Effect of Molecular Structure on Film Blowing Ability of Thermoplastic Zein

M. Oliviero,[1] E. Di Maio,[2] S. Iannace[1]

[1]Institute of Composite and Biomedical Materials, National Research Council of Italy, Naples 80125, Italy
[2]Department of Materials and Production Engineering, Faculty of Engineering, University of Naples Federico II, Naples 80125, Italy

ABSTRACT: The aim of this work was to explore the feasibility of preparing thermoplastic films from commercial zein by film blowing technology. Zein, a cereal protein extracted from maize, was plasticized directly in the extruder, without the time-consuming and expensive solubilization step, usually utilized in literature for this material. Four different batches of zein were investigated, for it has been observed that properties such as the film-forming ability of natural polymers strongly depend on several factors such as sources, extraction, and drying conditions. Thermal, rheological, and macromolecular structural characteristics were evaluated for the different materials to establish a correlation among molecular structure, rheological behavior in uniaxial elongation, and film blowing processability. Results indicate that the best films were made by using thermoplasticized zein characterized with a pronounced strain hardening and a large content of α-helices. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 277–287, 2010

Key words: protein; zein; secondary structure; elongational viscosity; film blowing

INTRODUCTION

During the last 20 years, an increasing interest in the use of natural polymers, such as proteins, lipids, and polysaccharides, for the production of new environmental friendly packaging materials has been noticed. In particular, several protein sources have been proposed for the preparation of new thermoplastics,[1] but, in general, mechanical and water vapor barriers properties of films produced from these materials are inferior to those of synthetic origin.[2,3] However, protein-based films can act as barriers to oxygen, carbon dioxide, oil, and fats, to prevent quality deterioration and increase the shelf life of food products. One of the film-forming proteins considered in literature as well as in the industry is zein, the prolamine of corn, mainly for its uniqueness in terms of thermoplasticity and hydrophobicity. The relatively hydrophobic nature of zein is related to its high content of nonpolar amino acids[4,5]: because of the hydrophobic nature, zein is a substantially better moisture barrier than other proteins such as casein, or polysaccharides such as starch[6] and is used in the food and pharmaceutical industry as coating material for candies, rice, dried fruits and nuts, pharmaceutical tables, and to encapsulate ingredients.[7–9]

In the scientific literature, two different methods are generally used to prepare zein films. The first method involves casting of aqueous alcoholic solutions of zein and a suitable plasticizer on inert, flat surfaces from where films are peeled off after the solvent is evaporated. Typical technical difficulties of this process are the control of thickness and homogeneity of the formed films.[10] In the second method, zein films are formed by stretching over rigid frames a soft solid mass, which is generally referred to as resin, obtained by precipitation of alcoholic solutions of zein and fatty acids in a stream of cold water. In general, resin films showed better mechanical properties than cast films.[11] However, both these processes are expensive and difficult to scale up, and more efficient technique, such as extrusion or film blowing, has to be investigated for massive production of zein films.

The application of extrusion technology for the production of zein films has been a challenge to researchers and few reports have been published. Wang and Padua[12] produced zein sheets plasticized with fatty acids by extruding a moldable, dough-like resin prepared by precipitating zein and oleic acid from aqueous-alcohol solutions. The same formulation was used by Wang and Padua[12] to prepare films by film blowing technology. In 2007, Selling

and Sella[13] prepared, instead, ribbons of zein plasticized with triethylene glycol (TEG) using the following procedure. Zein was first treated with water and TEG in a Hobart mixer to prepare a initial blend to fed to the extruder. After the extrusion, the thermoplastic material obtained was allowed to cool and then converted into pellets to again feed the extrusion for producing the final ribbons. These techniques, despite the relative simplicity, are time-consuming and quite expensive for massive production of films.

The main objective of this study was to explore the feasibility of preparing thermoplastic films of zein by film blowing without the time-consuming and expensive solubilization step. The zein powder was plasticized directly in the extruder, without the use of solvent and of a premixing phase. This process has the advantages of reduced environmental, health and safety problems, no need to handle the evaporation of ethanol, and reduced raw materials costs. However, to our point of view, the most important problem in the use of biopolymers is the difficulty in having "standard" raw materials. In fact, it is known that the natural polymers molecular structure strongly depends on several factors such as sources and extraction technique and drying conditions.[14] In this context, four different batches of zein from maize were investigated to determine the possibility of these materials to be processed by a film blowing line. Thermal and structural characterizations were performed on the different batches to evaluate the thermoplasticization degree and the protein conformation. Furthermore, it has been observed that the increase of the melt deformability and elongational properties is crucial in the film blowing processability of thermoplastic polymers.[15] For these reasons, extensional rheological behavior of zein films was analyzed and a correlation among molecular structure, rheological behavior in uniaxial elongation, and film blowing properties was determined.

