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Extrusion of soy protein with gelatin and sugars at low moisture content

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ABSTRACT

Soy protein-based materials modified with gelatin, lactose and sucrose were prepared by extrusion at low moisture content. The effect of composition on the extrusion parameters was investigated and specific mechanical energy (SME) was measured as an indication of extrusion processability, thus providing good characterization of the extrusion process in order to make it highly energy efficient and cost effective. Water content was the most important factor on the extrusion parameters and product properties. The incorporation of gelatin increased SME and the product obtained at the extruder die was not continuous. However, when lactose was added, SME decreased and the color of the product changed due to Maillard reaction. This reaction could be analyzed by Fourier transformed infrared spectroscopy (FTIR) where the changes of the amide I and amide II bands reflected that hydroxyl groups in sugars and amino groups in soy protein isolate (SPI) were consumed during extrusion. These results are in good agreement with total soluble matter (TSM) values, which were lower for mixtures with lactose than sucrose due to a higher degree of Maillard reaction. Moreover, X-ray diffraction (XRD) and scanning electron microscopy (SEM) results also showed the influence of Maillard reaction, which lead to more ordered and compact structures, respectively.

1. Introduction

Extrusion is widely used in the plastics industry and most of synthetic polymers are produced by this way. However, few reports related to proteins processed by extrusion have been published, so thus the application of extrusion technology to the production of protein films or biocomposites is a challenge for researchers (Ghorpade et al., 1997; Konstance et al., 2002). On the other hand, extrusion processing techniques for producing starch films have been developing for many years; however, there are few commercial products on the market, mainly because extrusion parameters such as barrel temperature, moisture content, screw speed, and feed rate have significant influence on processability and must be still studied (Chen et al., 2007; Fishman et al., 2000).

In a typical extrusion operation, the two main sources of energy associated with enthalpy change of the extrudate are convection heat transfer between the hot or cold barrel and the material, and viscous dissipation of the mechanical energy into heat inside the material (Harper, 1989; Hu et al., 1993). The rate of convection heat transfer is proportional to the amount of contact area between the barrel and the flowing material, whereas the heat generated due to viscous dissipation is proportional to the volume of the material. Twin-screw extruders have considerably more heat exchange capability than single-screw extruders. In addition, the direction of screw rotation, screw shape, screw configuration and relative position of screw sections minimize pressure and leakage flows (Harper, 1989). In a co-rotating twin-screw extruder, viscous dissipation of the mechanical energy predominates, especially at low moisture contents, thus making the extrusion process highly energy efficient and cost effective. Specific mechanical energy (SME) is the amount of mechanical energy dissipated as heat inside the material, expressed per unit mass of the material. Specifically, it is the work input from the drive motor into the material being extruded, and thus provides a good characterization of the extrusion process.

Thermal extrusion exposes the proteinaceous ingredients to high temperature, high pressure and mechanical shear, which converts soy protein into a continuous plastic "melt", resulting in protein denaturation and solubility reduction (Harper, 1989). Within the process, water soluble fractions of soy protein (7S and 11S globulins) undergo a complex pattern of association–dissociation reaction (Cheftel et al., 1985). The effect of extrusion is to disassemble proteins and then reassemble them together by disulfide bonds, hydrogen bonds and noncovalent interactions forming fibrous structure in extrudates. However, it has been shown that the extrusion processing of soy protein sheets containing low parts of glycerol resulted in very brittle materials after losing moisture (Zhang et al., 2001), so that the use of higher glycerol contents

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seems to be necessary in order to make the protein chains mobile. Breaking intermolecular linkages that stabilize the protein in their primitive structure, as well as the orientation and restructuring of the chains with the formation of new intermolecular linkages, stabilize the three-dimensional network formed.

Despite of the increasing use of extrusion technology, extrusion process is still a complicated multi-input–output system that has to be mastered. A simplified analysis model was proposed (Meuser and Van Lengerich, 1984), which sorted extrusion parameters into three groups, namely, process parameters (screw speed, moisture content, barrel temperature, screw configuration, die dimension, raw material characteristics, etc.), system parameters (energy input, residence time, etc.), and products properties (color, nutrition, texture, taste, etc.). Among these three kinds of parameters, process parameters have effects on the properties of final products by means of affecting extrusion system parameters. In order to better understand the effect of extrusion process on the characteristics of products and to obtain various extrudates with ideal structure and texture, it is imperative to research the correlation between process parameters, system parameters and product parameters. SME is also an important process parameter influencing the final product characteristics such as solubility, extrudate density, expansion index, hardness, etc.

