde in cacodylate buffer 0.1M for 90 min. To prevent the films from dissolving in the solution, 5% calcium chloride solution was added to the fixative solution. Films were rinsed four times with 5% $CaCl_2$ solution and once with milliQ water and postfixed with 1% osmium tetroxide overnight. The films were then rinsed with 0.1M cacodylate buffer before being

dehydrated in an ethanol series (50%, 70%, 90%, 96% and 100%, for 10 min each). They were infiltrated and embedded in Spurr's low-viscosity resin (EMS, Hatfield, USA). Sections of 500 nm in thickness were obtained using a UC6 ultramicrotome (Leica Microsystems, Vienna, Austria), dyed with 0.5% methylene blue and observed in an optic microscope Leica DM200 (Leica Microsystems, Vienna, Austria).
Sections of 60 nm in thickness were obtained using a UC6 ultramicrotome (Leica Microsystems, Vienna, Austria) and stained with 2% uranyl acetate and lead citrate. Sections were observed in a Tecnai Spirit microscope (EM) (FEI, Eindhoven, The Netherlands) equipped with a LaB6 cathode. Images were acquired at 120 kV with a 1376 x 1024 pixel CCD Megaview camera.

2.3 Statistical analysis

The average of the replicates was used for the statistical analysis. The effect of additive on the several alginate properties was tested with the General Linear Models procedure in the SAS program, version 9.2 (SAS Institute Inc., Cary, NC, USA), including casing/ film composition as fixed effect in the model. Least square means were calculated and the differences were tested with Tukey test.

3. Results and discussion

3.1. Effect of surfactants and double coating on water transfer properties (Experiment 1)

The calcium alginate films without additives had a permeance value of $1.74 \times 10^{-4} \pm 3.41 \times 10^{-5}$ g/m^2·s·Pa. The oil and surfactants added to the sodium alginate solution tended to reduce the permeance of calcium alginate films up to 19 % (Table 1). Studies with calcium alginate films (Benavides, Villalobos-Carvajal, & Reyes, 2012; Carulo & Kieckbusch, 2005) and other composites with alginates (Hambleton et al., 2009; Li Liu et al., 2006) showed a similar reduction of water transfer properties when lipids were added. The water transfer reduction was also tested comparing the weight loss of minced meat slices covered with alginate films containing the oil and/or surfactants (Table 2). The surfactant E475 without oil added into the alginate film can reduce the weight loss rate of the minced meat slices coated with the film. The effectiveness of this surfactant agrees with the lowest permeance shown in Table 1. All other combinations

348 of oils and surfactants did not reduce significantly the weight loss rate. Probably more
349 oil and surfactant would be needed to build up a lipid continuous phase that effectively
350 reduced the weight loss rate. Phan The et al. (2009) suggested the need of a high
351 hydrophobic/hydrophilic ratio in hydrocolloids emulsified films to avoid aggregation of
352 lipid particles and form a continuous ‘‘lipid layer’’ necessary for an effective water
353 transfer barrier.
354 When the surfactants were coated by brushing onto the alginate film (double coating of
355 the meat slice), the weight loss rate of the minced meat slices was significantly reduced
356 (Table 3). Surfactant with oil at low concentration (0.1 kg surfactant /kg oil) was easily
357 extended onto the alginate film due to its low viscosity and low amount of coating
358 applied (Table 3). At higher concentration (0.5 kg surfactant /kg oil) viscosity was
359 higher and higher amount of coating was applied. When surfactant was used without oil,
360 even higher amount of coating was used due to its increased viscosity. The amount of
361 coating is related to the thickness of the coat, which affects the weight loss rate. From
362 an industrial point of view, the most effective coatings would probably be the ones
363 made of oil with the surfactant E472a or E475 at low concentration, because less
364 surfactant and less amount of coating is required to have a significant reduction of the
365 weight loss rate. Surfactants E322 and E472c need to be used at the high concentration
366 to be as effective as the previous surfactants. Wu et al. (2001) also reported less weight
367 loss in the beef patties packaged with films double coated with tocopherol (hydrophobic
368 molecule) compared to the ones with the tocopherol into the emulsion of the film.
369 Karbowiak et al. (2007) described that emulsion-based films are less efficient against
370 water transfer than bilayer films because of the non-homogeneous distribution of lipids.
371 However, they have the advantages to require a single step during the manufacture and
372 application process against one step per layer for multilayer films. It has been shown for
373 emulsion-based films that the smaller and the more homogeneously distributed the lipid
374 globules are, the lower the water vapour permeability is.

375 ## 3.2. Properties of alginate film with proteins (Experiment 2)

376 After experiment 1 results, E475 was selected to be used in experiment 2.

377 ### 3.2.1 Water transfer

378 #### 3.2.1.1 Water vapour transfer rate (WVTR)

The water vapour transfer rate of the natural, artificial collagen casings, and calcium alginate films with or without additives (pea and collagen proteins and E475) were not significantly different (Table 4). Harper et al. (2013) also reported no influence of addition of most proteins to alginate composites films on WVTR.

3.2.1.2 Water activity and sorption isotherms

The water content at equilibrium before drying in natural and artificial casings were lower than in alginate films (Table 5). The differences are in part attributed to the different a_w, to the different type and concentration of salts in each film/casing and to the desalting and hydration processes of natural and artificial casings respectively. Calcium chloride was added to alginate films while sodium chloride was added to natural and artificial casings. Comaposada, Gou, and Arnau (2000) reported in meat isotherms the increase of water content with salt content. These authors also reported a breaking point in salted meat isotherms at a water activity below 0.75. The NaCl solution crystallizes below a water activity of 0.75 (its saturation point) and the crystalized NaCl absorbs little or no water. Though the natural casing were desalted, a higher salt content is expected in comparison to the collagen artificial casings. This can explain that the natural casing retains more water than the collagen artificial casing at high a_w, while at a_w below 0.75 it retains less water.

Alginate films tended to reduce the water content with the addition of proteins and with the addition of surfactant E475, which suggest a higher sorption capacity of alginate with respect proteins or E475.

3.2.1.3 Weight loss of salami coated with calcium alginate films

The weight loss rate of salami slices was higher with natural casing than with artificial collagen casing. With alginate films it was slightly higher than with natural casings when E475 was not added and slighly lower when E475 was added. However, it was higher with alginate films than with artificial collagen in all cases (Table 4). The addition of pea and collagen protein into the alginate film did not affect the weight loss rate of the salami slices during drying. Several publications have already reported the diminution of the water transfer in coatings when fats are used, while proteins are low efficient barrier against water transfer (Debeaufort et al., 1998; Hernandez-Izquierdo & Krochta, 2008).

