Characterization of Soybean Protein Concentrate—stearic Acid/Palmitic Acid Blend Edible Films

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ABSTRACT: The effect of incorporating commercial stearic acid/palmitic acid blend (SA/PA, 63/37 wt %) into hydrophilic soybean protein concentrate (SPC) film-forming solutions at neutral and alkaline pH on some selected properties of edible cast films was investigated. SA/PA-added SPC film exhibited a significant increase in translucency, being more relevant for films obtained at pH 7. This was associated with the more heterogeneous morphology of such films as observed by scanning electron microscopy. Calorimetric measurements and X-ray diffraction studies confirmed the presence of crystalline fatty acids in films at pH 7 and new crystalline structures at pH 10 due to interactions or reactions between SPC and SA/PA blend. Fourier Transform infrared spectroscopy results confirmed the incorporation of fatty acids into SPC and revealed the occurrence of interactions between both components, depending on the film-forming emulsion pH. Moisture absorption isotherms at high relative humidity (RH) were determined and experimental data were adequately fitted by Peleg's empirical equation. Control SPC films produced at pH 7 were distinctly more moisture resistant than those at pH 10 owing to the more charged protein molecules at alkaline pH. The increased moisture resistance of SA/PA-added-SPC film at pH 10 was related to the more homogeneous dispersion of fatty acid particles within the protein matrix. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 000: 000–000, 2011

Key words: biodegradable; soybean protein concentrate; edible films; differential scanning calorimetry; X-ray diffraction

INTRODUCTION

Polymers from biomass, such as proteins, carbohydrates and lipids have received considerable research attention as potential substitutes for certain conventional polymers in domains where derivatization from natural resources and environmentally sound disposability provide added value. Proteins represent one of the cheapest and most abundant biological feedstocks available in large quantities, and their use as starting materials offers numerous advantages, such as low toxicity and inherent biodegradability.[1]

Soybean proteins have been used to fabricate edible and environmentally sound films and coatings due to their film-forming ability, excellent gas barrier properties at low relative humidity, low cost, biogenic origin, and worldwide availability.[2–8] Soy beans are grown predominantly in North and South America where 33 and 49%, respectively, of the 2007/2008 world bean supply was harvested. Argentina is the third soybean producer (54 million tones 2009/2010) behind USA and Brazil[9] and most of the current production is intended for oil production for export (about 7.5 million tones in 2009/2010). The recycle of the soybean oil industry residue may result in the development of economically feasible new industrial products with more added-value. This in turn will give much return to agriculture, thereby reducing the burden of petroleum-based products.

Many different soy protein grades are commercially available such as defatted soy flour containing about 50% protein, soy protein isolates (SPI)[2] containing about 90% protein and soy protein concentrate (SPC) which is commercially obtained by removing the soluble sugars from defatted flour, being the remaining protein (about 65–70%) and insoluble carbohydrates.[10]

Based on ultracentrifugal sedimentation rates, protein fraction in SPC can be classified into four categories: 2S, 7S, 11S, and 15S, being 7S and 11S the largest and most important fractions corresponding to two globular storage protein fractions β-conglycinin (7S) and glycinin (11S). The globulin 7S is a trimer formed by four subunits with similar aminoacidic

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sequences. On the other hand, the glycinin 11S, is a hexamer of 300–380 kDa of molar mass.[11] The carbohydrate fraction in SPC is mostly composed by non starch polysaccharides, oligosaccharides and monosaccharides. This fraction contains ~ 8–10% cellulose and the remaining are pectic polysaccharides (linear hetero-polysaccharides which contain free or esterified galacturonic acid-based units).[10]

Since pure globular protein films by their self, are very brittle,[8] plasticizers must be added to increase the flexibility and handling of the final film.[8,12] Plasticizers overcome the brittleness in protein films by softening the structure and by inserting them into polymeric matrix, increasing the free space between protein chains.[12–14] Plasticizers for biopolymers include polyols such as sorbitol, glycerol, glucose, fructose or other polyols.[2–4,8,12] However, the hydrophilic character of such plasticizers contribute to increase the moisture sensitivity of the obtained films, which imparts undesirable changes in shape (i.e., swelling), mechanical and barrier properties.[8] Poor performance as water vapor barrier is one of the main limitations of protein films. The addition of hydrophobic components, such as lipids, waxes and long chain fatty acids has demonstrated great potential to improve at least some of the abovementioned issues[4,5,8,13–19] Edible protein films with lipid materials distributed in particles are produced by taking advantage of the good emulsifying and film-forming ability of proteins and exploit the excellent oxygen barrier of protein films with the excellent moisture barrier of lipids to design more suitable films for food preservation: superior moisture resistance, improved flexibility, transparency and gloss.[5,8] Blending proteins with fatty acids such as stearic ($C_{18:0}$), palmitic ($C_{16:0}$), lauric ($C_{12:0}$), oleic ($C_{18:1}$) and mixtures of fatty acids have been reported in the literature[3–5,8,13–20]. This can be accomplished by emulsifying the fatty acid in the film-forming solution[4,5,8,13–20] or by forming a plastic resin with the protein.[3,8,21] Emulsions are heterogeneous systems containing at least one immiscible liquid dispersed in another one that stabilizes in droplet particles.[22] In the presence of proteins, the characteristic aqueous lipid self-association can change due to the lipid-protein interactions. In the dry films, fatty acids could crystallize and remain dispersed within the protein matrix or as a nonseparated lipid as a result of more intense lipid-protein interactions[18] or reactions[3] with reactive groups in protein chains, giving rise to the formation of a more homogeneous network and, thereby reducing the moisture binding capacity of proteins films. Moreover, the acid long hydrocarbon chain can also be of help in reducing the moisture absorption and water vapor permeability[5,15–20].

Among the saturated fatty acids, stearic (SA) and palmitic (PA) acids are currently used as hydrophobic additives in edible films formulations because of their highly regarded characteristics reported in the literature,[3,4,5,8,13–21] especially their classification as generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) and the melting temperatures (c.a., 64°C for PA and 72°C for SA) which are compatible with denaturation ones of most proteins.[16]

Despite the extensive work reported in the literature about fatty acid or hydrophobic compounds-modified soy protein based films[5,7,8] most of the informed data are related to SPI and limited information is currently available on SPC. Therefore, the aim of this work was to explore the technical feasibility of using the more economically favorable SPC instead of SPI in the production of environmentally sound stearic acid/adipic acid blend (SA/PA) modified-SPC edible films with further moisture repellency, aimed to be used in food preservation.

EXPERIMENTAL

Materials

Soybean protein concentrate (SPC, Solcom S 110), isoelectric point (pI) near 4.5, with an average particle size passing through 100 mesh and 7% moisture, 69% protein, 1.05% fat, 3.5% fibers, 6% ashes and about 15% non starch polysaccharides (NSP, mainly cellulose, non cellulose polymers and pectin polysaccharides) as mean composition,[23] was obtained from Cordis SA (Villa Luzuriaga, Buenos Aires, Argentina). SA/PA blend 63 : 37 (MATER-560-2) was obtained from tallow and kindly supplied by Materia Oleochemicals (Mar del Plata, Argentina).