Screw extrusion is generally defined as a process to mix, homogenize, and shape material by forcing it through a specifically designed opening. Based on the considerations outlined above and the motivation of the fundamental research and potential industrial applications of biocomposites, the purpose of the present work was to investigate extrusion of SPI with gelatin and sugars using twin-screw extruder at low moisture content to study the effect of moisture, gelatin and Maillard reaction on system parameters and product properties.

2. Materials and methods

2.1. Materials

Soy protein isolate (PROFAM 974) with 90% protein on a dry basis was supplied by Lactotecnia S.L. (Barcelona, Spain). SPI has 5% of moisture, 4% of fat and 5% of ash. It has acid character and the isoelectric point is 4.6.

The commercial bovine gelatine (GE) type A (bloom 200/220) was obtained from Sancho de Borja S.L. (Zaragoza, Spain). The gelatin meets the quality standard for edible gelatin (1999/724/CE). Glycerol used in this study was food grade reagent obtained from Panreac, as well as sugars: sucrose (SUC) and lactose monohydrate (LAC).

2.2. Extruder and experimental configurations

A twin screw extruder (MPF 19/25 APV Baker) was used in this study. A schematic diagram of the extruder is shown in Fig. 1. The MPF 19/25 has 19 mm diameter barrel, a length to barrel diameter ratio of 25:1, and a barrel comprising four controlled temperature zones, resulting in a die temperature of 70–100 °C. The APV Baker MPF19 extruder is designed to generate the high pressures needed to extrude a wide range of products, in particular, edible/biodegradable products. The screw configuration used in the present study is shown in Table 1.

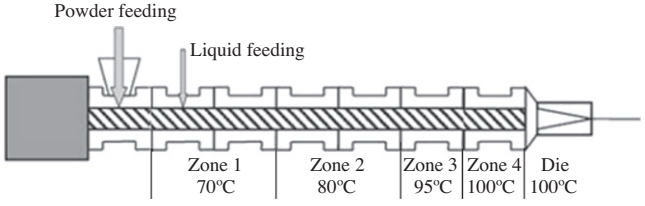

Fig. 1. Schematic diagram of the twin-srew extruder employed in this study.

2.3. Samples preparation

SPI and the gelatin, sugar and glycerol contents required to obtain the desired percentage were mixed. All components were mixed in a variable speed Stephan mixer, model UMC 5 (Stephan, UK) for 5 min at 1500 rpm.

Different glycerol contents (20%, 30% and 40% w/w) were employed and samples were designed as SPI20, SPI30 and SPI40. Gelatin was selected as 15% by weight on SPI dry basis, based on the results of a previous work (Guerrero et al., 2011). Samples were designed as SPI-GE. Different sugar contents (10%, 20%, 30% and 40% by weight on SPI dry basis) were also added and samples were designed as SUC10, SUC20, SUC30 and SUC40 in the case of sucrose, and LAC10, LAC20, LAC30 and LAC40 for lactose.

Mixtures were added into the feed hopper and mixed with water in the barrel of the twin-screw extruder. Based on a series of preliminary experiments, the following optimum processing parameters were used in this work: temperature profile of 70, 80, 95, and 100 °C along the extrusion direction; feeding rate of 1 kg/h, and screw speed of 250 rpm.

All trials were carried out using a water speed of 2.94 g/min (0.15 kg/h). Water was pumped directly into the extruder barrel zone 1 using a peristaltic pump (504U MK, Watson Marlow Ltd.), as shown in Fig. 1.

All samples were conditioned in a controlled biochamber (ACS Sunrise 700 V) at 25 °C and 50% RH (relative humidity) for 48 h before testing.

2.4. Specific mechanical energy (SME)

The specific mechanical energy was calculated using the expression (Hu et al., 1993):

$$\text{SME (kJ/kg)} = \frac{\text{Screw speed} \times \text{Power (kW)} \times \text{Torque (\%)}}{\text{Max. screw speed} \times \text{Throughput (kg/h)} \times 100}$$

The extruder was operated at a constant speed of 250 rpm. The feed rate of extruder was adjusted to 1 kg/h, and had a 2 kW/h motor power in practice. Dies were scaled up by maintaining a constant throughput per unit of orifice area. The extruder was equipped with a single die of 3 mm diameter giving a throughput per unit area of 0.141 kg/h mm^2.