Salami slices without any casing had lower weight loss rate than the salami slices coated with alginate films. The surface meat proteins of the salami slices could have changed its structure with the loss of water altering the water transfer properties, while the alginate coating could protect the salami surface avoiding the changes on the water transfer properties.

3.2.2 Oxygen transfer rate

The results obtained with the methodology of the equipment LabThink Model VAC-V1 showed that the oxygen transfer rate was the lowest in the natural casing and the highest in the alginate control film (Table 4). The presence of proteins and the E475 surfactant tended to reduce this rate of oxygen transfer. In fact, protein films exhibit better oxygen barriers than polysaccharide films (Bourtoom, 2008; Skurtys et al., 2011), while different studies reported different behavior of the lipids on the oxygen permeability (García et al., 2000; Hambleton et al., 2009; Kowalczyk & Baraniak, 2014; Ruban, 2009).

3.2.3 Film adhesivity

The loin matrix showed a tendency to better adhere to the different alginate films when compared to the other matrices, except to the pork back fat matrix when proteins were added to the alginate (Table 6). This fact is probably due to the highest cohesiveness of the matrix and the hydrophilic behaviour of the alginate and the loin. The adhesivity of alginate without proteins was lower in the fat matrix, as well as with the minced meat matrices like salami, which also contains fat. It was considered that the protein could act as a binding agent between the film and the meat matrix, in agreement with Nussinovitch and Hershko (1996) results, which demonstrated that chemical similarity can contribute to better adhesion or better compatibility between the support and the coating. The use of collagen improved adhesion of a batter mix onto meat and fish (Debeaufort et al., 1998), and similarly other studies concluded that proteins help adhesion (Mukprasirt et al., 2000; Suderman et al., 1981; Varela & Fiszman, 2011). However, the proteins generally did not improve the adherence of the films, neither the surfactant E475. Only in pork back-fat matrix the addition of proteins without E475 had significantly increased the adherence of the film.

The adherence of film/casing on dried salami was higher with natural and artificial casings than with calcium alginate films. In our calcium alginate films, only 1% is

protein, while in natural casing the protein content can reach 11%. This big difference on protein content could explain the imperceptible effect of the protein addition to the alginate solution on the adherence properties.

3.2.4 Puncture test

The maximum force at the puncture tended to decrease with the presence of proteins (pea and collagen), and the decrease was even higher with the presence of the surfactant E475 (Table 7). Other studies reported the decreasing puncture force due to protein addition to alginate films (Harper et al., 2013) and also due to lipid addition (Kowalczyk & Baraniak, 2014), as a consequence of the development of an heterogeneous structure, where lipid particles lead to discontinuities in the polymer network. Elongation was generally less affected by the addition of proteins, which agrees with the results reported by Harper et al. (2013). The addition of surfactant only reduced elongation in alginate film with pea protein. Different behaviours have also been found when lipids are added (Kowalczyk & Baraniak, 2014) to alginate films. The Fmax values of alginate films were much lower than those of natural (10.30±2.71 N) and artificial (18.97±3.61 N) casings. The elongation of natural casings was higher (80.62±14.92 %), while artificial casings (17.52±5.61 %) presented values closer to that of the alginate films. However, properties of artificial collagen casings may vary among different manufacturers (Harper, Barbut, Lim, & Marcone, 2012).

3.2.5 Colour measurements

Any of the casings or films increased the lightness (L*) of the salami slice without film (Table 8). Natural casing showed the highest L* values, while the artificial casing showed similar results to the alginate films. The surfactant with the proteins tended to increase the lightness and to reduce the redness (a*) and yellowness (b*). The redness of the salami slice without film was reduced when natural and artificial casing were used, but it was less affected with alginate films. The redness was not affected by the proteins used into the alginate emulsion. The yellowness is reduced by most of the casing and films used. Other studies developed in meat products reported results in a similar direction (L. Liu, Kerry, & Kerry, 2007; Santos, Müller, Laurindo, Petrus, & Ferreira, 2008). Lightness increase has been attributed to a high surface moisture of the alginate coating, while the a* and b* values is considered to be affected by the natural redness and yellowness associated with the different casing types (L. Liu et al., 2007).

In fact, alginate wet films are clear and transparent after formation but CIE Lab parameters may change with the alginate type and process parameters (Comaposada, Gou, Marcos, & Arnau, 2015; Marcos et al., 2016)

3.2.6 Fourier transform infrared spectroscopy (FTIR)

The FTIR analysis of the alginate films showed absorbance bands at around 1610 cm^{-1} (COO- asymmetric stretching), at 1420 cm^{-1} (C–OH deformation vibration with contribution of O–C–O symmetric stretching vibration of carboxylate group), at 1090 (attributed to C–O stretching vibrations), at 1033 cm^{-1} (C–O (and C–C) stretching vibrations of pyranose rings), at 946 cm^{-1} (indicative of uronic acid presence by the C–O stretching vibration), and the ones at around 900 and 815 cm^{-1} assigned to the α-L-gulopyranuronic asymmetric ring vibration and to the mannuronic acid residues, respectively (Figure 2). Fan et al. (2006); (Fenoradosoa et al., 2009) also showed similar bands in sodium alginate.

Xu and Dumont (2015) reported absorbance bands at 1416, 1082 and 1029 cm^{-1} of pea protein-calcium alginate beads like were observed in our calcium alginate film, but the bands were absent in the FTIR analysis of pea protein isolate or sodium alginate. Therefore, the absorbance of this bands appear in the formation of the calcium alginate structure. The addition of pea protein involved higher absorbance of this bands, indicating that the calcium alginate structure was modified.

In the spectra of the alginate film with surfactant, two strong bands from characteristic common lipid functional groups can be seen at about 2919 and 2851 cm^{-1}, where the absorbance is higher respect the alginate film. This bands would indicate the asymmetric and symmetric stretching vibrations of the acyl CH_2 groups (Herrero, Ruiz-Capillas, Pintado, Carmona, & Jimenez-Colmenero, 2017; Kumar et al., 2016). The addition of the pea protein and E475 involved structural changes of the calcium alginate films.

