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Structure and mechanical behaviour of corn flour and starch–zein based materials in the glassy state

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Abstract

Corn flour and starch–zein based samples were prepared by extrusion and thermomoulding and then analysed at a moisture content of 12.0% (wb). Starch–zein blends (5–50% zein, db) were used to study the influence of starch–zein ratio on material properties. Glass transition temperatures were determined by differential scanning calorimetry and molecular relaxations by dynamic mechanical thermal analysis. Behaviour at large deformations was examined by the three-point bending test.

The behaviour of materials made from glassy corn flour and starch–zein blends was compared to the behaviour of their components. Amorphous starch was ductile whereas blends and corn flour samples were brittle. This difference could not be explained by molecular mobility.

Blend morphology observed by confocal scanning light microscopy (CLSM) showed that proteins undergo aggregation during thermomechanical processing, which largely conditioned their mechanical properties. The consequences of processing on the morphology of the protein phase in the corn flour were also discussed.

Keywords: Amorphous starch; Zein; Corn flour; Mechanical properties; Thermomechanical treatment

1. Introduction

The texture of dry food made from cereals results from relationships between processing conditions, composition and ingredient properties. Ready-to-eat breakfast cereals made from corn flour, for instance, and mainly composed of starch, belong to a family of products whose textural properties depend on the reorganisation of their components when submitted to thermomechanical processing (Rokey, 1995). Among cereal processes, extrusion-cooking has been widely investigated, with emphasis on starch structural modifications (Colonna & Mercier, 1989). The influence of extrusion conditions and the role of ingredients like sugars on expansion have already been studied (Barrett, Kaletunc, Rosenburg, & Breslauer, 1995; Fan, Mitchell, & Blanshard, 1996), but only a few studies report the interrelationships of other major components present in flour, such as proteins (Batterman-Azcona & Hamaker, 1998). Structural changes are not well known for different scales. Proteins are generally known to undergo conformational and chemical changes during processing, particularly changes in ionic, hydrogen and hydrophobic bonding and cross-linking by disulfide or non-disulfide covalent bonds (Stanley, 1989). Zein aggregation due to the creation of disulfide bonds during thermomechanical treatment has been demonstrated by Batterman-Azcona, Lawton, and Hamaker (1999a,b) in corn flakes. When studying the effects of components, particularly starch and proteins in blends, the characterisation of expanded products begins with an investigation of the properties of the cell walls. Properties of dense materials containing no bubbles can also be used for solid foams, using the Ashby relationship (Gibson & Ashby, 1997).

Corn flour is composed of the endosperm, which generally contains between 75 and 87% starch and 6–8% protein (Shukla & Cheryan, 2001). Zeins, the storage proteins of corn, represent 60% of the proteins and are

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located in the protein bodies (Lending & Larkins, 1989). Zeins, belonging to the family of proteins known as prolamins, are divided into four different classes, α-, β-, γ- and δ-zein, and differ by their molecular weight and location in the protein body. Their different solubility behaviours are the result of their various amino acid sequences. α-Zein, the major component of zein (75–85% of the total zein), is composed of two polypeptides of 23,000–24,000 and 26,500–27,000 Da (Esen, 1987). β-Zein (17,500 Da) and γ-zein (21,800 Da) constitute 10–15% and 5–10% of the total zein, respectively. Located at the periphery, they are organised into clusters within the protein bodies (Lending & Larkins, 1989). Due to their hydrophobic characteristics, zein molecules present interesting film properties which have been described by many authors. Without any plasticiser, cast zein films obtained from aqueous alcohol solutions exhibit fragile behaviour (Lai & Padua, 1997; Yoshino, Isobe, & Maekawa, 2000).

For wheat products, particularly wheat dough and pasta, it is well known that gluten proteins can create a network (Amend, Belitz, Moss, & Resmini, 1991; Bache & Donald, 1998). Conversely, corn gluten meal does not have the same ability (di Gioia, Cuq, & Guilbert, 2000) and the morphology of zein phases in dry cereal-based foods is not well understood at this time. Confocal scanning light microscopy provides the opportunity to analyse the problem from another point of view. It has become a suitable tool for observing food structure in three dimensions and for focusing on particular components by specific staining (Dürrenberger, Hanschin, Conde-Petit, & Escher, 2001), as in the case of bread or spaghetti, for example. It could also be used to observe protein morphology in corn flour products.

Nicholls, Appelqvist, Davies, Ingman, and Lillford (1995) characterised the behaviour of starch and gluten in the glassy state in order to better understand the fracture mechanisms of dry wheat-based foods. The structure of these solid products has to be determined since they constitute the cell wall materials of expanded cereal products and contribute to their mechanical and fracture behaviour. The behaviour of the major components of corn flour, starch and zein and their blends, has not yet been studied in this way. Corn starch and zein blends were principally studied to obtain non-edible water-resistant films with mechanical properties and storage stability improvements (Lim & Jane, 1993, 1994).

The aim of this study is to better understand the relationships between the properties and the structure of materials made from corn flour. For this purpose, dense materials were prepared from corn flour and starch–zein blends by extrusion and thermomoulding under different thermomechanical conditions, and then analysed in terms of thermal and mechanical properties and morphology. Starch–zein blends may be considered as a model system for determining the influence of zein concentration.

2. Materials and methods

2.1. Materials

Corn starch was purchased from Roquette (F-62 Lestrem, France). The initial moisture content was 13.5% (wb). Zein powder, a mixture of two alcohol-soluble polypeptides with molecular masses of 25,000 and 29,000 Da, was purchased from Fluka BioChemika (Germany). Loss due to drying and ash was less than 4 and 1%, respectively. Corn flour was a gift from M.C. Technologies (F-63 Ennezat, France). Protein content (6.6%, db) was determined by the Kjeldahl method and starch content (83%, db), according to the method described by McCleary, Gibson, and Mugford (1997) and Planchot, Colonna, and Buléon (1997).