2.5. Differential scanning calorimetry (DSC)

DSC experiments were performed using a Mettler Toledo DSC 822. Sample weights were in the range of 3 mg, and all runs were

Table 1
Configuration of the twin-screw extruder employed (D = screw diameter).

Screw configuration	Screw function
5.0 D	Feed screw
6.0 × 60°	Forward paddles
5.0 D	Feed screw
5.0 × 60°	Forward paddles
2.5 D	Feed screw
3.0 × 60°	Forward paddles
2.0 D	Single lead screw
5.0 × 60°	Forward paddles
3.0 D	Single lead screw
3.0 × 60°	Forward paddles
2.0 D	Single lead screw

carried out from −50 °C up to 250 °C at a heating rate of 10 °C/min. Experiments were carried out under nitrogen atmosphere, and the nitrogen gas flow employed was 10 mL/min. Sealed aluminum pans were used to prevent mass loss during the experiment.

2.6. X-ray diffraction (XRD)

XRD studies of extruded pellets were performed with a diffraction unit (PANalytical Xpert PRO) operating at 40 kV and 40 mA. The radiation was generated from a Cu-Kα (λ = 1.5418 Å) source. The diffraction data were collected from 2θ values from 2.5° to 50°, where θ is the angle of incidence of the X-ray beam on the sample.

2.7. Fourier transformed infrared spectroscopy (FTIR)

FTIR spectra of the samples were carried out on a Nicolet Nexus FTIR spectrometer using ATR Golden Gate (Specac). A total of 32 scans were performed at 4 cm^{-1} resolution. Measurements were recorded between 4000 and 800 cm^{-1}.

2.8. Moisture content (MC) and total soluble matter (TSM)

TSM was expressed as the percentage of film dry matter solubilised after 24 h immersion in distilled water. Two methods of determination were used and compared. One of them was previously used in some studies of films from proteins (Cuq et al., 1996; Guerrero et al., 2011; Kunte et al., 1997). Using the first method (method 1), three specimens of each sample were weighed (m_w) and subsequently dried in an air-circulating oven at 105 °C for 24 h. After this time, samples were reweighed (m_0) to determine MC values. Afterwards, samples were immersed in 30 mL of distilled water in the presence of sodium azide (0.02%) in order to prevent the microbial growth. The beakers were stored in an environmental chamber at 25 °C for 24 h with occasional gentle stirring. After this time, specimens were dried in an air-circulating oven at 105 °C for 24 h and weighed (m_f). Using the second method (method 2), dry matter and soluble matter were not determined on the same sample in an effort to avoid heating sample prior to immersion in water. Instead, three samples were directly immersed in water and beakers were stored in environmental chamber at 25 °C for 24 h with occasional gentle stirring to determine soluble dry matter. Initial dry matter values needed for TSM calculation were the ones obtained from MC measurements for the same sample.

MC and TSM values were calculated as:

$$\text{MC}\ (\%) = \frac{m_w - m_0}{m_w} \times 100 \qquad \text{TSM}\ (\%) = \frac{m_0 - m_f}{m_0} \times 100$$

2.9. Scanning electron microscopy (SEM)

The morphology of the fracture surface of the pellets was visualized using a field emission scanning electron microscope (Hitachi S-4800) at an acceleration voltage of 15 kV. Samples were fractured under liquid nitrogen prior to morphology visualization. The fracture surfaces were mounted on a metal stub with double-side adhesive tape and coated under vacuum with gold (JFC-1100) in an argon atmosphere prior to observation.

2.10. Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) by means of a SPSS computer program (SPSS Statistic 18.0). Post hoc multiple comparisons were determined by the Tukey's test with the level of significance set at $P < 0.05$. All measurements were carried out in triplicate.

3. Results and discussion

3.1. Effect of water and glycerol on extrusion

Firstly, the effect of glycerol on SME values, both with and without water, was analyzed. SME value also indicates the extent of molecular breakdown or degradation that mechanical force undergoes during extrusion process, so this value is an important parameter influencing final product characteristics such as solubility, expansion index, etc. (Godavarti and Karwe, 1997).

When SPI/glycerol mixtures were extruded without water, it was not possible to obtain product from the die due to a high increase in torque, irrespective of the glycerol level employed. When water was added, SME decreased ($P < 0.05$) as the content of glycerol was higher (Table 2). Based on these results, 20% glycerol was fixed for the extrusion process in order to analyze the effect of the other additives employed in this study.