Calcium chloride ($CaCl_2$) was analytical grade from Aldrich (St. Louis, USA).

Preparation of SPC-based films

SA/PA-modified soybean protein concentrate (SA/PA-SPC) films were obtained by casting of the film-forming emulsions produced at two different pH values: 7 and 10. The solutions were prepared by dispersing SPC powder in distilled water (1 : 14 by weight of dry SPC) to provide a 10 wt % protein content in the film-forming solution. Dissolution was performed at 80°C under continuous stirring (Cole-Palmer, Vernon Hills, IL). The pH was adjusted to the desired values by adding 1*N* NaOH solution during dissolution and stirring. The variation of pH was monitored using an electronic pH-meter (Crison Basic-20, Barcelona, Spain). SA/PA blend was incorporated into the film-forming solution (20 wt % on soy protein dry basis). The SA/PA-SPC emulsion was maintained at 80°C for other 30 min under mild stirring. After lipid melting, the solution was homogenized for 10 min in a mechanical mixer and then cooled at room temperature. Subsequently, a

TABLE I
Opacity, Initial Moisture Content (w_0), Equilibrium Moisture Absorption (M_{eq}), Constant Values k_1 and k_2 and Coefficient of Determination (R^2) of Sorption Curve (eq. 2) for SPC Films at 25°C and 100% RH

Sample name	Thickness (μm)	Opacity/thickness (nm/μm)	k_1 (h/%)	$k_2 \times 10^3$ (per 1/%)	w_0 (%)	M_{eq} (%)	R^2
SPC7	316 ± 29 (*a*)	1.91 ± 0.10 (*a*)	2.13	15.62	11.9 (*a*)	75	0.98
SA/PA-SPC7	321 ± 36 (*a*)	5.46 ± 0.10 (*c*)	0.10	5.64	11.8 (*a*)	189	0.99
SPC10	319 ± 30 (*a*)	1.43 ± 0.14 (*a*)	0.20	6.71	11.6 (*a*)	161	0.99
SA/PA-SPC10	290 ± 27 (*a*)	2.70 ± 0.32 (*b*)	0.37	9.08	11.6 (*a*)	121	0.99

Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different according to Tukey test.

well known volume of the emulsion obtained was poured onto Teflon-coated Preti plates setting on a leveled surface to maintain constant area and uniform thickness (Table I) and dried at 23 ± 2°C in a air circulating-oven (Mettler AJ50, Greifensee, Switzerland) until reaching constant weight (about 20 h). The dried films were peeled from the plates and kept in desiccators before conditioning and testing. The SPC films modified with 20 wt % SA/PA blend at pH 7 and 10 were labeled as SA/PA-SPC7 and SA/PA-SPC10, respectively. Control SPC films without SA/PA blend were prepared at both pH values as above described and labeled as SPC7 and SPC10.

Methods

Film conditioning

All the films produced were dried in a dessicator containing Calcium Chloride (0% relative humidity, RH) at ambient temperature (c.a., 23 ± 2°C) until reaching constant weight (±0.0001 g), using an analytical balance (Mettler AJ50, Bradford, MA).

Scanning electron microscopy

The films failure and external surface (upper and lower) were observed with a Jeol JSM-6460LV (Tokyo, Japan) scanning electron microscope using 10 kV as accelerating voltage. Prior to the observation, the surfaces were sputter-coated with a gold layer of about 100 Armstrong to avoid charging under the electron beam.

Thickness measurements

The film thickness was measured with a 0–25 mm manual micrometer (Venier, China) with an accuracy of ± 0.01 mm. The reported values are the average of five readings taken randomly on each film sample. Values were used to assess the opacity of the produced films. Calcium chloride was analytical grade from Aldrich (St. Louis, USA).

Opacity

The visible light-barrier properties of films were determined by measuring their light absorption at wavelength ranging from 400 to 800 nm, using a UV-Visible spectrophotometer Shimadzu 1601 PC (Tokyo, Japan), according to the method described by Irissin-Mangata et al. (2001).[24] The film specimens were cut into rectangular strips and placed directly in the spectrophotometer test cell. Air was used as reference. Film opacity was expressed as the area under the integrated recorded curve. Opacity was expressed as absorbance units per thickness unit (AU nm/μm). Results were taken as the average of three replicates on each film.

Differential scanning calorimetry

DSC experiments were performed using a DSC-50 (Perkin–Elmer, Waltham, MA). Sample weights were in the range of 5–7 mg, and all runs were carried out from room temperature up to 200°C, under nitrogen atmosphere and at a heating rate of 10°C/min. The instrument was calibrated with high purity indium at 20°C/min, following the standard procedure.

X-ray diffraction

XRD patterns were recorded in the range of $2\theta = 2$–30° at a scanning rate of 1°/min, by using PW1700 X-ray diffractometer (Phillips, Eindhoven, The Netherlands) equipped with Cu Kα radiation source ($\lambda = 0.1546$ nm), operating at 45 KV and 30 mA as the applied voltage and current, respectively.

Fourier transform infrared spectroscopy

Spectra were recorded on a Thermo Scientific Nicolet 6700 spectrometer (Wisconsin, USA). All runs were performed between 400 and 4000 cm^{-1} using an attenuated total reflection (ATR) accessory with a diamond ATR crystal using 32 scans with 4 cm^{-1} resolution.

Moisture absorption

MA was gravimetrically determined. Five samples of each film type were weighed (100–200 mg) and dried for 24 h in an air-circulating oven at 105°C

(Mettler AJ50, Greifensee, Switzerland) to gravimetrically obtain the initial moisture content (w_0) (±0.0001 g) using an analytical balance (Mettler AJ50, Bradford, MA). The dried samples were then put in a climatic chamber (Dycometal CCK81, Barcelona, Spain) at 98 ± 2% RH to measure moisture absorption. The chamber temperature was maintained at 25°C throughout the experiment. Specimens were periodically removed and reweighted (±0.0001 g) in an analytical balance. The moisture content w_t as a function of time t was obtained from the total and partial (water) mass balance over the sample as a function of time:

$$w_t(\%) = \frac{m_0\, w_0 + (m_t - m_0) \cdot 100}{m_t} \quad (1)$$

where w_t is the moisture content at different times, m_t is the weight of the wet sample (g) after exposition, m_0 is the initial weight (g) and w_0 is the initial samples moisture content (%). Experimental data were correlated by using Peleg's empirical equation.[25] This equation has been used to successfully describe the sorption process of many biopolymers.[25–28] The expression relates the instantaneous moisture content (w_t) to the initial moisture content (w_0), as shown in the following equation:

$$w_t(\%) = w_0 + \frac{t}{k_1 + k_2\, t} \quad (2)$$

where t (h) is time, w_t (%) is the moisture content at time t and w_0 is the initial moisture content (%). The constants k_1 and k_2 are fitting parameters. As k_1 (h/%) is related to mass transfer, the lower the k_1 value the higher the initial absorption rate; k_2 (per 1/%) is a constant associated with the maximum moisture absorption capacity, therefore the lower the k_2, the higher the absorption capacity.[27,28] The equilibrium moisture content (M_{eq}), which is a function of k_2, can be estimated from eq. (2) as follows:

$$M_{eq} = \underset{t\to\infty}{Lim}\ w_t = w_o + \frac{1}{k_2} \quad (3)$$

where w_t (%) is the moisture content at time t, w_0 (%) is the initial moisture content, and k_2 (per 1/%) is a constant associated with the maximum moisture absorption capacity.