EXPERIMENTAL PART

Materials

Four different batches (A, B, C, and D) of maize zein were purchased from Sigma-Aldrich (Italy) (product No. Z3625). Lot numbers of the different batches were 20K0243 (A), 02K0054 (B), 065K0110 (C), and 093K1055 (D). Poly(ethylene glycol) 400 (PEG400) was used as plasticizer and was purchased from Fluka (Italy). "As received" materials (powder) were subjected to thermal analysis, X-ray diffraction, Fourier transform infrared spectroscopy (FTIR), and characterization by SDS-PAGE. Thermoplasticized materials (thermoplastic zein by internal mixer, TPZm) by using the procedure described in section

"thermoplasticization by internal mixer" were also subjected to rheological analysis. Finally, films produced by film blowing as described in section "film blowing of thermoplasticized zein materials" (thermoplastic zein by extrusion and film blowing equipment, TPZe) were subjected to mechanical tests. All of the materials, unless otherwise specified, were conditioned before testing at room temperature and 50% relative humidity (RH) for 2 days.

Thermoplasticization by internal mixer

Zein powder, as received by the supplier (moisture content, as determined by thermogravimetric analysis, equal to 7 wt % ± 0.3 for all batches), was mixed with the PEG 400 in a beaker using a spatula to provide a crude blend. The blend was then subjected to temperature and shear stresses in a twin counter-rotating internal mixer (Rheomix 600 Haake, Germany) connected to a control unit (Rheocord 9000 Haake, Germany) for thermoplasticization. The mixing chamber (volume of 50 cm^3) was filled with 50 g total mass for all experiments in ∼ 3 min at 5 rpm. Plasticizer content of 25 wt % was used. Mixing temperature, speed of rotation, and mixing time were 70°C, 50 rpm, and 10 min, respectively.[16] A P300P hot press (Collin, Germany) was then used to prepare slabs with thickness of 1 mm. Materials were heated to and kept at 70°C and pressed at 50 bar for 10 min and subsequently cooled to 30°C under pressure. From the central region of the slabs, specimens for different characterizations were cut.

Thermal analysis

Thermogravimetric (TGA) and differential scanning calorimetric (DSC) measurements were conducted on both the "as received" powders and TPZm from all of the batches, to evaluate the effect of the thermoplasticization on thermal properties for the different lots. In particular, TGA experiments were carried out in a TGA 2959 (TA Instruments, USA) over a temperature range from 30 to 1000°C at 10°C/min under inert atmosphere. The DSC analyses were conducted on a Q 1000, TA Instruments, USA. Samples were first heated from −70 to 200°C, and then were subjected to an isothermal stage for 10 min, quickly cooled to 30°C, and finally reheated to 200°C. The rate in both heating processes was 10°C/min.

Dynamic mechanical measurements

Dynamic mechanical analysis (DMA) was used to determine the glass transition temperature of the different batches of TPZm. Dynamic mechanical analysis in fact is complementary to DSC and is

particulary suited for studying the molecular motions that give rise to glass transition temperature, being, in general, more sensitive than DSC. The dynamic mechanical properties of the different slabs were evaluated by a Tritec 2000 (Triton Technology, Keyworth, Notts, UK). In the dual cantilever bending mode, an oscillation frequency of 1 Hz (displacement of 0.03 mm) was applied to the center of the sample (5 mm free length) and the temperature was scanned from -20 to $100°C$ at a heating rate of $2°C$/min. The glass transition temperature (T_g) was determined from the maximum of the tan δ.

X-ray diffraction

The effects of the mixing process on the structure of the zein from the different batches were investigated by X-ray diffraction. The X-ray patterns of the different zein batches (both "as received" powders and TPZm) were obtained by using a PW1710 diffractometer (Philips, Netherlands) with Cu-Kα radiation ($\lambda = 1.54$ Å) at a voltage of 40 kV and 30 mA. Samples were scanned in the range of diffraction angle $2\theta = 5–60$ with a scan speed of $1°$/min at room temperature.