2.2. Sample preparation

2.2.1. Extrusion

Samples of starch, corn flour and starch–zein blends were extruded as ribbons using a SCAMIA single-screw machine (Rheoscam Type 20.11d, France) with a flat die ($50 \times 30 \times 1\ mm^3$). Prior to extrusion, composite flours containing starch and zein were mixed in a laboratory kneading machine. Water was added to adjust the moisture content to 26% (wb) for all samples.

The extrusion profile temperature was 100–100–115 (Fig. 1) and 120 °C in the flat die, while the screw speed was set at 25 rpm. Specific mechanical energy was 400 J/g and calculated shear rate in the die was approximately $10\ s^{-1}$, for a feed rate of 300 g/h.

Only starch–zein blends with a zein content lower than 20% (w/w) could be produced by extrusion due to instabilities in the extruder for higher concentrations. Moulding was thus used as a complement to prepare samples with a wider range of zein content.

2.2.2. Thermomoulding

Blending and initial hydration of the flours prior to processing were carried out using the same method as

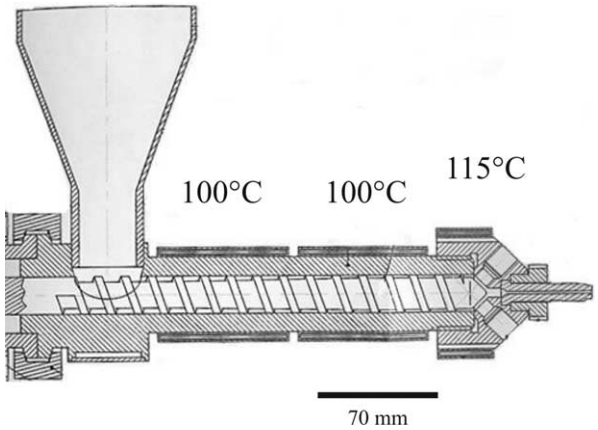

Fig. 1. Profile temperature in the Rheoscam single-screw extruder.

the one used for extrusion. The moisture content of the samples was 26% (wb) and powders were pressed into a plate mould ($100\times100\times1$ mm^3), heated for 10 min at 140 °C, at 20 MPa. Pressure was not released until after cooling in order to avoid the creation of bubbles and growth in the solid.

Thermomoulding makes it possible to produce starch–zein blends with a wide range of zein content (0–100%, w/w).

Extrusion and moulding temperatures were optimised in order to obtain amorphous starch, which was further checked by the absence of residual gelatinisation enthalpy measured by DSC in excess water (Planchot et al., 1997).

After cooling, strips of extruded and thermomoulded samples were stored in dessicators at relative humidity (NaBr, RH = 59% at 20 °C), for 30 days, in order to obtain complete equilibrium. The same conditioning was applied to samples prior to subsequent analysis.

2.3. Water determination

The Karl Fisher method was used to measure the moisture content of the samples (MC, wet basis) after storage at controlled relative humidity. Water was extracted from the samples at 150 °C for 30 min under a dry nitrogen flow. The water content in the gas was further measured.

2.4. Dynamic mechanical thermal analysis (DMTA)

Thermomechanical measurements were performed on a Dynamic Mechanical Thermal Analyser (DMTA MK IV, Rheometric Scientific, USA). Vibration frequency in the dual cantilever bending solicitation mode was 0.2 Hz, the strain was 0.05% and the heating rate 3 °C/min. Strips ($22\times10\times1$ mm^3) were coated with a silicone-based hydrophobic grease to limit dehydration during experiments at high temperatures. It was verified beforehand that the thin coating of grease had no effect on thermomechanical properties by making the same measurements with a coating of thin plastic film. The temperature of the glass-rubbery relaxation was determined at the maximum of tan $\delta = E''/E'$, which corresponds to the molecular mobility associated with the calorimetric glass transition of the material.

2.5. Differential scanning calorimetry

The temperature of glass transition of the moulded and extruded strips was measured by differential scanning calorimetry on a DSC 121 apparatus (Setaram France). Strips were reduced to powder by a cryogrinder and stored at constant relative humidity before being placed in pressure-tight cells (about 70 mg of matter per cell). After a first scan from 5 to 150 °C to delete any thermal events due to ageing phenomena occurring during the storage, samples were rapidly cooled to 5 °C (60 °C/min) in order to maintain starch in the amorphous state. Measurements were performed during a second scan at 3 °C/min from 5 to 150 °C. Graphic determination of glass transition temperature was carried out according to the procedure recommended by Wunderlich (1992), $T_g^{1/2}$, corresponding to a half-variation in calorific capacity during transition. The procedure was similar for all samples, regardless of composition.

2.6. Mechanical testing

Mechanical tests on material strips ($e\times50\times10$ mm^2) were performed with a three-point bending test on an Instron machine, model 1122. The thickness ($e\approx1$ mm), was precisely measured with a vernier calliper for each piece of tested strip. The distance between supports was $L=38$ mm and crosshead speed was 20 mm/min. Tests were performed until samples broke.

Mechanical behaviour of the samples was characterised by calculating rupture stress, fracture strain and elastic modulus from the force vs. crosshead displacement curves obtained.

Stress σ (Pa) was calculated from values of measured force F and the rupture stress corresponded to the maximum of the curve

$$\sigma = F\frac{3L}{2e^2h}$$

where F, force (N); h, width of the sample. Strain ε (%) depended on the sample dimensions and was determined at maximum stress

$$\varepsilon = \frac{6de}{L^2}$$

where d, displacement of crosshead. The flexural modulus E (Pa) was defined by the slope of the linear part of the curve, stress vs. strain, for a slight strain

$$E = \frac{\sigma}{\varepsilon} = \frac{FL^3}{4e^3hd}$$

For each composition, measurements were performed on 10 samples and the mean value was calculated. Error bars correspond to a confidence interval of 95%.