3.2.7 DSC analysis

In all the thermograms a wide and intense endothermic transition with very large enthalpy values with variable peak were obtained. Figure 3 shows the thermogram of alginate film with surfactant E475 as an example. This transition corresponds to the evaporation of the water present in the films. The endothermic peak temperatures for

alginate, alginate with protein, alginate with surfactant, and alginate with protein and surfactant were 112.6, 114.7, 114.3 and 113.2 °C, respectively. As a reference system (Bellich, Borgogna, Carnio, & Cesàro, 2009), dehydration thermogram of bulk pure water in open pan is characterized by a continuous exponential increase of the heat flow up to the sharp peak with an abrupt decrease of the signal to the baseline. In the case of the alginate films, the decrease is not as rapid as for bulk water. Bellich et al. (2009) considered that the evaporation rates of free-water from the alginate films was delayed by the calcium alginate polymeric network. The stiffer molecular chains may have a significant effect on the overall chain mobility (El-Din & El-Naggar, 2011). Gohil (2011) also suggested that the peak observed might either be due to overlapping of peaks from water evaporation and polysaccharides or just from water. According to Xu and Dumont (2015) the interactions between the proteins and the polysaccharides increased the thermal stability of the hydrogels. This fact would explain the tendency of increasing endothermic peak temperature of the alginate with protein.

After the transition and heating the samples up to 400 °C, a slight exothermic transition occurs in all samples corresponding to the beginning of material degradation.

In addition, alginate films with surfactant E475 showed a small additional melting point at 58.8 ±0.3 ºC. Protein addition to the films with E475 did not modify the melting point (58.2 ±0.3 ºC). Strasdat and Bunjes (2013) reported melting points at lower temperatures (44 – 53 ºC) for calcium alginate beads with lipid nanoparticles.

3.2.8 Microscope analysis

Images at x20 magnification (Figure 4) of alginate films with pea protein showed irregular bodies that could be protein granules. Images of alginate films with surfactant showed oval bodies that could be air bubbles. This air bubbles must have been included during the preparation of the solution, and retained during film formation. The air bubbles presence in alginate films with surfactant could explain in part the reduction of adhesiveness and Fmax in puncture test.

At higher magnification (Figure 5 and 6), alginate films, with or without protein (pea or collagen) and with or without surfactant, showed some differences mainly due to the microstructure compactness, like the ones observed by Wright et al. (2009). Although the main threads observed in the images are attributed to the alginate, the pure calcium alginate film had a denser and tighter network than the films with protein or surfactant,

which could explain the higher Fmax of this film in the puncture test. All the films exhibited an entangled texture in which linear/bent filaments delimited roughly polygonal voids. Brun et al. (2011) reported similar pattern with the voids sized according to a pseudohierarchy, where the axes of the largest ones reaching about 500 nm.

Although Figures 5 and 6 do not show structures attributed to protein, other micrographs (not shown) showed heterogeneities that could be attributed to aggregates interfaces like the ones observed by Mession et al. (2013), who evidenced that during gelation, the pre-aggregated proteins were mainly associated into large agglomerates. The expected increase in the binding sites due to protein addition can be much lower if protein is distributed in large agglomerates, which would explain the small effect of pea or collagen protein addition on the adhesivity properties of the film. An increase of protein concentration in the solution could have a positive effect in this direction, although the mechanical properties of the films should be considered since a tendency on decreasing the Fmax of the films was observed. In such case, the use of an alginate with higher viscosity or an increase of alginate concentration should be suggested to keep similar mechanical properties.

4. Conclusions

Water transfer properties and OTR of alginate films can be reduced by the addition of E475, reaching values between those shown by natural and collagen artificial casings. The addition of E475 produces films with colour similar to collagen artificial casing and a slight reduction on adhesivity and mechanical resistance. The mechanical resistance could not be improved by the addition of pea or collagen proteins in the conditions of the present study.

The optical microscope images and TEM micrographs allows to understand the weaker structure of the composite coating matrix, due to the air bubbles absorption and lower microstructure compactness, when surfactants and proteins are added.

Results of this study are pointing out a potential use of wet alginate films with E475 as substitute of natural and collagen artificial casings in the stuffed meat products industry.

However, the impact of the differences in the mechanical properties needs to be studied on real meat products.

Acknowledgements

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705 hydrogels in simulated gastrointestinal fluids. *Journal of Food Engineering, 165*, 52-59.
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Tables

737 Table 1. Variation of water permeance (%) of calcium alginate films (A) due to the presence of
738 additives in relation to alginate films without additives.

	kg/kg solution		n	%
	Oil	Surfactant		
A+Oil	0.005	-	8	92.2
A+Oil+E471	0.005	0.005	12	90.6
A+E322$_{\text{high grade}}$	-	0.01	9	85.8
A+E472c	-	0.01	9	84.9
A+E472a	-	0.01	9	86.6
A+E475	-	0.01	9	81.5

739 A: alginate Protanal RF6650. Root mean square error: 9.99 %

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760 Table 2. Weight loss rate (kg/s x10^{-7}) of meat slices coated with calcium alginate films (A) with
761 surfactants and with or without oil.

	Surfactant	without oil		with oil	
	kg/kg solution	n	kg/s x10^{-7}	n	kg/s x10^{-7}
A	0	30	-1.057 ^{b}	9	-1.056 ^{b}
A+E322$_{\text{high grade}}$	0.002	6	-1.086 ^{b}	6	-1.086 ^{ab}
	0.01	9	-1.126 ^{ab}	6	-1.026 ^{ab}
A+E472c	0.002	6	-1.107 ^{b}	6	-1.115 ^{ab}
	0.01	9	-1.115 ^{ab}	6	-1.157 ^{a}
A+E472a	0.002	6	-1.084 ^{b}	6	-0.970 ^{ab}
	0.01	9	-1.037 ^{ab}	6	-0.798 ^{bc}
A+E475	0.002	6	-1.117 ^{b}	6	-1.070 ^{ab}
	0.01	9	-0.526 ^{c}	6	-1.014 ^{ab}

762 A: alginate Protanal GP3350 (η-low) 0.02 kg / kg solution; Oil 0.02 kg / kg solution; the average
763 thickness of the films was 0.399±0.095 mm. The diameter of the films was 89 mm. The average
764 thickness of the meat slices was 3 mm. ^{abc} means without a common letter are significantly
765 different (P<0.05). Root mean square error: 0.160 kg/s x10^{-7}.
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783 Table 3. Weight loss rate (kg/s x10^{-7}) of meat slices double coated with calcium alginate films
784 (A) and with oil or surfactants or mixture surfactant/oil (0.1, 0.5 kg surfactant/kg oil), and
785 weight (kg x10^{-3}) of the coat (oil, surfactant, mixture) applied on the alginate film.