The addition of water resulted in accelerating the flow speed of extrudate coming from the extruder. The water content was the most important factor influencing extrusion parameters and product properties. Water played an important role in extrusion processing due to its effect on the heat transfer during extrusion. Increasing water content resulted in increased heat transfer from the extruder barrel to the feed material and consequently decreased viscosity, shear, and friction during extrusion. Normally, the higher the water content, the lower the torque and SME because of the reduction of the force required to push wet mass through the die, thus decreasing the friction between raw material and screw shaft and extruder barrel (Chen et al., 2010) strictly linked to water lubricating effect. Therefore, increasing water content results in the decrease of dough viscosity in the extruder barrel, so thus lower force is required to pump the melt through the die, and the conversion ratio of extruder mechanical energy into heat energy, and consequently the SME become lower due to a lower viscous dissipation. As the water content greatly influences final properties such as tensile strength, hardness and chewiness (Chen et al., 2010; Lin et al., 2000), the optimum water content chosen in this study was 16% due to the fact that the glycerol concentration used also helped to retain moisture.

3.2. Effect of gelatin and sugars on extrusion

The effect of gelatin and sugars on SME is shown in Table 3. SME increased with the incorporation of gelatin due to a frictional increase in the extruder barrel, thereby increasing the force required to extrude wet mass through the die and converting the ratio of extruder mechanical energy into heat energy. Consequently SME became higher as did temperature in the die and as a result, the viscous dissipation was higher. The product obtained at the extruder die was not continuous, indicating that gelatin could unfold

Table 2
Effect of water and glycerol on SME values.

Sample	Glycerol content (%)	Water content (%)	SME (kJ/kg)
SPI20	20	0	∞
SPI20	20	16	1188 ± 22[a]
SPI30	30	0	∞
SPI30	30	16	972 ± 18[b]
SPI40	40	0	∞
SPI40	40	16	612 ± 15[c]

[a–c] Two means followed by the same letter in the same column are not significantly ($P > 0.05$) different through the Tukey's multiple range test.

and align during extrusion. As it was shown in previous work (Guerrero et al., 2011), this fact may be attributed to protein–protein interactions, which are determined by hydrogen bonds, electrostatic and/or hydrophobic interactions (Cheftel et al., 1985; Sothornvit and Krochta, 2001). These interactions are ultimately influenced by both the sequence of amino acid residues and the three dimensional size of the entire network. The gelatin can reacquire part of the triple helix structure with a high degree of organization, and consequently can show more organized networks compared to those made from SPI (Cao et al., 2007; Denavi et al., 2009).

As it can be seen in Table 3, the incorporation of sugars caused lower SME values compared to those samples without sugars. This change in SME value is due to the fact that torque decreased with increasing sugar content. Sugars in the system could alter the conformation of proteins, interact with them by binding to protein side groups through Maillard reaction, and influence physicochemical properties (Gu et al., 2009). The Maillard reaction rate depends on the type of sugar involved in the reaction, as it was found by Rich and Foegeding (2000). Fig. 2 shows the products obtained at the extruder die. As it can be seen, the color of the product changed depending on the sugar type employed. Lactose is a reducing disaccharide which can react with protein through Maillard reactions. Reactions that can take place during extrusion are controlled by the composition of samples, temperature, and residence time. However, these reactions are accelerated during extrusion processing because of shear forces. High barrel temperatures and low moisture contents promote Maillard reaction during extrusion. The early stage of the Maillard reaction involves the formation of the protein–sugar conjugates between the carbonyl group of a reducing carbohydrate with an available amino group in the protein, which leads to a Schiff base with the release of water (Yasir et al., 2007). The Schiff base subsequently cyclises to produce the Amadori compound and, in the final stage, highly colored, insoluble polymeric compounds, referred to as melanoidins, are formed.

3.3. Thermal properties of extruded SPI

Differential scanning calorimetry (DSC) can be used to characterize thermal properties of food proteins, including heat-induced denaturation. The denaturation process is a molecular change involving the destruction of internal order and, in some cases, the complete unfolding of protein chains. Heating can change soy protein from its native state to a denatured state (Kitabatake and Doi, 1990), which can be seen as an endothermic peak in DSC thermograms. The two denaturation peaks of SPI, corresponding to the low molecular weight 7S globulin and the high molecular weight 11S globulin, were shown in a previous work (Guerrero and de la Caba, 2010), indicating that a significant amount of the SPI used in this study remained in its native conformation with globular structure after processing by compression. In the case of extrusion,