Fourier transform infrared spectroscopy

FTIR was used to investigate the secondary structure of different zein batches "as received" and thermoplasticized samples TPZm. FTIR measurements were performed at room temperature by using a Nexus Spectrophotometer (Thermo Electron Corp., USA) with a wavenumber resolution of 2 cm^{-1} for 128 scans from 4000 to 500 cm^{-1}. The FTIR spectra were collected in absorbance mode on transparent pellets obtained by dispersing the sample powders 1 wt/wt % in KBr. Samples were prepared and analyzed in triplicate. To estimate the content of the various secondary structures, the resultant spectra were normalized and then deconvoluted in the range of frequency 1700–1600 cm^{-1} using the OriginPro 8.0 software. The normalization was performed by dividing all of the values by intensity of the invariant peak at 3290 cm^{-1}. The deconvolution was made by using the best fits by Lorentzian sum and specifying the position of the peaks corresponding to the different secondary structural elements (α-helix and β-sheet).

Characterization by SDS-PAGE

The different batches of zein powder were subjected to sodium dodecyl sulfate-polyacrylammide gel electrophoresis (SDS-PAGE) according to the procedure of Gao et al.,[17] to evaluate the molecular sizes of polypeptides present in the different zein batches. In particular, two different amounts of powders for each batch were loaded to investigate the mass effect on the obtained results.

Rheological measurements

Measurements of extensional viscosity

Generally, the processing performance of polymer melts is related to shear flow properties. However, the number of instances when two polymers have the same shear properties but perform differently during certain type of processing operations (e.g., film blowing and sheet extrusion) indicate that shear properties alone may not be sufficient to characterized polymeric fluids.[15] In these cases, the different processability of the polymers may be attributed to differences in molecular architecture due to factors such as branching and/or the presence of a high-molecular-weight tails[18–20] that are not readily detected in shear but are more visible in extensional deformations. In fact, one of the key properties in extrusion blown film is extensional viscosity.[21,22] Indeed, it is well recognized that bubble stability, biaxial stretching, and thickness uniformity are very sensitive to extensional viscosity.

Measurements of elongational viscosity were performed by using an ARES LS (Rheometric Scientific, USA) rheometer equipped with a SER-HV-A01 tool (Xpansion Instrument, USA) to establish a correlation between rheological behavior in uniaxial elongation and film blowing ability for different zein batches. The temperature was kept at $95°C$, whereas the elongational rate $\dot{\varepsilon}_0$ was changed from 0.03 to 0.1 s^{-1}.

Measurements of haul-off force

Another important rheological property in the blown film process is the haul-off force.[22] It is defined as the force necessary during processing to sustain the polymer weight when the bubble is formed.