Double coating	Surfactant/oil mixture kg surfactant/kg oil	n	Weight loss kg/s x10^{-7}	Coating weight kg x10^{-3}
A	-	30	-1.057 ^{a}	-
A - Oil	-	6	-0.910 ^{ab}	0.302 ^{cd}
A - E322$_{\text{high grade}}$	-	6	-0.568 ^{c}	1.895 ^{a}
	0.1	6	-0.707 ^{bc}	0.272 ^{d}
	0.5	6	-0.183 ^{ef}	0.428 ^{cd}
A - E472c	-	6	-0.274 ^{def}	0.992 ^{b}
	0.1	6	-0.798 ^{ab}	0.177 ^{d}
	0.5	6	-0.132 ^{ef}	0.402 ^{cd}
A - E472a	-	6	-0.279 ^{def}	0.815 ^{bc}
	0.1	6	-0.297 ^{def}	0.148 ^{d}
	0.5	6	-0.075 ^{f}	0.692 ^{bcd}
A - E475	-	6	-0.471 ^{cde}	1.217 ^{b}
	0.1	6	-0.252 ^{def}	0.272 ^{d}
	0.5	6	-0.118 ^{f}	0.998 ^{b}
RMSE			0.160	0.269

786 A: alginate Protanal GP3350 (η-low) 0.02 kg / kg solution; the average thickness of the films
787 was 0.296 ± 0.069 mm (without oil, surfactant, mixture). The diameter of the films was 89 mm.
788 The average thickness of the meat slices was 3 mm. $^{a-f}$ means within a column without a
789 common letter are significantly different (P<0.05). RMSE: Root mean square error.

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Table 4. Water vapor transfer rate (WVTR) and oxygen transfer rate (OTR) of conventional casings and calcium alginate films (A), and weight loss rate of salami slices coated with standard casings and calcium alginate films formulated with/without proteins and surfactant E475.

Casing/film	Surfactant E475 kg/kg solution	Film WVTR g/m^2 day	Film OTR $ml/m^2 \cdot day \cdot 0.1MPa$	Weight loss rate of salami slice kg/s $x10^{-7}$
Without film	-	-	-	1.007 [bc]
Natural casing	-	627	102.3 [c]	1.138 [ab]
Artificial casing	-	483.3	362.3 [ab]	0.790 [d]
A	0	710.3	469.3 [a]	1.238 [a]
	0.01	576.3	330.3 [ab]	0.967 [c]
A+Pea	0	620	267.3 [bc]	1.204 [a]
	0.01	511.7	370.7 [ab]	0.950 [c]
A+Collagen	0	674.7	331.0 [ab]	1.164 [a]
	0.01	693	304.7 [ab]	0.992 [bc]
RMSE		83.09	57.2	0.102

A: alginate Algogel 6021 (η-medium) 0.02 kg / kg solution; Proteins: pea or collagen 0.01 kg/kg solution; Addition method of the surfactant E475: emulsion alginate/protein solution (0.01 kg / kg solution); The average thickness of the films was 0.307±0.107 mm. The diameter of the films was 89 mm. [a-d] means within a column without a common letter are significantly different (P<0.05). RMSE: Root mean square error.

812 Table 5. Equilibrium water content (%) at different water activities of standard casings and
813 calcium alginate films (A) formulated with/without proteins and surfactant E475.

Casing/film	Surfactant E475 kg/kg solution	Water activies				
		[1]0.994 ±0.005	[1]0.972 ±0.016	0.756	0.568	0.334
Natural	-	-	80.29 [a]	44.43 [b]	9.29 [e]	7.28 [c]
Artificial	-	-	52.57 [b]	21.11 [d]	18.48 [d]	14.63 [bc]
A	0	95.42 [a]	-	51.45 [a]	33.85 [a]	22.74 [a]
	0.01	94.26 [bc]	-	44.15 [b]	30.4 [ab]	25.07 [a]
A+Pea	0	94.29 [b]	-	44.12 [b]	31.24 [ab]	24.7 [a]
	0.01	93.49 [c]	-	40.47 [bc]	26.67 [bc]	23.08 [a]
A+Collagen	0	94.47 [b]	-	43.71 [b]	23.62 [cd]	24.25 [a]
	0.01	93.73 [bc]	-	37.77 [c]	26.63 [bc]	21.31 [ab]
RMSE		0.5	1.24	2.64	2.97	4.15

A: alginate Algogel 6021 (η-medium) 0.02 kg / kg solution; Proteins: pea or collagen 0.01 kg / kg solution; Addition method of the surfactant E475: emulsion alginate/protein solution (0.01 kg / kg solution); [1]average water activity ± standard deviation of hydrated films/casings. RMSE: root mean square error; [a-d] means within a column without a common letter are significantly different (P<0.05).

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828 Table 6. Adhesivity (N) of standard casings and calcium alginate films (A) formulated
829 with/without proteins and surfactant E475 onto several meat matrices surface.

Casing/film	Pork back fat		Loin		Undried salami		Dried salami	
Natural casing	-		-		-		0.546	cde
Artificial casing	-		-		-		0.418	efg
A	0.496	def	1.040	a	0.280	fgh	0.154	gh
A+E475	0.652	cde	0.727	bcd	0.227	gh	0.128	gh
A+Pea	0.973	ab	0.949	ab	0.271	fgh	0.245	fgh
A+Pea+E475	0.633	cde	0.582	cde	0.174	gh	0.115	h
A+Collagen	0.889	ab	0.634	cde	0.232	gh	0.175	gh
A+Colagen+E475	0.756	bc	0.542	cde	0.140	gh	0.155	gh

830 A: alginate Algogel 6021 (η-medium) 0.02 kg / kg solution; Proteins: pea or collagen 0.01
831 kg/kg solution; Surfactants: E475; Addition method of the surfactant: emulsion
832 alginate/protein solution (0.01 kg/kg solution); Adhesivity: average force (N); $^{a-h}$ Lsmeans
833 without a common letter are significantly different (P<0.05). Root mean square error: 0.309
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Table 7. Puncture test of calcium alginate films (A) formulated with/without proteins and surfactant E475.