Statistic analysis

Experimental values were statistically analyzed by one-way analysis of variance (ANOVA). Differences between pairs of means were assessed on the basis of confidence intervals, using Tukey's test. The significance level was $P < 0.05$.

RESULTS AND DISCUSSION

Film—Forming conditions and opacity

SA/PA-SPC films were produced by incorporating SA/PA blend to the film-forming emulsion produced at two different pH values and at 80°C. Emulsion film formation was considered to depend on pH, protein and lipid concentration, homogenization and drying conditions.[14,18–20] Preliminary studies allowed finding out the best ratio of protein content and SA/PA to obtain edible film. The study involved two protein contents (5 and 10 wt %), different SA/PA concentrations (10, 20, and 40 wt %) and two pH values. Proteins film formation is a pH dependent process. The pH level affects the protein charge and degree of unfolding, which in turn determine the type of interactions involved in the network formation.[29–31] At pH near isolectric point (pI ≈ 4.5), fatty acid can act largely as hydrophobic components ($pKa_{SA} \approx pKa_{PA} \approx 4.7$), more that at pH 7 or 10. However, near the pI, hydrophobic interactions between alkyl chains of fatty acids with soy protein are restricted due to the higher compactness of protein films in the vicinity of the isolectric pH.[29,30] At pH levels away from the isoelectric region, protein denatures and unfolds, exposing sulfhydryl and hydrophobic groups. Such groups associate upon drying to form disulfide and hydrophobic bonding forces.[29–31] Reportedly, alkaline conditions favor soy protein film formation, presumably by aiding protein dispersion in film-forming solutions.[29] It has been also reported that unfolded soy proteins at basic pH levels produced structures that were more capable of stabilizing emulsion droplets than at acidic pH.[22,32] According to this and to ensure film formation, two pH values above the isolectric point were chosen, that is, 7 and 10. Results showed that at least 10 wt % protein was required to obtain freestanding films by drying the film-forming solutions at 23 ± 2°C. At protein content lower than 10 wt %, the cohesive strength may be low resulting in the inability to form strong bonds at room temperature.[30]

Emulsion films with 10 wt % protein and containing SA/PA concentration lower than 20 wt % gave rise to films difficult to take off the anti adherent surface, behaving similarly than the unmodified counterparts. For SA/PA concentrations higher than 20 wt %, intact films were difficult to obtain may be because the higher lipid content may have prevented the protein–protein interactions necessary for film formation. Considering all these observations, the level of SA/PA blend was fixed at 20 wt % and the concentration of protein was 10 wt %, for both pH values. Emulsion temperature was chosen above the melting point of SA/PA blend (c.a., 63°C) though lower than the solvent volatilization temperature (i.e., 100°C). Soy protein denaturantion temperature

was not taken into account since, as discussed later in the text, DSC results revealed that proteins in commercial SPC were completely denatured. This fact also determined the drying temperature which was fixed at 23°C. Upon drying water is progressively eliminated, favoring the mutual approach of the unfolded protein chains to form a protein network through a combination of disulfide, hydrophobic, and hydrogen interactions, as reported for gluten,[14,29] soybean[29,30] and whey protein.[31]

The film opacity values were used to assess the transparency of the films. With increasing pH from 7 to 10, opacity of SPC control films slightly decreased ($P > 0.05$). At higher pH values, solubility of the soy protein increased and chains are more evenly dispersed enabling the formation of a homogeneous film. Therefore, more light penetrates through the film giving lower opacity values. Addition of fatty acid to SPC films led to changes in transparency of films (Table I). Film opacity is sensitive to various factors including film thickness.[24] In this study, no significant differences ($P > 0.05$) in the average thickness were detected, being close to 300 μm for control SPC film and to 316 μm for fatty acid-containing films. The slight increment in thickness in fatty acid-containing films has been previously reported for SPI-fatty acid composite,[4] gelatin–fatty acid[7] and wheat gluten-solid lipid films.[14] Furthermore, the presence of disperse nonmiscible phase promotes opacity due to the differences in the refractive index of the phases and the concentration and particle size of the dispersed phase.[4,5,14] Opacity results showed that SA/PA-SPC films exhibited significantly ($P < 0.05$) higher opacity values when compared to SPC films (Table I), suggesting that there was strong scattering due to a dispersed solid phase. This was more relevant for SA/PA-SPC7 film, most likely due to the limited dispersion capability of solid fatty acid and the poor stability of the emulsion at pH 7,[29,32] as previously described for other protein-lipid emulsion films.[2,5,18–21] Formation of fatty acid particles and their development during film drying suppose the interruption of the protein matrix, increasing the internal heterogeneity and decreasing the transparency the film.[18,19]

SEM observations of film cross section and surface

All films analyzed in the present study were smooth, flexible and apparently free of pores. SPC films obtained at pH 7 appear less flexible and easy to disintegrate than films formed at pH 10. Under alkaline conditions proteins are completely or at least partially denatured, and once extended, protein chains in SPC films are mainly stabilized by disulfide bonds, since the pK values of proteins thiols in the unfolded state are generally in the range of 8.7–9.[29–31] The lower reactivity of free thiol groups at pH 7 diminishes the disulfide mediate polymerization and hydrophobic interactions prevail over disulfide bonds in SPC7 films. Even though the same type of interactions (covalent and noncovalent) are involved in protein networks,[30,31] the contribution of each type of bond is different and this would be reflected in film properties and microstructure. Cross sections of the obtained films are presented in Figure 1. Both films displayed dense structures, typical of protein films in consistency with previous results reported form other proteins.[21,29,31] SPC10 films exhibited an aligned fibrous-like pattern [Fig. 1(a)] and compact structure, while SPC7 film displayed a more entangled and less ordered arrangement [Fig. 1 (b)]. Denser protein structures are reported to take place at extreme pH values due to an enhanced gel formation manly due to thiol/disulfide interchange reaction.[29,31] In the present case, fibrous structure was though to be responsible for the higher malleability and flexibility of SPC10 films compared with SPC7 counterpart. When the pH of the film forming solution was closer to pI, a greater interaction of protein could result in more condensed and less elastic film structure.[29] With the addition of SA/PA blend, the fracture surfaces of the modified-SPC films become increasingly rougher. SA/PA-SPC7 films appeared less dense than before adding fatty acids [Fig. 1(c)] meanwhile SA/PA-SPC10 films maintained their cohesiveness and fibrous aspect with low amount of discontinuous zones (Fig. 1 d). This microstructure ensured the protein film having a certain deformation while the more discontinuous microstructure depicted by SA/PA-SPC7 led to a reduction in such property. The better incorporation of SA/PA into SPC10 can be explained by the presence of interactions between the hydrophilic end of the fatty acid with the hydrophilic groups in SPC, and the lipophilic end of fatty acid with the hydrophobic side-chain groups of SPC, favored by a more aligned protein structure and the exposure of hydrophobic side-chain groups, which were originally buried into the globular region of soy proteins resulted from alkaline treatment,[3,32] these interactions might lead to complexes between fatty acids and proteins, as previously reported for other lipid-biopolymer systems.[5,18–20] Fabra and coworkers[18] proposed that oleic acid interact with sodium caseinate through polar groups, modifying the protein network and film functional properties. The formation of such complexes might lead to only a fraction of fatty acids forming a separate phase, while the other fraction would be involved in protein-fatty acid interactions. At alkaline pH, reactions between fatty acids (or their salts) and SPC may occur,[3,15] favoring the more homogeneous incorporation of SA/PA blend into the proteinaceous matrix. It is presumed that the presence of soybean cellular polysaccharides in SPC