2.7. Confocal scanning light microscopy

CSLM (Zeiss LSM 410, Carl Zeiss, Germany) was used for examining the organisation of the protein in extruded and moulded corn flour and starch–zein blends. For each sample, three sections of different strips were prepared using a cryotome (20 μm section thickness). The sections were placed on flat glass slides and protein coloration was performed by staining for 5 min in a 0.01% (w/v) acid fuchsin solution diluted in 1% acetic acid (v/v). Sections were rinsed three times with distilled water and dried at room temperature. For the observation, water

and a coverslip were added, and the preparation was sealed with nail varnish to prevent it from drying.

Samples were examined in the epifluorescence mode of the microscope, excited by a green laser beam at 543 nm and the emitted light was selected by a long-pass filter (>570 nm). The laser focused on a plane, 1 μm thick, inside the sample. Images were acquired with CSLM parameters of pinhole (9), objective (40) and zoom (2).

Three-dimensional images of the samples were obtained by observing 20 planes of 1 μm, every 1 μm. Each image corresponds to the projection of these 20 planes of a sample area of $159.7 \times 159.7\ \mu m^2$.

3. Results and discussion

3.1. Moisture content and water distribution

At a relative humidity of 59% (NaBr salt at 20 °C), zein and starch materials contained 7.1 and 12.0% water (wb), respectively, whereas starch–zein- and corn flour-based materials contained about 12.0% water (wb). These results are consistent with data from the water vapour sorption isotherms of starch and zein published by Bizot et al. (1997) and Madeka and Kokini (1996), respectively, which show that starch and zein present different moisture contents under the same humidity conditions.

Table 1 presents the moisture-dependence of samples on relative humidity and material state (powder or solid). In the present work, solid and powder samples were analysed when they were stored at 59 and 33% RH, before DMTA and DSC measurements, respectively. Samples of pure starch and pure zein were tested at the moisture content they have in the blend, in order to make it possible to compare material behaviour.

Moreover, moisture content depended on zein content in the blends (Table 2) when they were stored at 59% RH. Due to the different water sorption of starch and zein, moisture content of starch–zein blends decreased when zein content increased. The additivity of moisture contents in blends observed by Labuza (1975) was extended to the starch–zein blends.

3.2. Morphology of starch–zein blends and corn flour materials

3.2.1. Model systems

Blend morphology was observed through protein staining by CSLM. Zein was homogeneously distributed throughout the entire extruded sample (Fig. 2a and b) for both zein concentrations (5 and 15%, w/w). The images suggest the aggregation of zein molecules with a phase separation between starch and zein. Blends are not homogeneous at the molecular level. When zein concentration increased, the size of aggregates also increased, from 2 to 10 μm in the 95/5 blend, to a maximum of 50 μm in the 85/15 blend. Zein was randomly mixed with starch and did not present any orientation, probably due to the low value of die shear rate ($\sim 10\ s^{-1}$). No trace of network or fibres was observed, in contrast with the observations of Lawton (1992). Moulded 95/5 and 85/15 blends presented the same morphology as extrudates, i.e. aggregation (Fig. 2c and d). However, the extrudate aggregates were more dispersed, probably due to the applied shear in the screw during extrusion.

Since blends with larger zein content (>30%, w/w) cannot be extruded, microscopy observations were made on moulded samples for higher concentrations. Zein aggregates were interconnected (Fig. 2e). Zein morphology was more and more continuous when the zein concentration was increased. For the 50/50 blend (Fig. 2f), the zein phase was continuous and delimited amorphous starch domains (about 10–20 μm). Starch granules may have melted without being dispersed since they were embedded in the zein matrix.

These observations clearly show that interfaces between starch and zein will depend on zein content. SZ 95/5 and SZ

Table 1
Sample moisture (%, wb) for two conditions, relative humidity and material state

Relative humidity (%)	Solid materials				Powder			
	Zein	Starch	SZ 85/15	Corn flour	Zein	Starch	SZ 85/15	Corn flour
59	7.1[a]	12[a]	12[a]	12[a]	8	14	14	14
33	–	–	–	–	4.5[b]	10[b]	10[b]	10[b]

[a] Storage conditions for DMTA and mechanical measurements.
[b] Storage conditions for DSC measurements.

Table 2
Experimental and theoretical moisture content of starch–zein solid blends for different zein contents, stored at 59% RH

Zein content (%, w/w)	Solid starch–zein blends						
	0	5	15	30	50	85	100
Moisture content (%, wb) (±0.5%)	12.0	12.0	12.0	10.5	9.5	7.1	7.1
Theoretical moisture content (additivity)	–	11.8	11.3	10.5	9.6	7.8	–