Haul-off force of the different TPZm was measured using a capillary rheometer (RH7, Bohlim Instruments, Germany) equipped with a melt strength apparatus. The thermoplastic materials were preconditioned at RH = 15% and room temperature, for 60 h. The temperature of the rheometer was set at $102°C$ for the batch A and at $115°C$ for the other batches. The temperature test for the different TPZms was chosen to simulate the conditions used in the film blowing process. The barrel had a diameter of 15 mm, whereas the capillary die diameter was 2 mm. The polymer melt was extruded from the die at a piston speed of 20 mm/min. The polymer melt strand extruded from the capillary die was gripped between two counter-rotating wheels and
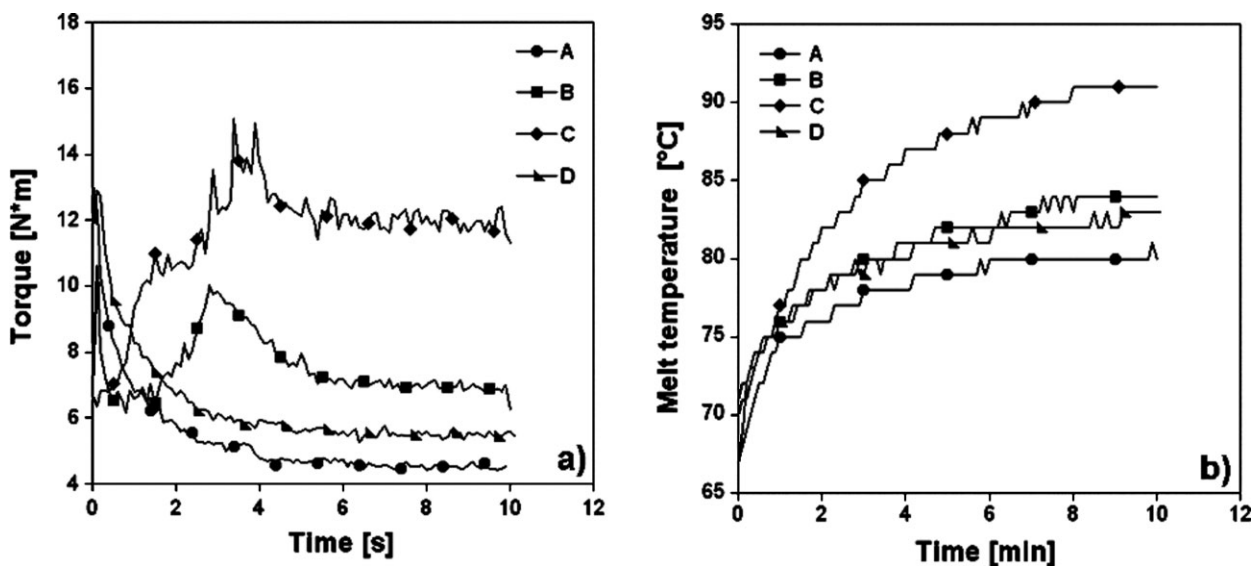
Figure 1 Torque (a) and melt temperature (b) evolution during the mixing process of different zein batches with PEG400 (25 wt % plasticizer content, 50 rpm, 70°C).

the take-up speed increased from 5 to 100 m/min at a constant acceleration. The haul force was measured as a function of the draw ratio until the strand breaks.

Film blowing of thermoplasticized zein materials

The technology consists in extruding a tube of small diameter that, before solidification, is blown with pressurized air. The pressure inside the bubble determines the increase of the bubble diameter and a corresponding strong decrease of the thickness. The bubble is closed at the top by the action of two rolls and, in this way, the gas is kept inside the bubble. During this process, the film is cooled and it solidifies before reaching the two rolls. The material is subject to elongational flow in both longitudinal and radial directions (biaxial elongational flow). The operation is controlled by the supply of cooling air, the amount of air inside the bubble, the velocity of the nip rolls, melt temperature, die diameter, die gap, and also by polymer properties. The material, in fact, must posses suitable rheological properties and, in particular, a suitable melt strength. Zein powder and PEG 400 (at constant composition of 25 wt % of plasticizer) were first mixed in a beaker using a spatula to provide a crude blend and then fed into a counter-rotating, laboratory twin-screw extruder (Haake model CTW100, Germany). Temperatures in the extruder were as follows: 20°C (water cooled) in the feeding section, to prevent the staking on the screws, 60°C in the mixing/plasticizing section, whereas the temperature of the two final zones of the extruder was 80°C. The screw speed was 80 rpm. The product coming out from extruder

die was cylindrically shaped and was cut into short pieces, that were conditioned at room temperature and 15% RH for 60 h to obtain an extrudate suitable for film blowing. Film blowing was then performed on a lab-scale equipment (Collin E 20T, Germany). The temperature of the four heating sections of the extruder barrel and die (optimized temperature profiles for the different batches are given in the Results and Discussion section), the velocity of the take-off rolls, and the volume of the bubble were adjusted to achieve a stable bubble with the lowest final thickness that was possible to achieve with the different batches. The screw speed was set at 43 rpm for all materials.

Film characterization—Tensile testing

The static tensile properties (Young modulus, stress and strain at break) were measured at room temperature with a 1 kN load cell on an Instron model 4204 tensile test machine according to ASTM D 1708-02.

RESULTS AND DISCUSSION

Thermoplasticization

Figure 1(a,b) shows the evolution of torque and melt temperature versus time during the mixing process of zein with PEG 400 at 25 wt % for the batches A, B, C, and D.