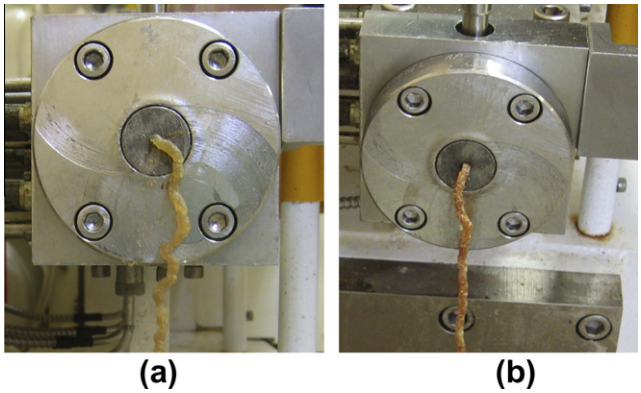

Fig. 2. Products obtained at the extruder die for (a) SPI with sucrose and (b) SPI with lactose.

Fig. 3 shows a broad peak for SPI20, indicating that processing conditions did not cause total denaturation of the protein. However, when gelatin was added, this peak disappeared, which is an indication of protein denaturation due to the conformational changes that produce a certain degree of interaction between gelatin and SPI, such as hydrogen bonds, dipole–dipole and hydrophobic interactions, characteristic of natural proteins. These results are in good agreement with the increase of SME with gelatin addition. It is also worth to note that the crystallization peak for pure lactose, which occurs at 124–135 °C (Drapier-Beche et al., 1997), could not be observed in the presence of SPI. The Maillard reaction could reduce the rate of nucleation and growth of crystals in the solid phase, showing changes in the crystallinity of sugars (Adhikari et al., 2009).

When soy protein is extruded under high pressure, high temperature, and low moisture conditions, the sudden release of pressure upon exiting from the die caused instant water evaporation from the extrudates, creating expanded and spongy structures, quite like that of common textured vegetable proteins. According to Kitabatake and Doi (1990), the temperature needed for texturization in the extruder was the denaturation temperature of soy protein, which is dependent on water content. After denaturation, viscosity decreased and the fluidity of soy protein dough might give rise to a certain order under some shearing conditions, which resulted in texturized protein after cooling.

3.4. X-ray diffraction (XRD)

XRD was used to determine the structure for the samples with different additives. Fig. 4 shows that the diffraction patterns of the SPI samples exhibited a dominant amorphous halo, a broad band with a maximum at $2\theta = 20°$, characteristic for pure SPI, which

Table 3
Effect of gelatin and sugars on SME, MC and TSM values.

Sample	Gelatin content (%)	Sucrose content (%)	Lactose content (%)	SME (kJ/kg)	Moisture content (%)	TSM method 1 (%)	TSM method 2 (%)
SPI20	0	0	0	1188 ± 22^{a}	15.8 ± 0.1^{a}	21.5 ± 0.1^{a}	21.5 ± 0.2^{a}
SPI-GE	15	0	0	1512 ± 19^{b}	15.9 ± 0.4^{a}	21.6 ± 0.2^{a}	22.3 ± 0.4^{a}
SUC10	15	10	0	828 ± 23^{c}	17.4 ± 0.1^{b}	25.9 ± 0.2^{b}	27.7 ± 0.2^{b}
SUC20	15	20	0	$720 \pm 22^{d,e}$	16.5 ± 0.2^{c}	31.3 ± 0.5^{c}	32.7 ± 0.2^{c}
SUC30	15	30	0	$684 \pm 20^{e,f}$	15.2 ± 0.1^{a}	35.8 ± 0.1^{d}	36.9 ± 0.4^{d}
SUC40	15	40	0	648 ± 18^{f}	14.2 ± 0.2^{d}	39.7 ± 0.2^{e}	40.6 ± 0.3^{e}
LAC10	15	0	10	1368 ± 24^{g}	20.0 ± 0.2^{e}	18.3 ± 0.1^{f}	22.4 ± 0.2^{f}
LAC20	15	0	20	900 ± 17^{h}	19.9 ± 0.2^{e}	$18.5 \pm 0.1^{f,g}$	24.8 ± 0.5^{g}
LAC30	15	0	30	$792 \pm 15^{c,i}$	19.8 ± 0.2^{e}	$19.1 \pm 0.1^{f,g}$	27.7 ± 0.4^{h}
LAC40	15	0	40	$756 \pm 16^{d,i}$	19.5 ± 0.5^{e}	19.3 ± 0.1^{g}	29.9 ± 0.4^{i}

[a–i] Two means followed by the same letter in the same column are not significantly ($P > 0.05$) different through the Tukey's multiple range test.