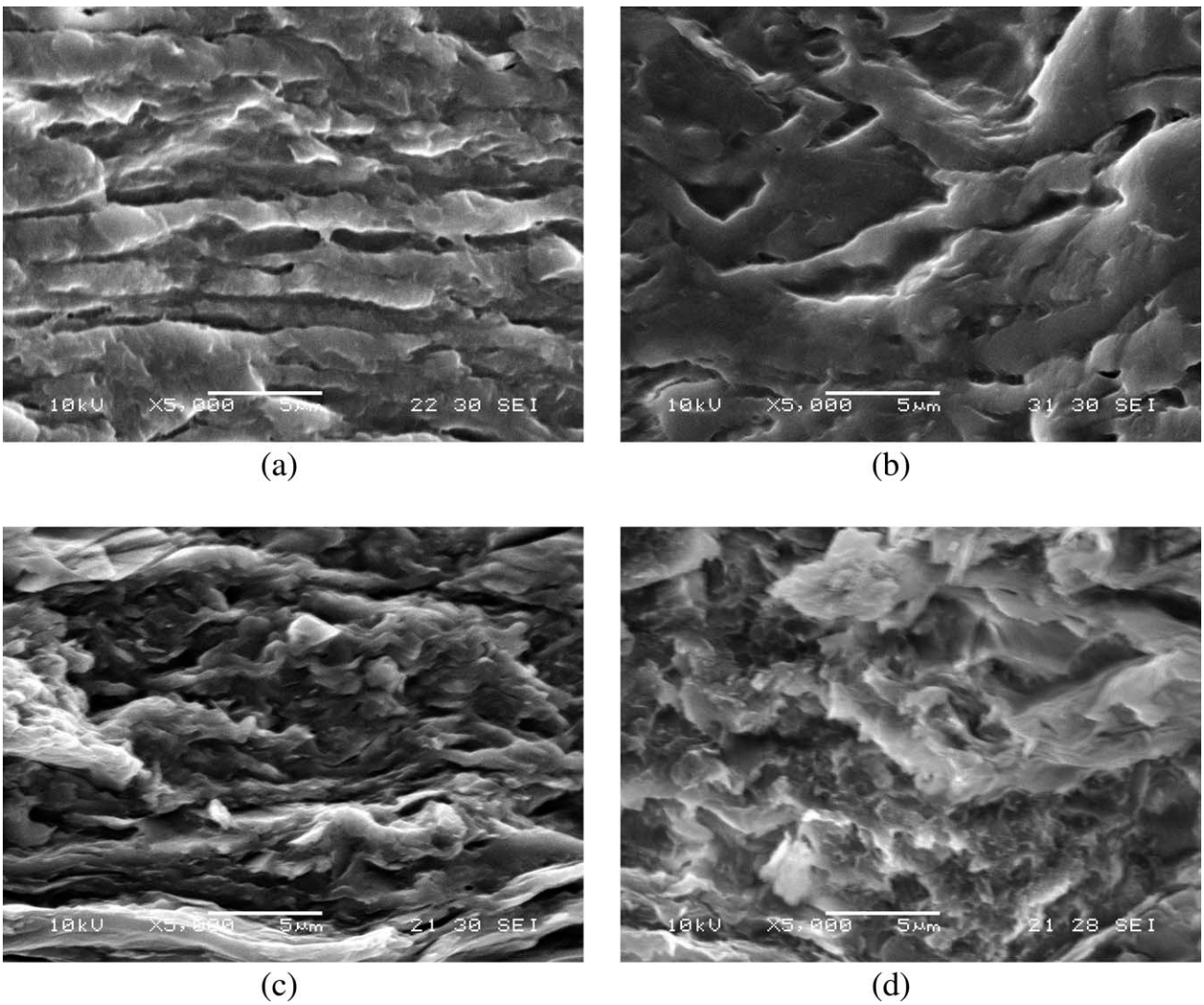

Figure 1 SEM observations of cross sections of SPC-based films (a) SPC10, (b) SPC7, (c) SA/PA-SPC7, and (d)SA/PA-SPC 10 (magnification 5000×).

would provide additional stability to the fatty acid-containing films produced at alkaline pH levels by acting as emulsion stabilizers.[22]

Figure 2 shows SEM micrographs of both sides of SA/PA-SPC films. The glossy side of both films (which is in contact with the casting-supporting surface) was smooth and featureless [Fig. 2(a,b)] whereas, the upper surface (which exposes the hydrophobic side-chain groups[5,29,30]), was rougher and uneven [Fig. 2(c,d)]. A similar effect was previously observed in SPI,[5] sodium caseinate[18–20] and zein films containing oleic acid[21] and gelatin films with hydrophobic compounds.[7,15–17] SEM observations revealed the presence of crystalline fatty acid particles not uniformly distributed on the upper surface of the SA/PA-SPC7 film [Fig. 2(c)]. This was attributed to the creaming of lipid droplets during film drying, which promotes the destabilization of the emulsion structure, including the flocculation and coalescence of hydrophobic globules.[5,17–20] This heterogeneous structure may contribute in increasing opacity and reducing the water vapor permeability of protein films, depending on the size and distribution of the dispersed phase.[13,14,17–20] The better miscibility observed in SA/PA-SPC 10 (Fig. 2 d) was postulated to result from protein-fatty acid interactions and/or reactions, already observed for other fatty acid-protein systems,[5,15,17–20] therefore decreasing the interfacial tension which play a key role in reducing the size of the dispersed phase. To get a better insight into structural changes in SA/PA-modified SPC films, control and modified-SPC samples were analyzed by DSC, XRD, and FTIR.