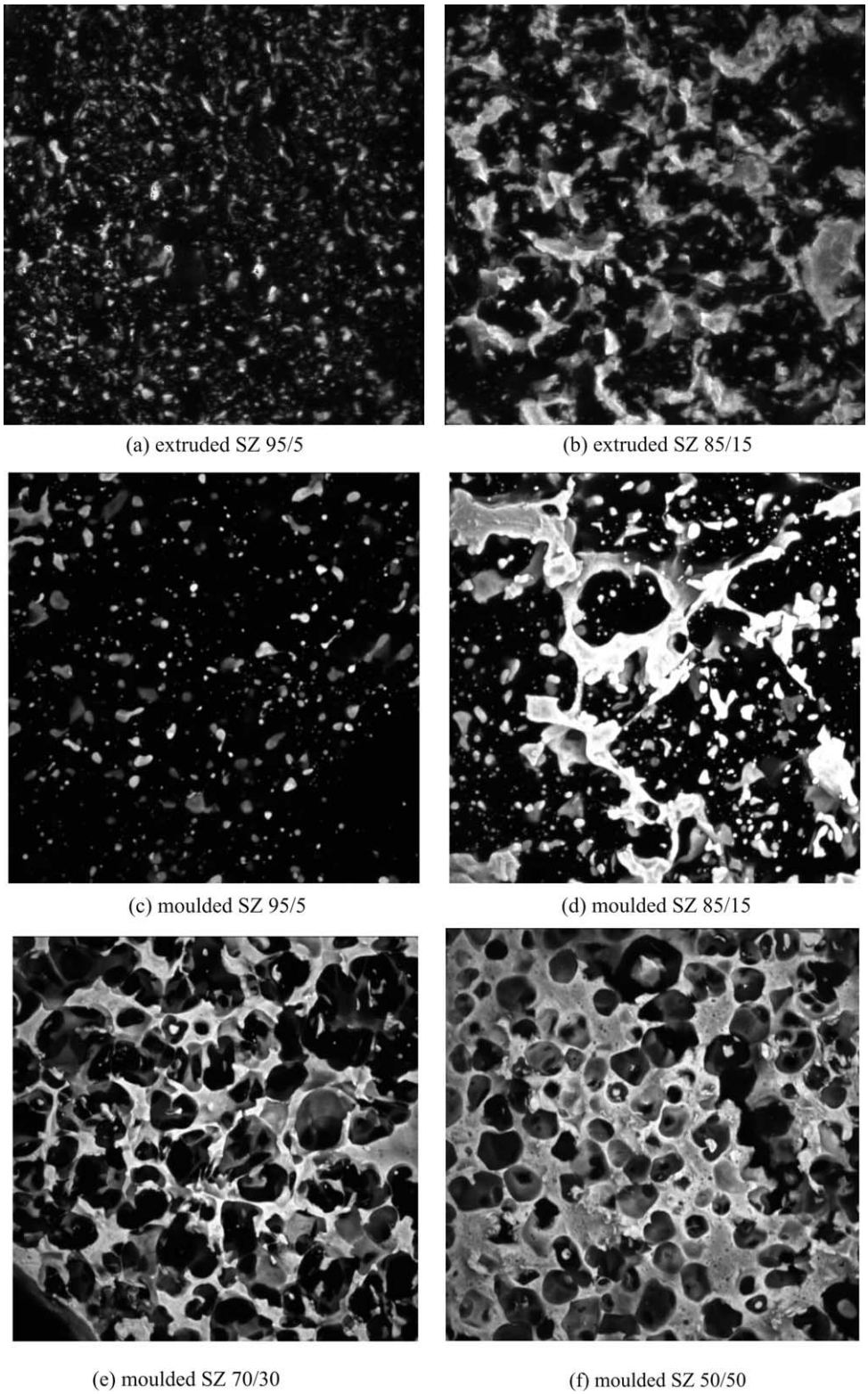

Fig. 2. CSLM observations of zein in extruded and moulded samples ($159.7 \times 159.7\ \mu m^2$).

70/30 do not present the same kind of interfaces in terms of amount of interface and morphology. Interfacial adhesion will thus depend on these factors and certainly influences the mechanical behaviour of the materials. Interfacial adhesion between phase domains is indeed known to play a key role in polymer blends, particularly when they are immiscible, due to the probable presence of voids in the interfacial domain (Wu, 2003).

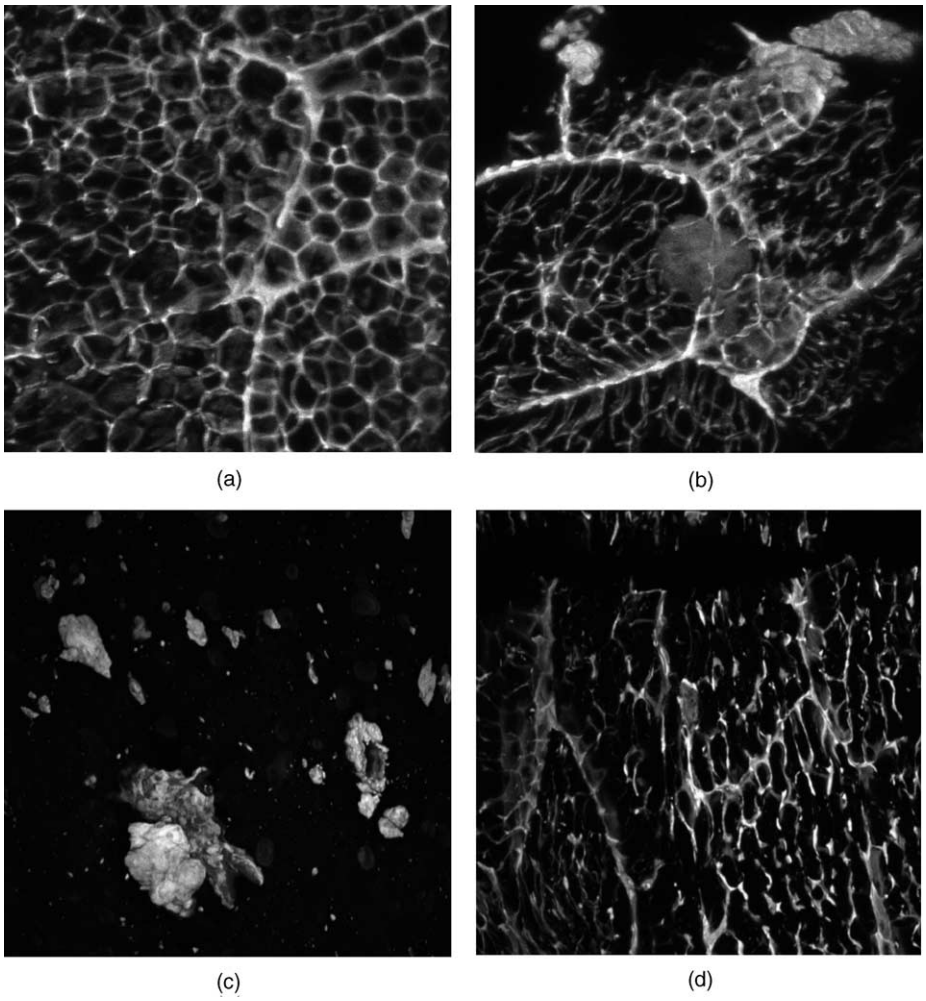

Fig. 3. CSLM observations of proteins in native corn flour (a), in extruded corn flour (b) and (c), and in moulded corn flour (d). ($159.7 \times 159.7\ \mu m^2$ except (c), $319.4 \times 319.4\ \mu m^2$).