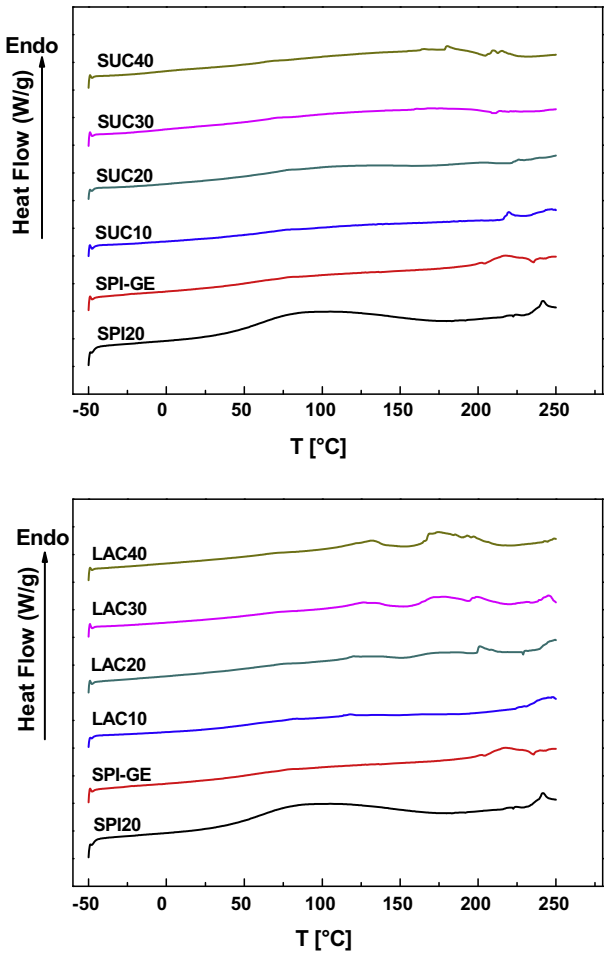

Fig. 3. DSC thermograms for SPI-based products with different additives.

has 7S and 11S amorphous globulins as main components. The intensity of this band increased when gelatin was added, indicating a higher order in the structure. The addition of sucrose did not affect the intensity of the peak obtained for SPI-GE; however, the addition of lactose caused a significant increase of crystallinity, indicating that the presence of lactose affect the amorphous structure of the SPI-based samples. It is worth to note that SPI can not totally inhibit the crystallization of lactose, although protein–lactose covalent bonds formed by Maillard reaction prevent crystal nucleation and growth (Forbes et al., 1998). These results are in accordance with the ones shown by DSC thermograms, where no crystallization peak was observed for lactose.

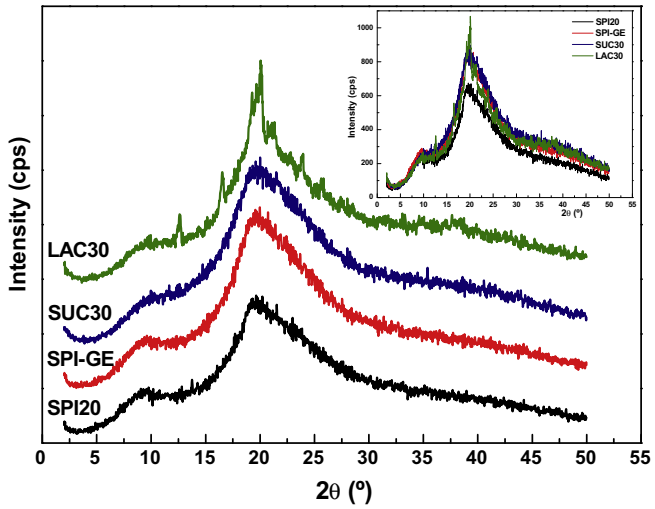

Fig. 4. XRD patterns for SPI-based products with different additives.