DSC characterization

The DSC curves of pure components and SA/PA-SPC films are depicted in Figure 3. The melting temperature of SA/PA blend was observed at 63°C, being lower than the lowest melting temperature of

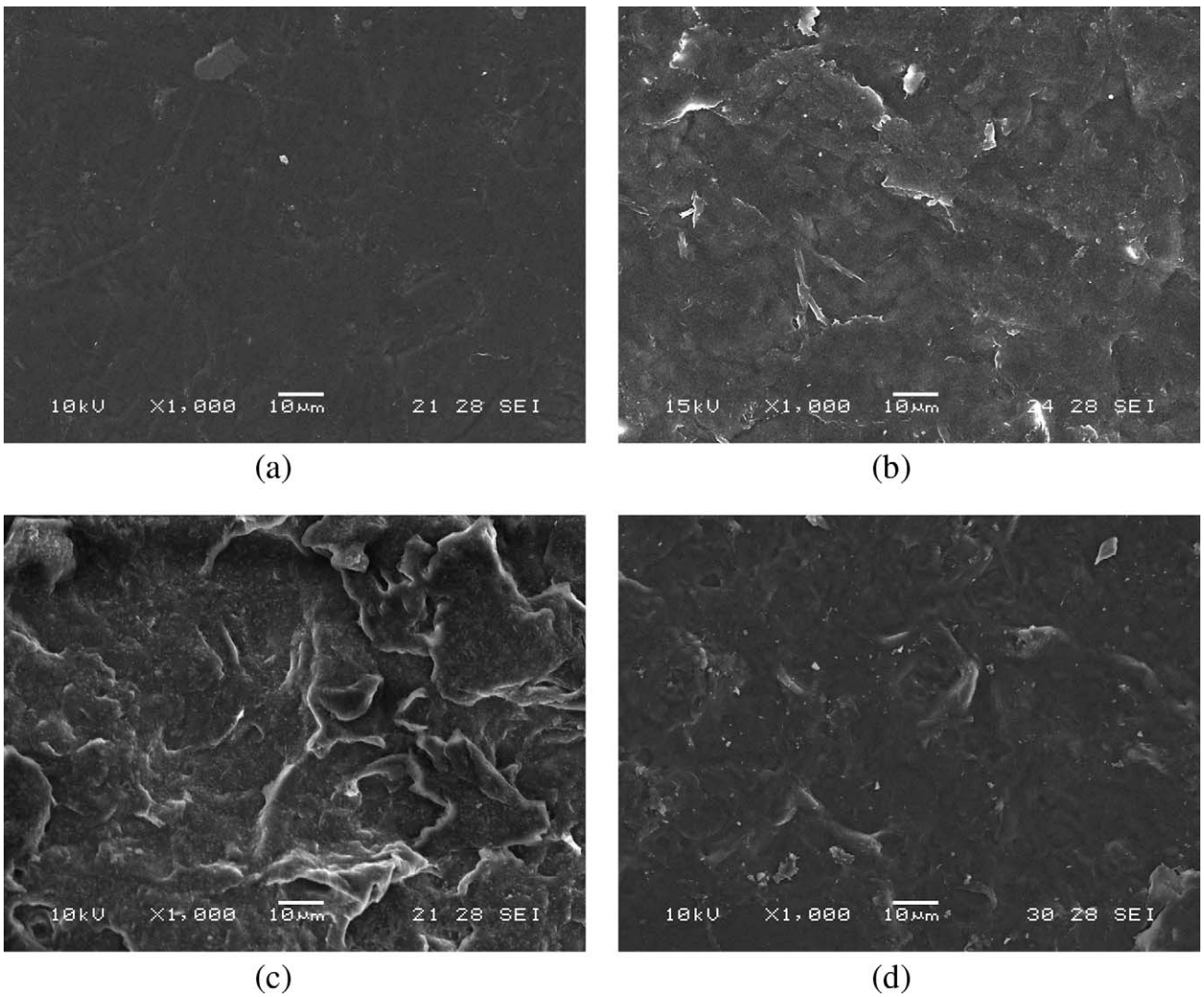

Figure 2 SEM observation of the supporting surfaces (a,b) and upper surfaces (c,d) of SA/PA-SPC7 and SA/PA-SPC10 films (1000×).

the blend components, that is, 64°C for PA and 72°C for SA [Fig. 3(a)]. Results were in good agreement with data published by Markley,[33] highlighting the existence of a eutectic point in a binary mixture of two fatty acids. Transition of a protein from a native to a denatured state is accompanied by the rupture of inter- and intramolecular bond and is characterized by the denaturation temperature (T_d) and the energy required or enthalpy of denaturation (ΔHd).[30] As observed from DSC thermograms of control and SA/PA-containing films shown in Figures 3(b–d), all thermograms were characterized by broad endothermic bands in the first scan in the range of 70–100°C assigned to the removal of residual water tightly bonded to the proteins after drying,[30,34] and no other thermal event was observed up to 200°C even in a second or third scan. No denaturation transitions were detected in control SPC in consistency with the standard industrial thermal processes applied to produce soybean concentrate from soybean flour, giving extensively denatured soy proteins.[34] The absence of denaturation transitions in SPC7 and SPC10 might suggest no further changes induced by varying the pH of the film forming solutions.

The addition of SA/PA to SPC at pH7 resulted in the appearance of two endothermic peaks at 57°C and 70°C together with water loss [Fig. 3(c)]. These two peaks were linked with the melting of unreacted fatty acids which could crystallize upon cooling. For SA contents lower than 60% in the mixture with PA, each fatty acid crystallizes in its own crystal lattice.[35] Therefore, one can hypothesize that after mixing with SPC, fatty acid phase separation, complexation[19,20] or reaction[3] with SPC could possibly change the initial SA/PA proportion. This result gave evidence of the non homogeneous incorporation of fatty acids blend to SPC matrix at pH 7. A similar effect was observed for gelatin-hydrophobic