3.2.2. Corn flour materials

Microscopic observations of native, extruded and moulded corn flour are presented in Fig. 3. In native corn flour (Fig. 3a), protein was organised as a continuous phase surrounding starch granules. This protein phase may contain protein bodies like those observed in corn flour by Wilson (1987). In our case, protein bodies were not visible because of the resolution. All corn flour particles had this typical structure. After extrusion, proteins in the corn flour materials exhibited two different types of morphology, randomly dispersed throughout the sample: (i) native organisation remnant (Fig. 3b) and (ii) protein aggregates dispersed in a starch matrix (Fig. 3c). Proteins of the aggregates are supposed to come from the protein bodies. The disruption of these proteins bodies, attributed to the influence of mechanical energy (Batterman-Azcona et al., 1999a,b), is supposed to be concomitant to starch melting at high temperatures. As for model systems, interfaces between aggregates and the continuous phase will depend on the way starch and protein are blended during extrusion, considering that other components existing in the corn flour could be involved in the interfacial adhesion. Moulded samples exhibited an intermediate morphology with a deformed protein network, or native remnant (Fig. 3d). Although samples were submitted to lower shear, the apparent orientation of the protein matrix may be due to a flattening perpendicular to the applied stress.

3.3. Molecular mobility and glass transition temperatures of starch–zein materials

A starch–zein blend containing 85% starch and 15% zein (SZ 85/15) was studied in order to compare its behaviour to a corn flour material. Although corn flour presents a concentration of 5.7% protein, a zein content of 15% was chosen for the hand-made blend in order to favour the dispersion of 15% zein rather than 5% zein.

On the basis of the dynamic behaviour of pure moulded zein and pure amorphous corn starch, a $\tan\delta$ peak, corresponding to the main relaxation, was observed at about 100 °C for moulded zein (7% MC, Fig. 4). With 12% MC, amorphous corn starch presented a main relaxation

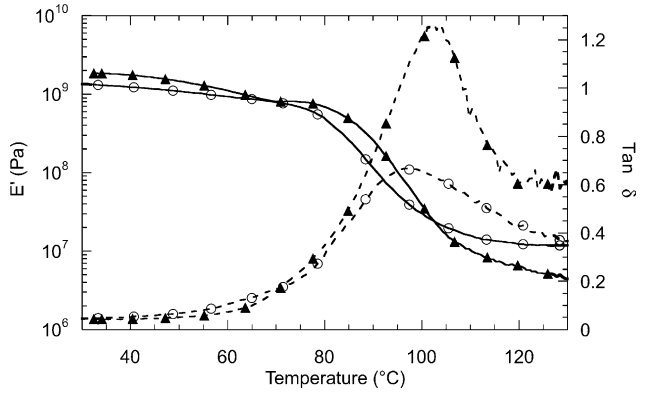

Fig. 4. Dynamic mechanical behaviour of extruded corn starch (○) and moulded zein (▲) (12 and 7% water content, wb, respectively): storage flexural modulus (E') and tan δ vs. temperature (dotted line) at 0.2 Hz, 0.05% strain.

located at 98 °C. Both pure samples were in the glassy state at room temperature and their flexural storage modulus E' values at 30 °C were 1.8 and 1.3 GPa for zein and starch, respectively.

Dynamic mechanical measurements of extruded SZ 85/15 and corn flour were performed under the same conditions as for the pure components (Fig. 5). Mechanical responses were similar for both materials which were in the glassy state at room temperature. Their flexural storage modulus E' value at 30 °C, which was about 2.2 GPa, was on the same order of magnitude as the value for pure zein and pure starch. The tan δ peak temperature for both samples was located at 98 °C, the same value as for starch, but with a slightly lower peak amplitude, 0.5 instead of 0.6. The major difference concerned the evolution of E' vs. temperature (Fig. 5): the drop in E', about one decade, was less pronounced for SZ 85/15 than for pure starch, which was close to two decades. Starch extrudate modulus E' (1.3 GPa) was lower than for the starch–zein blend (2.2 GPa) at 30 °C. Even though starch and zein are immiscible molecules, only one tan δ peak was detected in extruded samples, probably because peaks due to the main relaxation of starch and zein were superimposed. The same trends were observed for moulded samples.

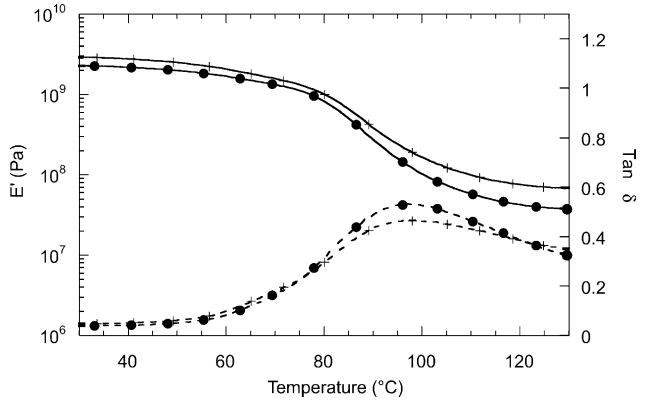

Fig. 5. Dynamic mechanical behaviour of extruded SZ 85-15 (●) and corn flour (+) (12% water content, wb): storage tensile flexural modulus (E') and tan δ (dotted line) vs. temperature at 0.2 Hz, 0.05% strain.