Film	Surfactant E475 kg/kg solution	Fmax N	Elongation %
A	0	0.951 [a]	16.31 [a]
	0.01	0.816 [b]	16.36 [a]
A+Pea	0	0.91 [ab]	15.88 [a]
	0.01	0.662 [c]	12.15 [b]
A+Collagen	0	0.828 [b]	15.55 [a]
	0.01	0.796 [b]	15.81 [a]
RMSE		0.122	2.84

A: alginate Algogel 6021 (η-medium) 0.02 kg / kg solution; Proteins: pea or collagen 0.01 kg/kg solution; Surfactants: E475; Addition method of the surfactant: emulsion alginate/protein solution (0.01 kg/kg solution). The average thickness of the films was 0.376±0.136 mm. F_{max}: maximum force required to break the film; E: elongation at break; [abc] Lsmeans without a common letter are significantly different (P<0.05). RMSE: root mean square error.

873 Table 8. Color parameters L *, a *, b * of standard casings and calcium alginate films (A)
874 formulated with/without proteins and surfactant E475.

Casing/film	Surfactant E475 kg/kg solution	L*	a*	b*
Without film	-	38.8 ^{d}	14.5 ^{a}	10.5 ^{a}
Natural c	-	62.1 ^{a}	1.8 ^{f}	4.9 ^{e}
Artificial c.	-	43.9 ^{cd}	11.3 ^{cd}	7.7 ^{bcd}
A	0	41.2 ^{cd}	13.8 ^{ab}	9.5 ^{ab}
	0.01	45.1 ^{c}	11.1 ^{cd}	7.3 ^{cd}
A+Pea	0	41.2 ^{cd}	13.4 ^{abc}	9.5 ^{ab}
	0.01	51.6 ^{b}	8.5 ^{e}	4.7 ^{e}
A+Collagen	0	41.2 ^{cd}	13 ^{abcd}	8.9 ^{abc}
	0.01	46 ^{c}	10.8 ^{de}	6.5 ^{ed}
RMSE		2.21	0.99	0.82

875 A: alginate Algogel 6021 (η-medium) 0.02 kg / kg solution; Proteins: pea or collagen 0.01
876 kg/kg solution; The average thickness of the films was 0.307 ± 0.107 mm. The diameter of the
877 films was 89 mm. Color parameters: L*: lightness; a*: redness; b*: yellowness. $^{a-f}$ Lsmeans
878 without a common letter are significantly different (P<0.05). RMSE: root mean square error.

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Figures

Figure 1. Illustration of the adhesivity test performed with the texture analyser: a) gauze, b) alginate coating, c) meat, d) metacrylate support, e) elastic grip rubber, f) test area definition lines, g) PVC strip.

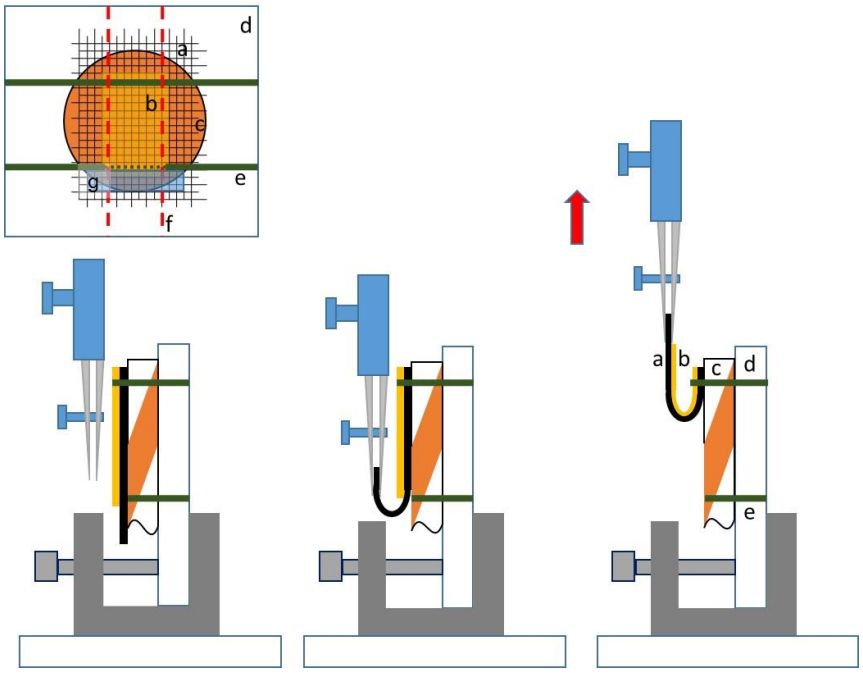


Figure 2. Overlap of reflection infrared spectra of alginate films (C) (red line), alginate with pea protein (C + P) (dark blue line), alginate with surfactant E475 (C + V) (blue line) and alginate with pea protein and surfactant E475 (C + P + V) (black line) after 24 hours of drying.