3.5. Fourier transformed infrared spectroscopy (FTIR)

The main absorption peaks of SPI are related to C=O stretching at 1630 cm^{-1} (amide I), N–H bending at 1530 cm^{-1} (amide II) and C–N stretching and N–H bending (amide III) at 1230 cm^{-1} (Lodha and Netravali, 2005; Schmidt et al., 2005). The broad band observed in the 3500–3000 cm^{-1} range is attributable to free and bound O–H and N–H groups, which are able to form hydrogen bonding with the carbonyl group of the peptide linkage in the protein. As was shown in previous work (Guerrero et al., 2011), when gelatin was added, some gelatin peaks in the 1300–1000 cm^{-1} range disappeared, indicating a certain degree of interaction between gelatin and SPI, which would be in agreement with the increase of SME values in the extrusion process.

Fig. 5 shows FTIR spectra of blends with various weight ratios of sugars. It can be seen that the intensity of the bands at 3100–2900 and 1600–1400 cm^{-1} decreased when sugars were added. These changes of the amide I and amide II bands reflected that hydroxyl groups in sugars and amino groups in SPI were consumed during the blending process at elevated temperatures, which was attributed to Maillard reaction. Changes were more apparent for the products processed with lactose (Fig. 5b), where the three peaks that appeared at 1050–950 cm^{-1} for the products with sucrose became a single entity for the products with lactose when sugar content increased. These results show that the degree of Maillard reaction was higher for the materials mixed with lactose than the ones with sucrose due to the presence of the free hydroxyl group in the anomeric carbon of the lactose.

3.6. Moisture content (MC) and total soluble matter (TSM)

Moisture content (MC) and total soluble matter (TSM) values for SPI blends are shown in Table 3. MC values for the blends without lactose are lower than those with lactose and these MC values are similar ($P > 0.05$) when the lactose content increased, being around 20%. However, when sucrose content increased, MC values decreased significantly ($P < 0.05$), which would indicate that hydrophilic sites were less accessible to water molecules.

TSM values for SPI-based blends calculated by method 1 were determined by drying specimens in an oven before immersion in water. It is well known that heating, along with the reagent chemical structure, influence Maillard reaction. Therefore, heating of specimens prior to immersion in water could cause cross-linking, leading to underestimation of TSM values. For this reason, TSM values were also measured by method 2, using different specimens to determine initial and soluble dry matter. As shown in Table 3, the samples without sugar did not show significant difference ($P > 0.05$) between the two methods employed due to the fact that only physical interactions take place between protein and gelatin. For the blends with sucrose, the results obtained by the two methods were also similar; however, method 2 resulted in greater TSM values than method 1 for the blends with lactose due to the fact that lactose is a reducing disaccharide that presents a higher degree of Maillard reaction than sucrose, which leads to insoluble polymeric compounds. On the other hand, TSM values increased when sugar content was increased ($P < 0.05$), which was more pronounced for blends with sucrose where the degree of Maillard reaction is lower.

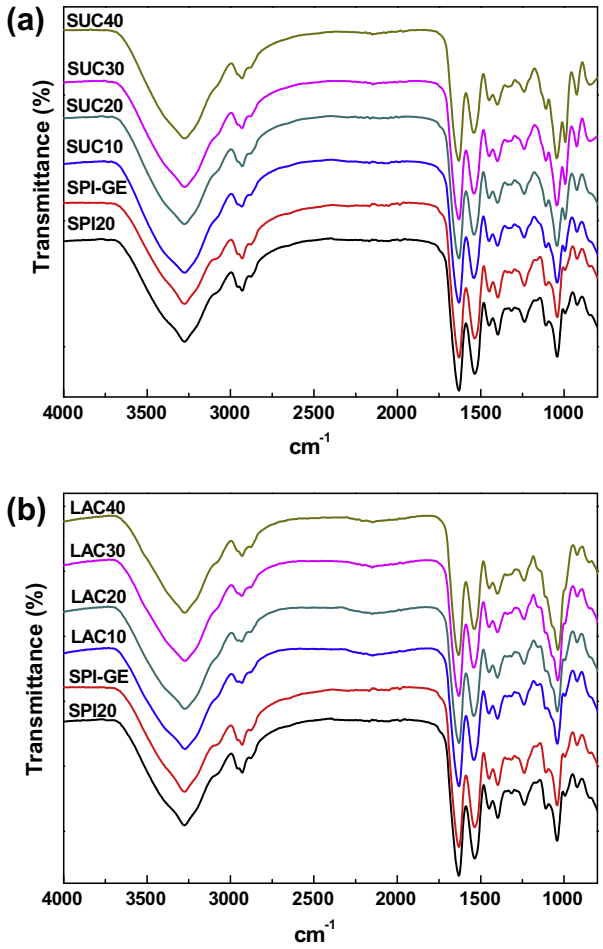

Fig. 5. FTIR spectra of SPI-based products with different contents of (a) sucrose and (b) lactose.