The variations of heat capacity of extruded corn starch, corn flour and SZ 85/15, for which the starch was verified to be totally amorphous, presented two events at a moisture content of 10% (wb): one at 55 °C showing a peak and a variation of the C_P values between 80 and another one at 110 °C (Fig. 6). The peak at 55 °C, which disappeared during the second scan performed after heating to just above T_g, was due to the physical ageing of the samples, depending on $\Delta T(=T_g-T_{ageing})$ and on the time of ageing of the materials (Shogren, 1992). The influence of the moisture content measurements on the accuracy of T_g determination was estimated using the Couchman–Karasz model (Couchman & Karasz, 1978). A variation of 0.5% in the moisture content induced a variation of 5 °C in T_g values. For zein, the variation was about 4 °C. Thermograms were superimposed and glass transitions for corn flour and starch–zein samples occurred in the same range of temperatures, i.e. 100 °C at a moisture content of 10% (wb). Just like for the DMTA results, state changes occurred over the same range of temperature values, with amorphous corn starch presenting a glass transition temperature at about 107 °C with 10% moisture content (wb). This moisture content was reached after equilibrium with a relative humidity of 33% (Table 1). For this RH, zein had a moisture content of 4.5% (Table 1), for which the T_g was 85 °C (thermogram not shown), in agreement with the one measured by Lawton (1992). This transition was located in the range of the C_P variation of pure starch and was not detectable on the thermogram of SZ 85/15. Despite the difference of protein content between corn flour and SZ 85/15, the fact that the main relaxation and the glass transition were found at the same temperature value was a first step towards validating this choice of zein concentration.

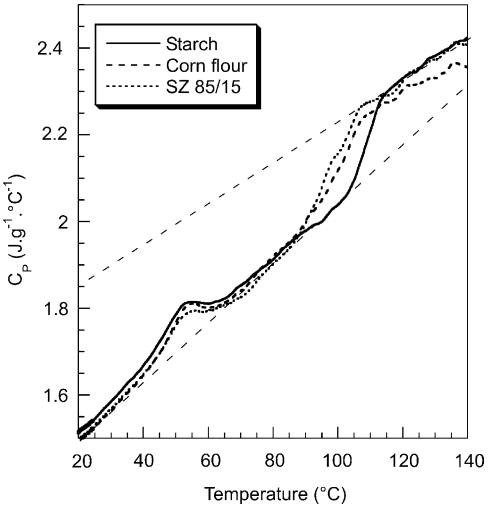

Fig. 6. Thermograms obtained on extruded corn starch, corn flour and SZ 85/15 (by DSC, 3 °C/mn) (10% MC, wb).

3.4. Influence of zein content on the mechanical properties of glassy starch–zein materials

Tensile tests are difficult to perform on this kind of sample, so the three-point bending test was used, although it results in various deformations of the sample: compression, traction and shear. A finite element model (ANSYS® simulation software) was used to create a volume corresponding to the dimensions of the samples. The volume, considered as isotropic, was divided into 10 pieces in length, width and thickness (volumetric element: Solid 45). The test was done within the configuration of the experiment and made it possible to check the linearity of the three-point bending test and to verify that theoretical values of force at specific deformations are similar to experimental values. This simulation confirms that results from the three-point bending test are as valid as those from the tensile test .

Typical shear–stress curves of moulded glassy zein, amorphous starch, SZ 85/15 and corn flour are presented in Fig. 7. Under the studied conditions (ambient temperature), starch sample did not show any failure, its behaviour was ductile and it presented an elastic limit (Fig. 7), although it was in the glassy state as suggested by the DMTA results (Fig. 4). This result is in agreement with Nicholls et al. (1995), who demonstrated a brittle–ductile transition of glassy starch. Under the studied conditions, corn starch with 12% moisture content was glassy and ductile. Glassy zein, SZ 85/15 and corn flour were fragile. They all broke under these conditions, although they did not all display the same stress/strain curves. An elastic limit was observed for glassy zein and rupture occurred in the plastic zone, whereas rupture of starch–zein blend and corn flour samples occurred in the elastic domain (Fig. 7). Contrary to the starch sample, these samples presented a fragile rupture. These differences in the mechanical behaviour cannot be explained by a variation of the glass transition, since transition temperatures are very close for all samples.

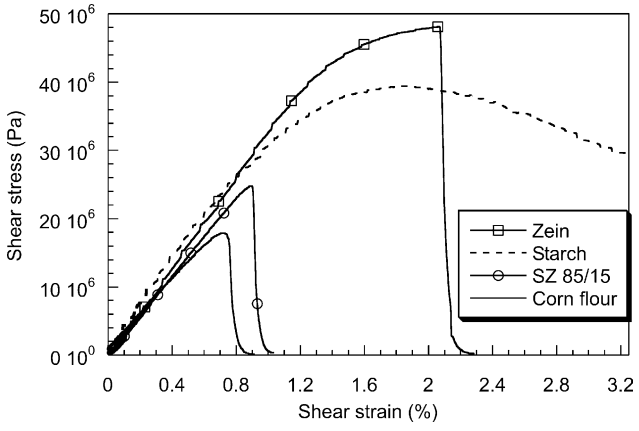

Fig. 7. Typical shear–stress–strain curve (obtained by three-point bending) of moulded glassy zein (□) (8% MC, wb), amorphous starch (dotted line), SZ 85/15 (○) and corn flour (continuous line) (12% MC, wb).