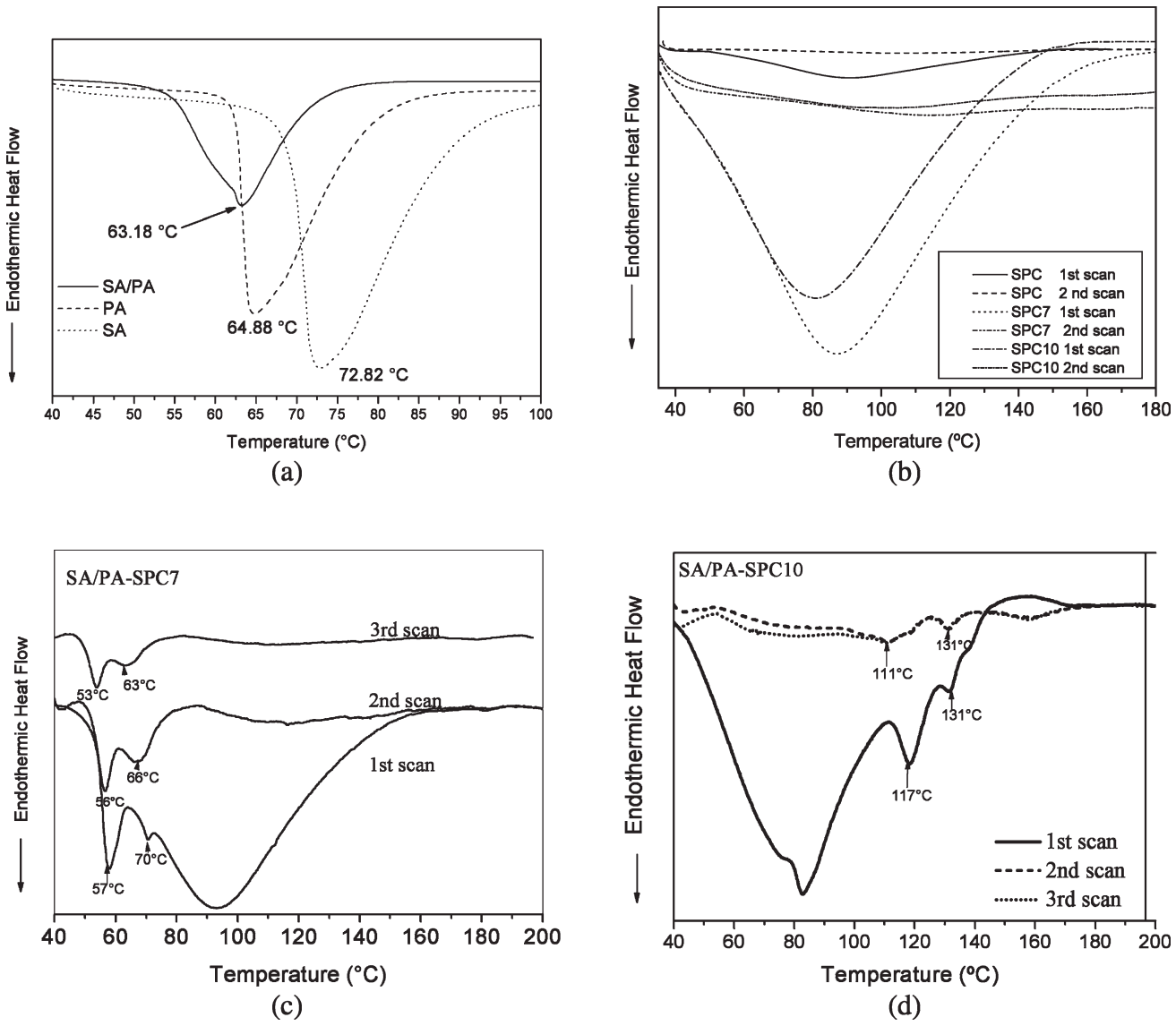

Figure 3 Dynamic DSC thermograms performed at 10°C/min under nitrogen atmosphere. (a) SA/PA blend, pure SA and PA, (b) control SPC, SPC7, and SPC10, (c) SA/PA-SPC7 film, and (d) SA/PA-SPC10 film.

compounds.[17] After re-heating crystallization seemed to be restricted to quite an extent as concluded from the shift to lower temperatures and reduced peak areas may be due to the restricted mobility of fatty acid chains in the absence of moisture which led to less perfect and stable crystals.[35] This could also an indication solid-solid transition between polymorphic forms of stearic acid induced by palmitic acid in quenched samples.[35] Calorimetric results agreed well with SEM observations and opacity results and confirmed that the dispersed phase in SA/PA-SPC7 was mainly constituted by the unreacted fatty acids. Similar results were reported by other authors regarding SPI films modified with 50% of stearic acid[3] and oleic acid-beeswax-SPI mixtures.[5] Unlike the results above described, SA/PA-SPC10 thermogram revealed two new endothermic peaks at 117 and 131°C (see arrows on DSC traces in Fig. 3 days) and no melting peaks for SA/PA. The absence fatty acid melting peaks suggests that strong protein-fatty acid interactions would take place leading to the bonding of SA/PA molecules which are not able to crystallize. High temperature endothermic peaks were absent in control SPC thermograms [Fig. 3(b)] and remained invariable when reheated. Despite the fact that fatty acids are ionized at pH > 6, these temperatures could not be associated with the sodium salts of stearic (245–255°C) and palmitic acid (283–290°C)[33] but rather to new crystalline structures developed owing to fatty acid complexation[19,20], reaction[3] or both with SPC. New crystalline structures at 77, 92, and 107°C have been reported in SA-modified SPI resins; however, the nature of such new structures has not been elucidated.[3]

XRD patterns

XRD results of control and SA/PA- modified SPC films are shown in Figure 4. Pure SA and PA as well as the 70/30 SA/PA blend exhibited crystalline structures [Fig. 4(a)]. It is worth mentioning that the pattern of SA/PA blend superimposed that of pure SA with peaks at $2\theta = 20.2$, 21.5, and 24°, as well as by long spacing at $2\theta = 6.64°$, corresponding to pure stearic acid. This last peak was found at $2\theta = 7.62°$ in pure PA. Frede and Precht[35] stated that shorter chains of PA molecules could be incorporated in the crystal lattice of pure SA in mixtures containing 60–95%SA, without significant changes in the unit cell parameters. The XRD patterns of SA/PA blend-containing SPC films are depicted in Figure 4(b). Pure SPC showed a typical amorphous XRD-pattern meanwhile SA/PA-added-SPC films exhibited the main characteristic peaks of SA/PA at 22.4, 20, and 21°, being more relevant for films modified at pH 7. This suggests that nonionized fatty acids remained distributed within SPC in a nonhomogeneous but crystallizable form as it was inferred from SEM and DSC results. SA/PA-SPC7 film featured higher crystallinity degree than that at pH 10. The reduction in the proportion of crystalline and amorphous phases in SA/PA-SPC10 films implies a greater amorphous phase content which can efficiently blend with the amorphous SPC matrix acting as external plasticizer. Moreover, new peaks appeared centered around 19, 23, and 25°, which could have originated from new crystalline structures, being more significant in the SA/PA-SPC10 XRD pattern.