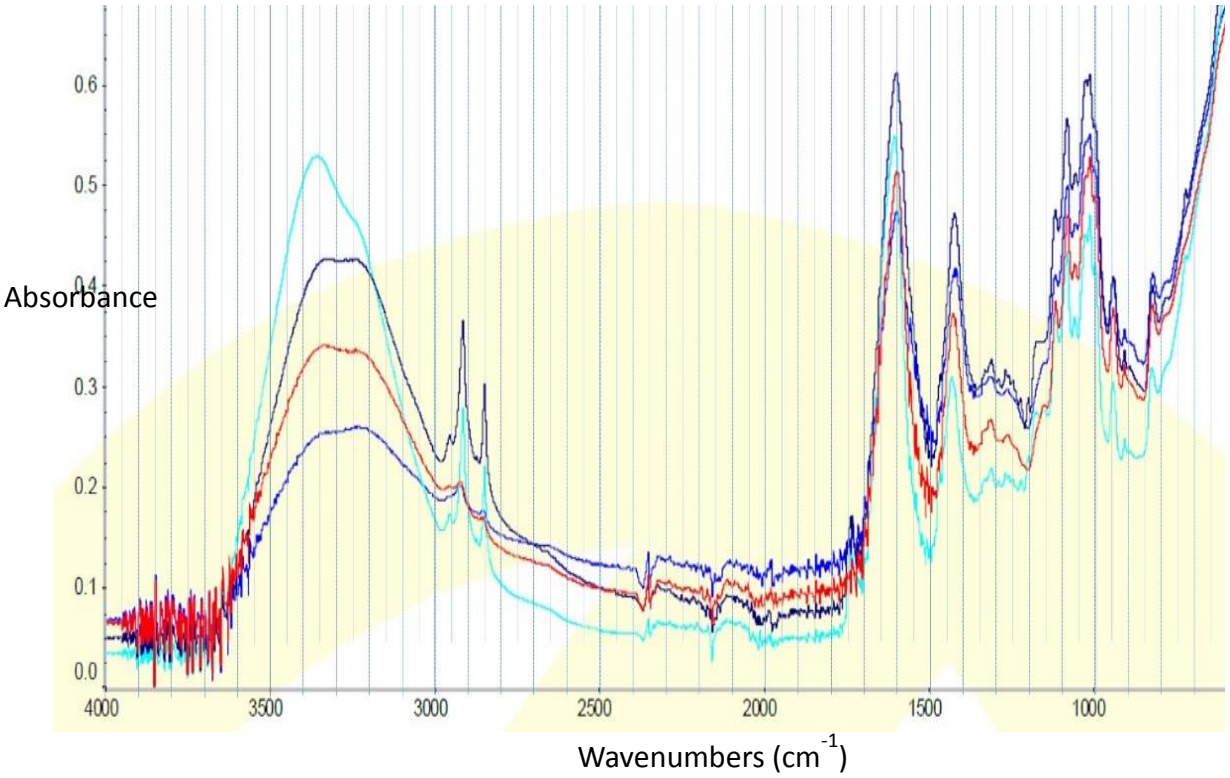
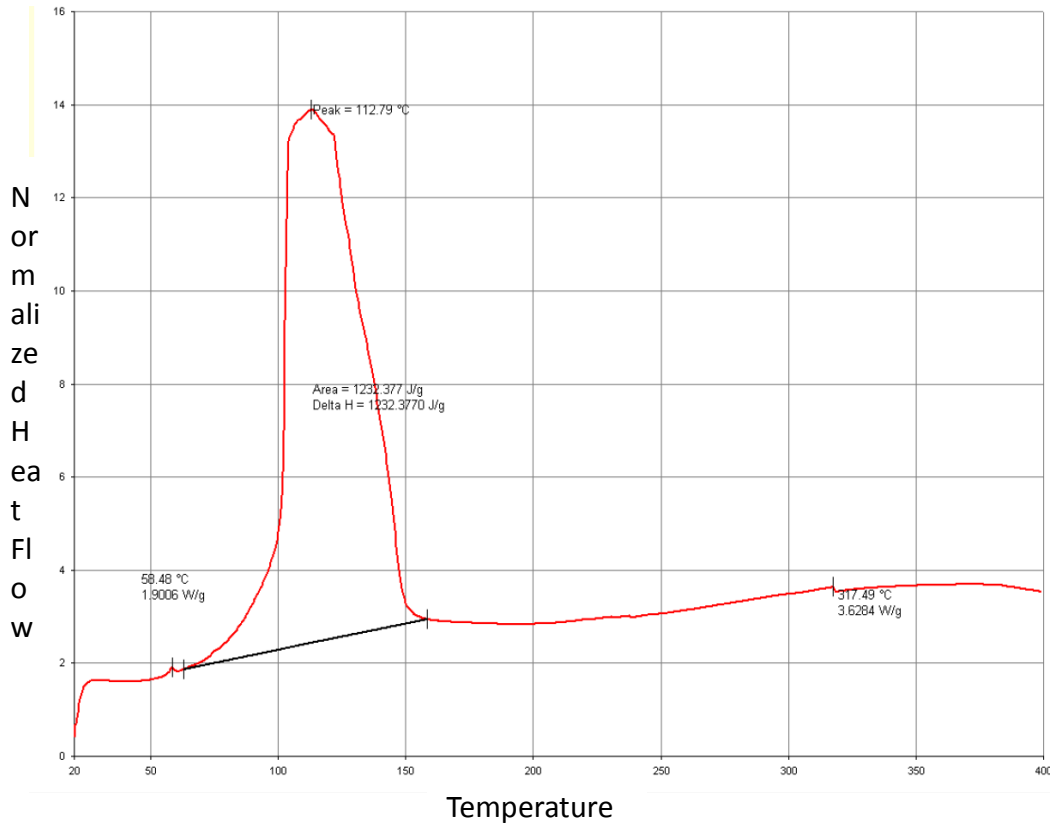
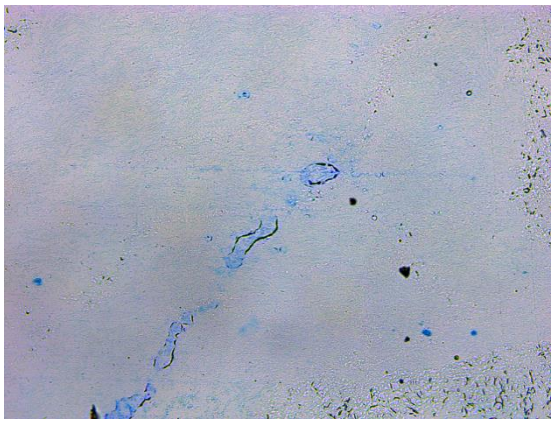


Figure 3. DSC thermogram of alginate film with surfactant E475.

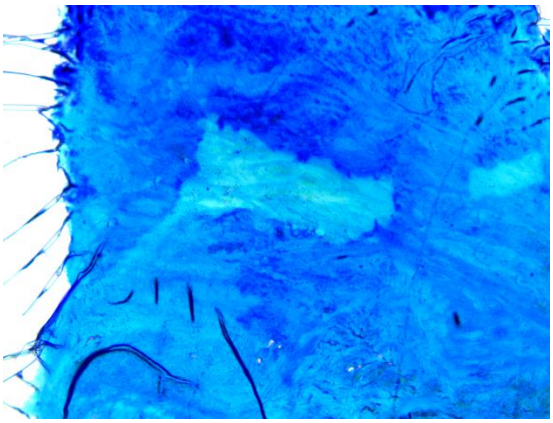


961 Figure 4. Photographs of natural casing, artificial casing, control alginate films (Algogel
962 6021), and alginate films with pea protein, and surfactant E475 obtained with optical
963 microscope at x20 magnification.