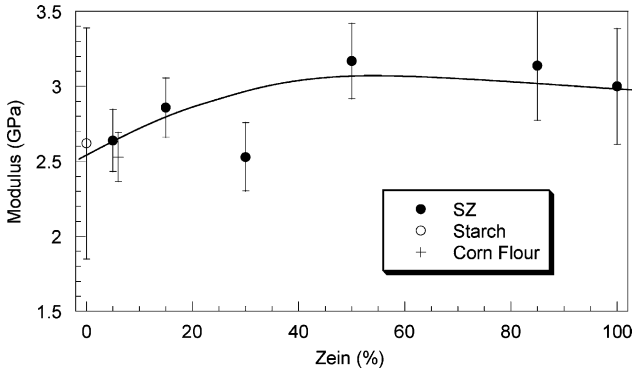

Fig. 8. Variation of flexural modulus vs. zein content (v/v) of starch–zein blends (●), starch (○) and corn flour (+).

Indeed, mechanical testing was carried out at ambient temperature and values of $T_g - T_a$ were similar.

Since the fragile behaviour of starch–zein blend cannot be explained by a glass transition temperature that is different from that of the pure components, the mechanical properties of starch–zein materials with various zein concentrations were studied more closely. The values of the modulus as a function of zein content were dispersed in a range of 2–3 GPa without any clear evolution and no significant difference (Fig. 8). The variations of the strain at failure (%) as a function of zein are shown in Fig. 9. Since the starch sample did not break under the studied conditions, the value presented for this material corresponds to the strain at maximum stress. This result clearly confirms that starch–zein blends are more fragile than starch samples whose strain values were the highest: the sample did not break at 8% strain, whereas the other samples presented an average strain value at failure that was lower than 2%. Corn flour materials showed the lowest strain value at failure, 0.7%, which was close to the values obtained for 15, 30 and 50% zein, and experimental uncertainty was remarkably low. Although the sample with 5% zein had a higher strain value, it exhibited fragile behaviour, similar to ones for

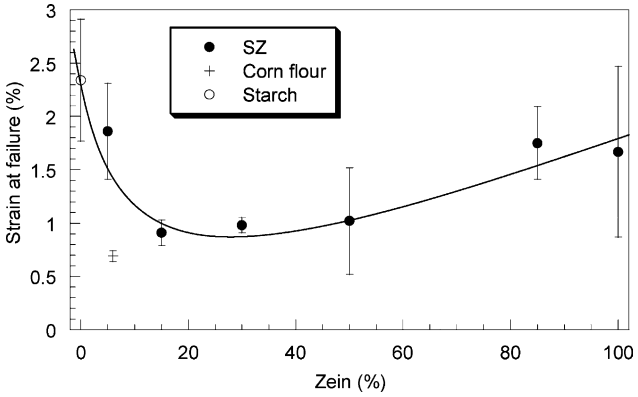

Fig. 9. Strain at failure vs. zein content (v/v). The value obtained for starch corresponds to the strain at maximum stress since the sample did not break. Dynamic mechanical behaviour of extruded SZ 85-15 (●) and corn flour (+) (12% water content, wb): storage tensile flexural modulus (E') and tan δ (dotted line) vs. temperature at 0.2 Hz, 0.05% strain.

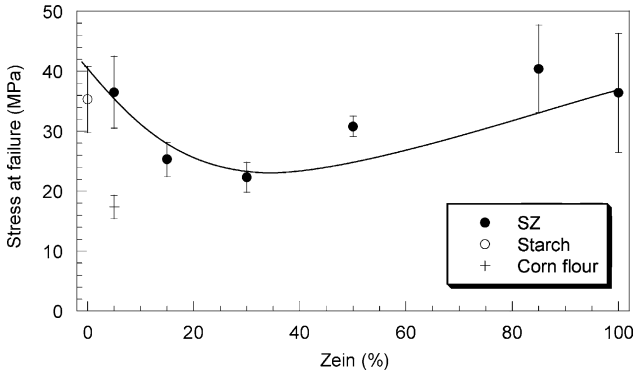

Fig. 10. Stress at failure vs. zein content (v/v). The value for starch corresponds to the maximum stress since the sample did not break. Dynamic mechanical behaviour of extruded SZ 85-15 (●) and corn flour (+) (12% water content, wb): storage tensile flexural modulus (E') and tan δ (dotted line) vs. temperature at 0.2 Hz, 0.05% strain.

larger zein contents (85–100%). These observations were reinforced by the trends obtained for extrudates, for which strain varied from 2.4% for SZ 95/5 to 1.5% for SZ 85/15. These results confirm that starch–zein blends are fragile and show that the ability of the blends to deform under an applied stress depends on the zein content. Fig. 10 indicates that the stress at failure depends on zein content as well. Although starch and SZ 95/5 could withstand similar stress (36 MPa), the blend broke and this value corresponds to the stress at failure. SZ 85/15 and 70/30 presented the same stress at failure at about 25 MPa, which was close to the value obtained for the corn flour sample which presented the lowest value of stress at failure (18 MPa), just like for the strain at failure. Materials made from pure components were more resistant to failure than the blends. The same trends were obtained for the extruded samples with stress values varying from 25 to 19 MPa for SZ 85/15 and corn flour, respectively, due to similar morphology, although extrudate aggregates were more dispersed, probably due to the applied shear in the screw during extrusion. But, this difference neither affected the state changes nor the mechanical properties.

The behaviour of blends with one component at low concentration is similar to the behaviour of the pure major component. Nevertheless, just a few percent of zein influenced the behaviour of the starch matrix, since 5% zein resulted in the fragile behaviour of the blend. Although corn flour contains 5.7% protein, it behaves very similarly to starch–zein blends with 15–30% zein, which justifies the choice of these blends as model systems. Therefore, protein content is not the only factor that influences mechanical behaviour, which suggests that it could be due to the way the components are mixed together.

4. General discussion

In this work, the preparation of model systems made it possible to analyse the behaviour of blends of two biopolymers, starch and zein, by scanning a large interval of zein content. This was a good method for making observations on the morphology and behaviour of corn flour products, although only the two major components were considered in a first step.