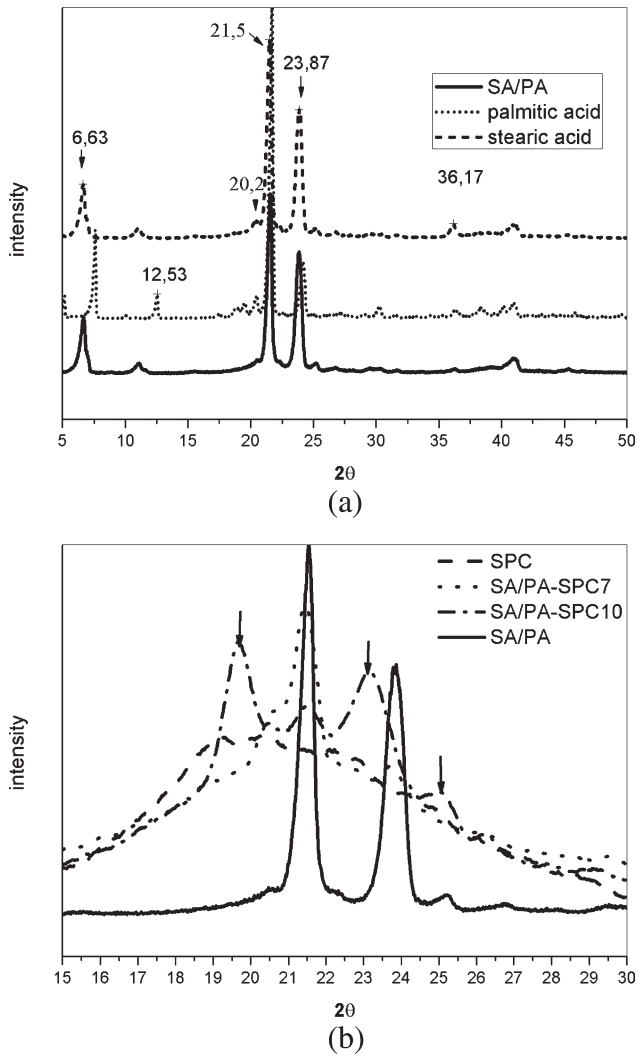

Figure 4 XRD diffraction patterns (a) SA/PA blend, stearic acid and palmitic acid and (b) control and SA/PA-SPC films.

ATR-FTIR analysis

A better comprehension of the film structure can be obtained by analyzing FTIR spectra of fatty acid blend, control, and emulsion films. The spectrum SA/PA blend [Fig. 5(a)] was dominated by relevant peaks at 3400 cm^{-1} arising from O-H stretching, the characteristic C=O stretching of the carboxylic acid functionality around 1700 cm^{-1} and strong absorption peaks in the range of 2800–3000 cm^{-1} associated with C-H stretching in the aliphatic chain of fatty acids.[15] Particularly, bands at 2925 and 2854 cm^{-1} resulting, respectively, from the asymmetric and the symmetric stretching vibrations of the acyl CH2 groups are visible.[36] The spectral feature of such peaks may change when mixing with proteins[22] and will be analyzed later with further detail.

Control SPC powder [Fig. 5(b,c)] showed peaks of relevance at 1632, 1532, and 1230 cm^{-1}, characteristics of amide I (C=O stretching), amide II (*N*-H bending) and amide III (C-N and *N*-H stretching).[37] The peak at 1064 cm^{-1} and its shoulder at about 1000 cm^{-1} was ascribed to C-OH stretching of carbohydrate fraction in SPC,[38] while the broad absorption band between 3500 and 3200 cm^{-1} (amide A band) was attributed to free and bound O-H and *N*-H groups in SPC.[37] The region between 1600 and 1700 cm^{-1} is the most often used to obtain information about the protein secondary structure since this region corresponds to β-sheets, α-helix, turns, and random coils.[39] The amide II region between 1600 and 1500 cm^{-1} is similarly dominated by chain oscillations, but the correlation between protein secondary structure and frequency is less straightforward than for the amide I vibration. According to amide I region, the denatured state of SPC (as determined by DSC) still preserves native like structures such as β-sheets (1629–1639 cm^{-1}), α-helix or random coil (1648–1655 cm^{-1}).[37] After film formation at both pH values, amide I band experienced a slight shift toward lower wave-numbers [Inset II, Fig. 5(b,c)] revealing a structural reorganization of soy proteins during drying with different distribution of

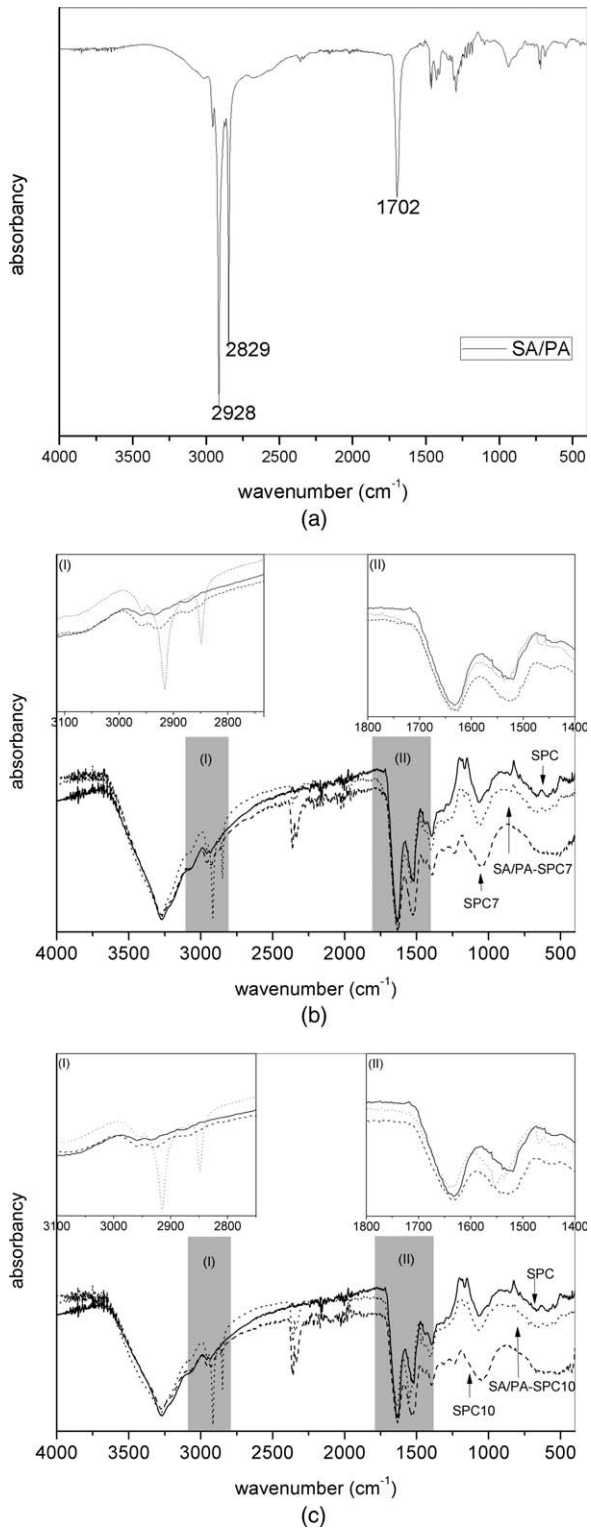

Figure 5 ATR-FTIR spectra (a) SA/PA blend and (b) control SPC, SPC7, and SA/PA-SPC7, and (c) SPC, SPC10, and SA/PA-SPC10.

hydrogen-bonding pattern,[37] among other interactions (*S*-S bonds, hydrophobic interactions, ionic) that can be established between protein chains.[29–31,37] Changes in amide II band were also evident [Fig. 5(c)], but this band is considered to be more sensitive to hydration than to secondary structure changes.[40] The broadening and slight shifting toward lower wave-number suffered by the carbohydrate peak around 1064 cm^{-1} may be attributed to the hydroxyl possible involved in hydrogen bonding. Similarly, the occurrence of inter- and intramolecular hydrogen bonds in dry film could be also confirmed by the small broadening in the absorption band in the frequency range 3600–3000 cm^{-1}.