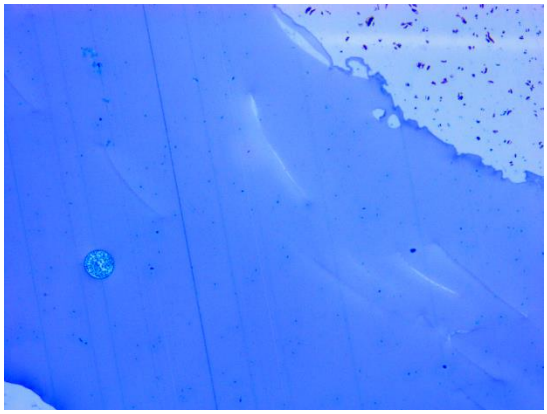
Natural casing



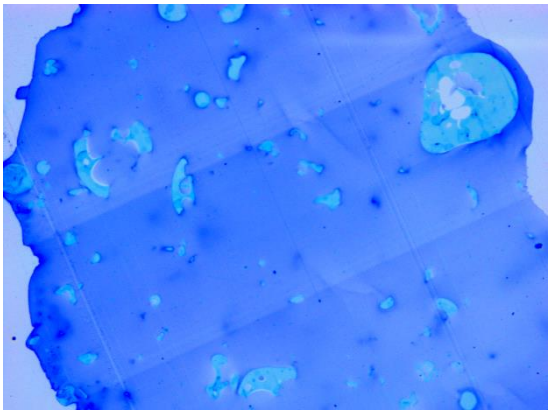
Artificial casing



Alginate film



Alginate film with pea protein



Alginate film with surfactant E475

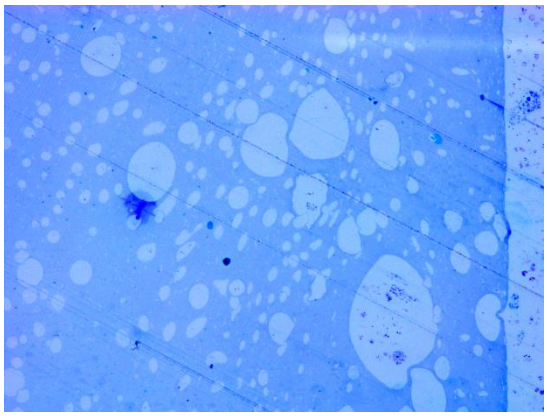


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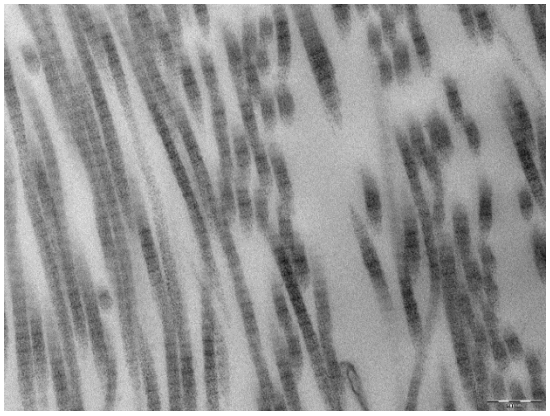
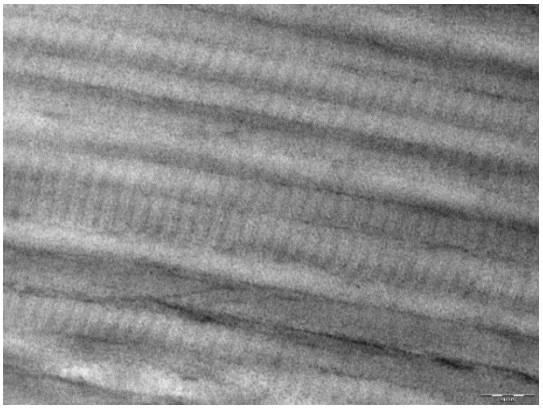
Figure 5. Micrographs of natural casing, artificial casing, control alginate films (Algogel 6021), alginate films with pea protein, collagen protein, and surfactant E475 obtained with electron microscope at x46000.