Confocal scanning light microscopy was used and proved to be the most appropriate microscopic technique for the observation of product morphology: three-dimensional images make it possible to examine the internal structure. Qualitative information from images, reflecting the morphology in the whole sample, were obtained, and further quantitative analysis may be possible by image analysis. Such observations can be extended to other blends. Limits were reached for the accurate observation of the interface, since starch staining would have been too destructive for the samples.

4.1. Aggregation

Fig. 5 underscores the similar behaviour between the corn flour and the SZ 85/15 materials, which can be explained by observations of the morphology of the proteins. Both samples present protein aggregates, dispersed throughout the materials (Figs. 2b and 3c), associated with a phase separation between starch and protein. It is well known that starch is hydrophilic whereas zein is a hydrophobic protein, which clearly explains why they are immiscible. An aggregation of zein in starch–zein blend products was already observed by Batterman-Azcona et al. (1999a,b), who used MET to observe labelled proteins. The observation of corn flour samples by CSLM not only confirms the aggregation but also provides information about the distribution and size of the aggregates.

4.2. Fragility of the blends

The study of the mechanical properties of the samples by a three-point bending test showed a difference of behaviour, between the pure components and the starch–zein blends. The presence of zein in a matrix of amorphous starch results in fragile behaviour, whereas pure starch is ductile when studied under the same conditions (Fig. 7). The results from DMTA and T_g measurements clearly showed no significant differences in the main relaxation and the values of T_g between the blends and the pure components. Thus, molecular mobility cannot explain why blends are weaker than the materials containing the pure components. This fragility is assumed to be related to the morphology of the blend and could be attributed to the phase separation between starch and zein, and to the aggregation of zein. Zein could be compared with particles, which fill a continuous polymer matrix. Guerrica-Echevarria, Eguiazabal, and Nazabal (1999) showed that a filler incompatible with a polymer continuous phase induces a decrease in large-strain mechanical properties. Furthermore, immiscible binary polymer blends are known to have different mechanical

properties, depending on the phase morphology. They generally show a variation in the Young modulus of the composite and an increased fragility in the inversion phase, i.e. when polymers are co-continuous (Leclair & Favis, 1996). Fragile behaviour of starch–zein blends can thus be characterised as a negative deviating behaviour, i.e. decreased mechanical behaviour of the blend compared to the pure components. The variations of flexural modulus values of the blends are weakly influenced by zein content but materials are not reinforced by zein (Fig. 8), compared, for instance, to starch–cellulose composites, which are strengthened by the increase of the filler content. The main difference comes from the compatibility of starch and cellulose (Averous, Fringant, & Moro, 2001). Moreover, blends with a zein content from 50 up to 100% have a continuous zein phase (Fig. 2e and f) and exhibit behaviour similar to glassy zein materials, in terms of values of the strain and the stress at failure (Figs. 9 and 10). Since zein has a fragile behaviour, materials whose continuous phase is zein are fragile as well. The samples which present an amorphous continuous starch matrix are much more influenced by a small amount of zein, suggesting that the interface between starch and zein should be taken into account to explain the weakening of starch by zein. The impact of zein on the mechanical properties of the blends is strongly related to the structure and a threshold close to 20–30% zein content seems to exist: above 50%, zein behaviour predominates because of the continuity of the protein, whereas the mechanical behaviour is much more complex underneath. When zein is the dispersed phase, the properties depend on the amount of aggregates and their morphology, and on the surface of contact with starch until the two phases become co-continuous at about 30% (w/w) zein, as can be observed from the various micrographs obtained by CSLM. Starch–zein blends prepared by extrusion or thermomoulding display the same trends in the mechanical behaviour, i.e. decreased mechanical properties due to similar phase morphology for the same zein content. In the starch–zein blends, like in incompatible binary polymer blends, immiscibility and phase morphology also have a strong influence on the mechanical properties.

4.3. Corn flour behaviour

Referring to the micrograph obtained by CSLM from extruded corn flour, two kinds of protein structure can be observed: one presents aggregates (Fig. 3c) and the other presents a damaged structure (Fig. 3b). We might further argue that, by extrusion, corn flour undergoes various transformations: first of all, a mechanical effect tending to disrupt the native protein matrix by destroying it and, afterwards, an aggregation of the constitutive proteins of the initial protein matrix, certainly due to the effect of the temperature.

The protein morphology of corn flour and particularly the size, is close to that of SZ 85/15 (between 25 and 40 μm). This analogy can explain the fragile mechanical behaviour of corn flour materials. Dispersed aggregates of zein weaken the mechanical properties of SZ 85/15 materials. Native organisation remnants and aggregates of corn flour protein dispersed in the continuous starch phase also lead to the fragile behaviour of corn flour. In both cases, interfaces between protein particles and the starch matrix may play a key role. Indeed, in corn flour materials, other minor components have to be taken into account as well. In other words, other molecules of corn flour not added in the model blends could influence the interfaces, which suggests that, for further studies, corn flour could be supplemented by proteins and the starch–zein system could include other components.

5. Conclusions

This work has shown that the mechanical properties of glassy starch–zein-based materials are weakened by zein, as compared to the behaviour of starch. It was also demonstrated that this phenomenon was not due to variations in the state change temperatures. Starch–zein systems with different zein content could be used as a model system to better understand the behaviour of corn flour-based products. The study of a wide range of zein contents in the blends made it possible for us to demonstrate how the mechanical behaviour of extruded corn flour samples is related to their morphology after thermomechanical treatment. The weakened behaviour of the starch–zein blends is a consequence of a phase separation between the two components and, concomitantly, a zein aggregation. The failure behaviour of the blends depends on the interface between starch and zein, which should be further studied. However, considering these solid materials as cell walls, this work already provides a basis for the study of the properties of expanded corn products.

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