Spectra of SA/PA-SPC films showed both the distinctive peaks of SA/PA blend and the characteristic bands attributed to SPC [Fig. 5(b,c)]. The presence of strong absorption 2850–2960 cm^{-1} [Inset I, Fig. 5(b,c)] confirmed the incorporation of hydrocarbon segments from fatty acids into the SPC matrix.[15] The broadening of 2925 cm^{-1} band in both SA/PA-added films [Inset I, Fig. 5(b,c)] compared to pure SA/PA [Fig. 5(a)], could be a result of the disorder of the SA/PA chains due to hydrophobic protein-fatty acid interactions.[18,22] It is worth noticing that protein structure also suffered some changes when mixing with SA/PA, being more relevant at pH 10. The shift of amide I band in SA/PA-SPC10 spectrum from 1629 to 1634 cm^{-1} suggests hydrogen interactions between fatty acid molecules and peptide bonds.[37] The reduced intensity of amide II band (Inset II Fig. 3 d), is an indication of a less hydrate protein structure.[40] It was also postulated that alkaline pH values could favor preferential reaction of fatty acids with amino-side chain groups of soybean proteins to produce new amide linkages[3] (ester linkages with OH groups from protein or carbohydrates in SPC can be neglected at highly alkaline pH).[3] Unfortunately, no evidence of such new amide moieties was found by FTIR since they are undistinguishable from peptide bonds in protein backbone.

Moisture absorption

The study of edible films moisture resistance is of great relevance given its influence on products performance and consumer acceptance.[8] Experimentally derived moisture sorption isotherms would offer suitable in-use performance to predict the in-use behavior of fatty acid modified SPC films at a given relative humidity environment. Therefore, the influence of adding SA/PA blend to SPC on the moisture resistance of the resultant films was investigated at 25°C and 98 ± 2% RH. Figure 6 illustrates the sorption isotherms. Experimental moisture sorption data of up to 100 h were fitted with Peleg's empirical equation [eq. (2)]. Estimated values correlated adequately with experimental data even within the curvilinear segment of the absorption curves[27–28] and yielded reasonable performance, as evidenced by the high value of the coefficients of determination, R^2 values (Table I). Constant values related to

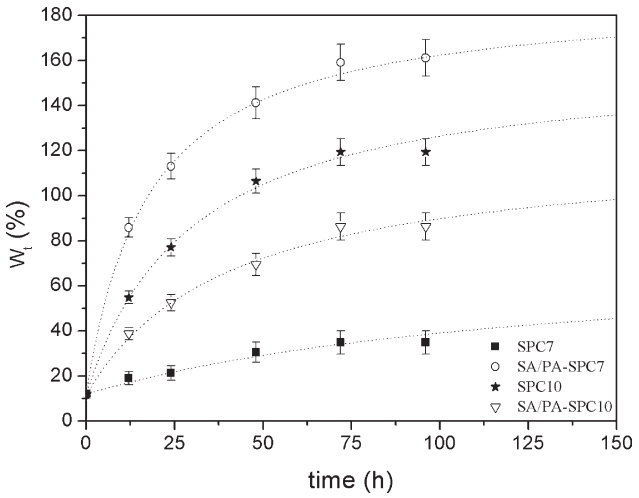

Figure 6 Moisture absorption curves at 25°C and 98 ± 2% RH of control and SA/PA-modified SPC films as a function of exposition time. Symbols: experimental data, lines: fitted with Peleg's empirical equation (eq. 2).

the mass transfer rate, k_1, and to the maximum water absorption, k_2 [25,27,28], were obtained from the linear fit and are summarized in Table I as well. By analyzing the experimental curves, a strong effect of pH and SA/PA blend addition on $M_{eq,}$ k_1 and k_2 values was verified. Among the un-modified SPC films, SPC7 was the most effective in restricting moisture absorption as evidenced by the moisture content at the equilibrium (M_{eq}) (i.e., $M_{eq\ SPC7}$ = 75% versus $M_{eq\ SPC10}$ = 161%). SPC7 films yielded the highest k_1 and k_2 values, implying that these films absorbed less moisture at a slower rate during storage at 98% RH. Even though SPC 10 films showed denser microstructure than SPC 7 films (Fig. 1) did, protein molecules were also more negatively charged at alkaline pH, increasing their moisture binding capacity. The addition of SA/PA blend to SPC at pH 7 resulted in an adverse effect on water resistance, as reflected by a significant decrease in both, k_1 and k_2 parameters, whereas SA/PA-SPC10 films exhibited higher k_1 and k_2 values compared to control SPC10 films (Table I). As a general trend, the simple addition of hydrophobic compounds cannot improve the moisture resistance of hydrophilic films unless a homogeneous and continuous lipid layer is formed.[5,18,19] The faster absorption rate and higher moisture affinity of SA/PA-SPC7 could be explained in terms of the more inhomogeneous microstructure which restricts fatty acid-protein interactions compared with the pH10 counterpart. With regard to other biopolymers, SA/PA-SPC 10 films absorbed faster but similar amounts of moisture than unplasticized wheat starch films exposed to 100% RH (k_1 = 32.645 h/%; $k_2 = 9.046/10^2$/%).[28]

This suggests that nonionized fatty acids remained distributed within SPC in a nonhomogeneous but crystallizable form as it was inferred from SEM and DSC results. SA/PA-SPC7 film featured higher crystallinity degree than that at pH 10. The reduction in the proportion of crystalline and amorphous phases in SA/PA-SPC10 films implies a greater amorphous phase content which can efficiently blend with the amorphous SPC matrix acting as external plasticizer.

CONCLUSION

The addition of 20 wt % SA/PA blend to SPC film-forming solution gave rise to edible films with different characteristics depending on the selected pH conditions. SEM observations revealed the presence of lipid globules unevenly distributed in the upper surface of SA/PA-SPC7 films, ascribed to the creaming of lipid droplets during the film drying process. The more homogeneous morphology of SA/PA-SPC10 film was related with the ability of establishing fatty acid-protein interactions at alkaline pH as probed by FTIR. This favored the integration of fatty acids hydrophobic segments into SPC, generating more even films with reduced moisture absorption rate and absorption capacity but with increased opacity due to the light blockage of lipid particles. The potential of SA/PA-SPC10 as protective coating materials for perishable foods (i.e., fresh broccoli) remains under study.

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