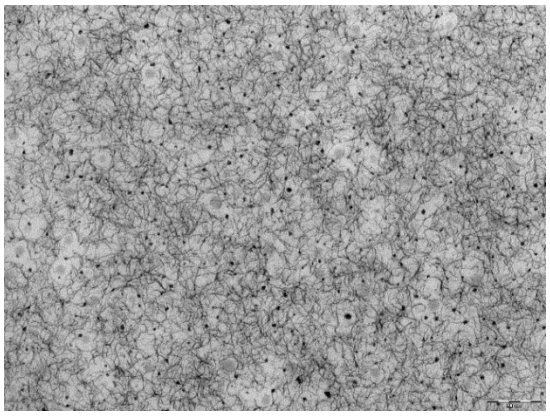
Natural casing



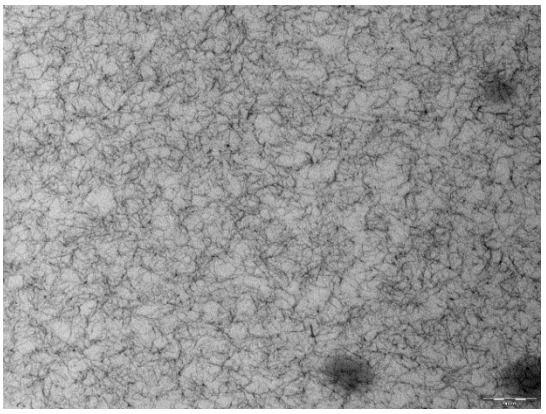
Artificial casing



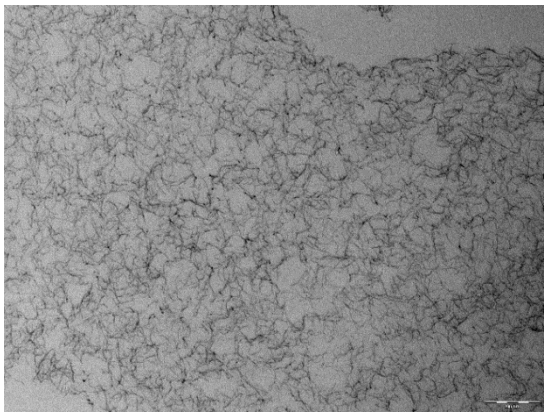
Alginate film



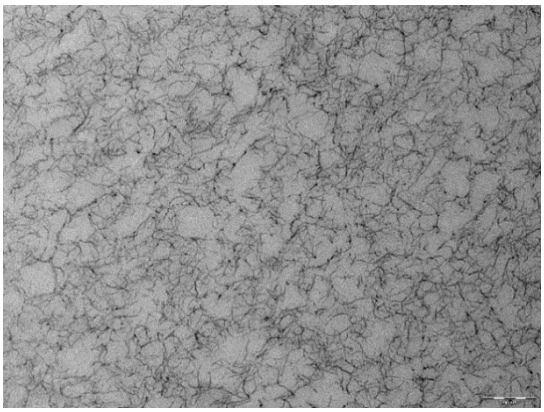
Alginate film with pea protein



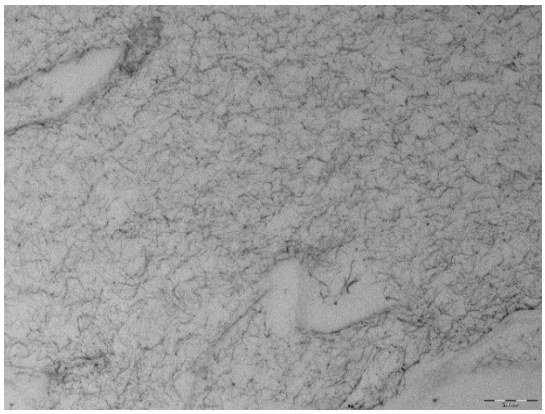
Alginate film with surfactant E475



Alginate film with collagen protein



Alginate film with pea protein and surfactant E475



Alginate film with collagen protein and surfactant E475

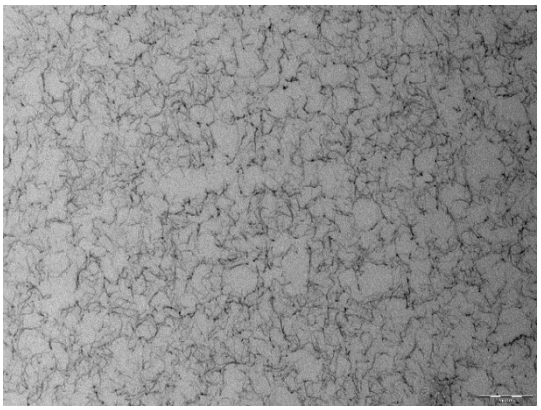
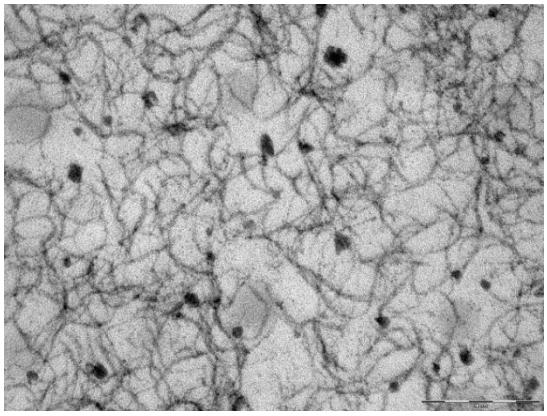
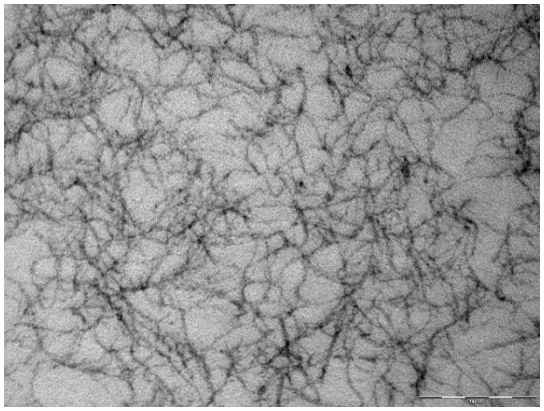


Figure 6. Micrographs of control alginate films (Algogel 6021), alginate films with pea protein, collagen protein, and surfactant E475 obtained with electron microscope at x195000.

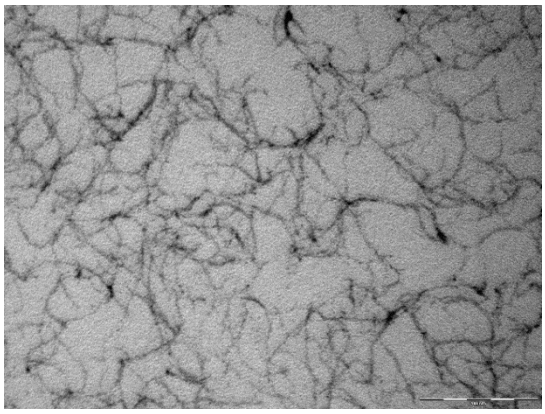
Alginate film



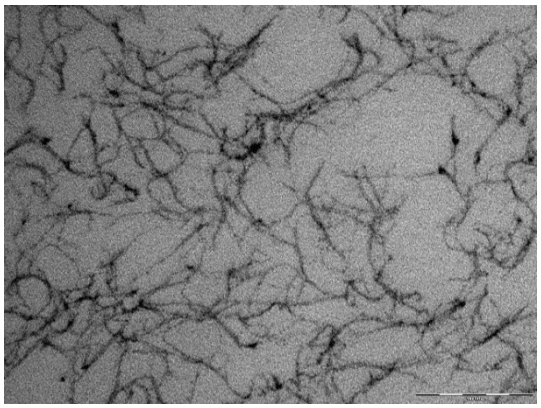
Alginate film with pea protein



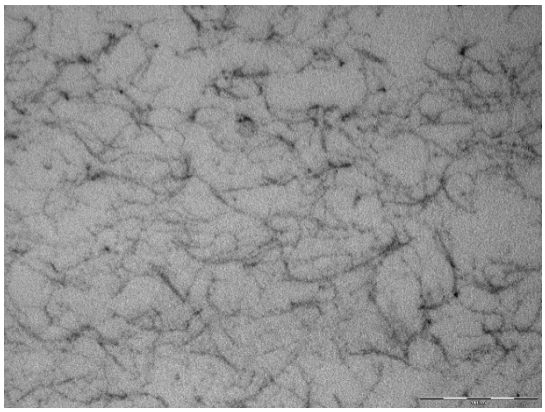
Alginate film with surfactant E475



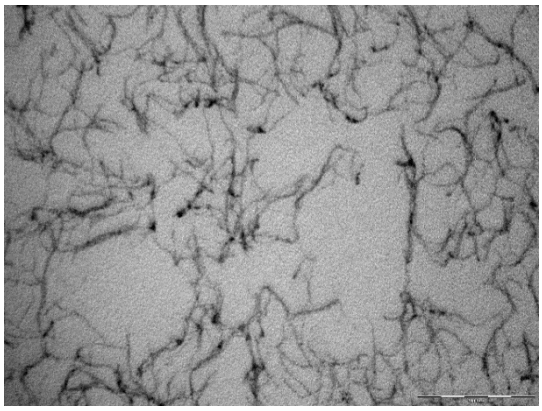
Alginate film with collagen protein



Alginate film with pea protein and surfactant E475



Alginate film with collagen protein and surfactant E475



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