3.7. Scanning electron microscopy (SEM)

Fig. 6 shows SEM micrographs taken at the break surface of SPI samples fractured in liquid nitrogen. Samples are characterized by minor surface heterogeneities, which are typical in a brittle fracture. Some porosity was detected in SPI samples, although these cavities seem to be closed pores (Fig. 6a). Porosity decreased with the incorporation of gelatin (Fig. 6b), indicating that blending with gelatin cannot completely eliminate the structural defects of SPI. These micrographs are in accordance with the increase of SME values in the extrusion process and the increase of the intensity peak in XRD.

Morphologies for SUC30 and LAC30 showed more compact structures. Moreover, crosslinking reactions (Maillard reaction) occurred between lactose and SPI during extrusion lead to more compact structures for LAC30 than SUC30 (Fig. 6c). In the case of lactose (Fig. 6d), white spots indicate some local heterogeneities that lead to some surface irregularities where lactose particles comprise dense agglomerates of smaller subunits combined together to form large clusters.

4. Conclusions

Extrusion parameters for SPI-based materials with gelatin and sugars using twin-screw extruder at low moisture content were optimized. When SPI/glycerol mixtures were extruded without water, it was not possible to obtain product; however, SME decreased when only 16% water was added due to the fact that the 20% glycerol used in this study also helped to retain moisture. The incorporation of sugars also resulted in a decrease of SME values and the color of the product changed depending on the type of sugar employed. In the case of lactose, cross-linking reactions occurred between sugar and protein due to Maillard reaction. In the early stage of the reaction, the formation of the protein–sugar conjugates lead to highly colored and insoluble polymeric compounds, which showed a more ordered structure, as it was shown by XRD and SEM.

Maillard reaction could be evaluated by the changes of the amide I and amide II bands which reflected that hydroxyl groups

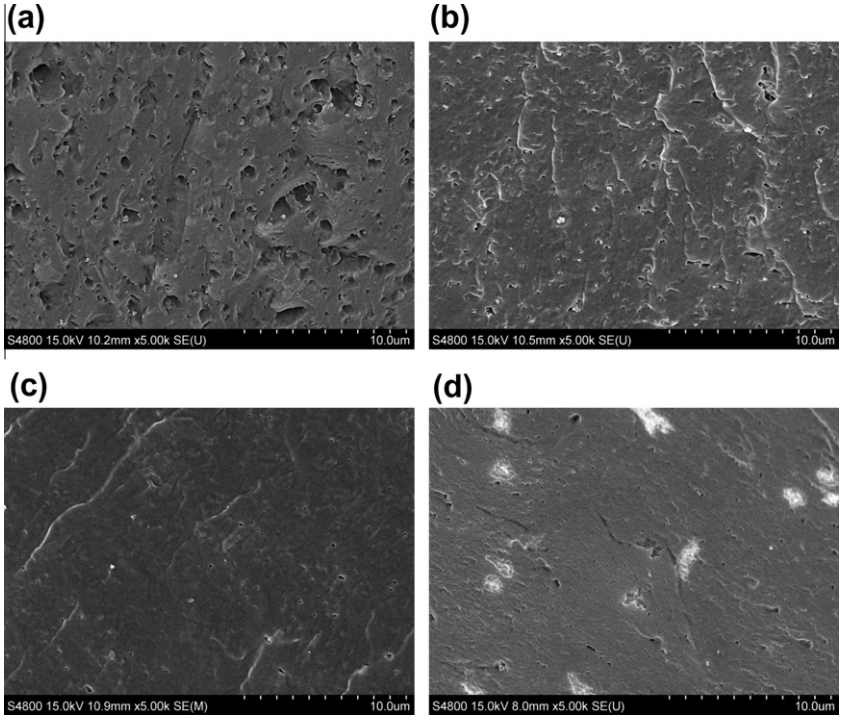

Fig. 6. SEM micrographs taken at the break surface of SPI-based products fractured in liquid nitrogen: (a) SPI20, (b) SPI-GE, (c) SUC30 and (d) LAC30.

in sugars and amino groups in SPI were consumed during the blending process at elevated temperatures. It was observed that the degree of Maillard reaction was higher for the materials with lactose than for the ones with sucrose due to the presence of the free hydroxyl group in the anomeric carbon of the lactose. These FTIR results were in accordance with the lower TSM values obtained for materials with lactose.

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