Typically, the curves obtained during thermoplasticization of protein-plasticizer systems[23] [Fig. 1(a)]) are characterized by an initial induction time followed by a steep increase of the torque to a maximum and then a continuous decrease to a rather stationary value, when also the melt temperature
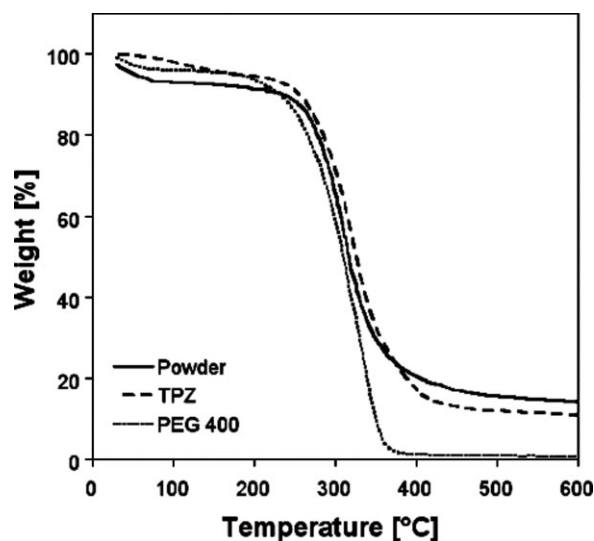
Figure 2 TGA curves for PEG 400 and for powder and TPZm from batch A.

Figure 3 Curves of tan δ versus temperature of TPZm from different batches.

becomes constant [Fig. 1(b)]). However, after the plasticization is complete, the torque can decrease indicating degradation, or it can increase, indicating crosslinking or loss of plasticizer.[24,25] As shown in Figure 1(a), this behavior was more evident for batches B and C, whereas for batches A and D, the torque increase was immediate, with the absence of the induction time. After plasticization, the value of the torque was rather constant and no evidence of plasticizer loss, crosslinking, or degradation was observed for all batches. These results suggest that the diffusion of the plasticizer inside the protein macromolecules and the corresponding formation of entangled molecules of zein-PEG400 was easier for batch A and D, having, as will be seen in the following, a higher content of α-helices and, therefore, a less aggregated secondary structure, with an improvement and a shortening of the whole thermoplasticization process for these materials.

Thermal properties

Thermogravimetric analysis

Figure 2 reports TGA results for PEG 400 and for pure and TPZm from batch A. TGA curves for the other batches were similar and are not reported for simplicity.

TGA curves of the powder form showed a small weight loss in the range 30–170°C, associated to evaporation of water, whereas a significant weight decrease started at 270°C and can be associated to the degradation of the protein. Results of the TGA for TPZm showed a water loss similar to the powder form but shifted at higher temperatures (range 100–170°C) because of the interaction between water and TPZm. However, in this case, the presence of the
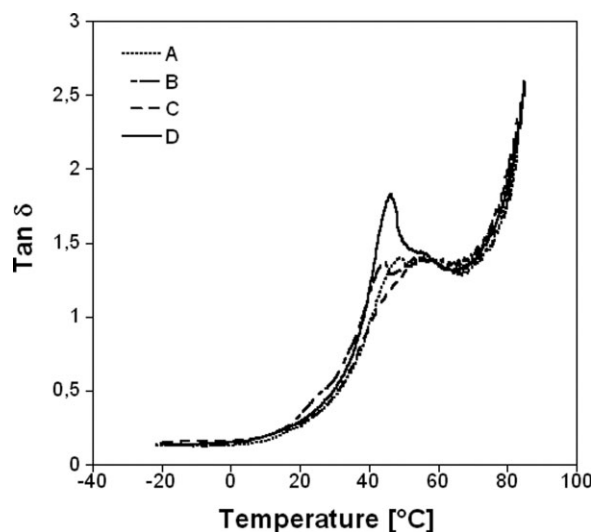
plasticizer and, possibly, the mixing process was responsible for the earlier weight loss starting at 240°C. These results indicated that during the extrusion process of these materials, attention should be paid to avoid loss of water in the melt and degradation phenomena above 240°C.

Glass transition temperatures

The glass transition temperature of powder zein from batch A, B, C, and D was calculated by DSC as midpoint temperature associated to the baseline change in the heat flow signal. The glass transition temperature of TPZm was measured by DSC and also by DMA, from the tan δ peak. In particular, two tan δ peaks were observed for all batches excepted for the C (see Fig. 3). The double peaks could be associated to the glass transition of two zein subfractions with different molecular weights[26] or to a glass transition of PEG400-rich fraction and zein-rich fraction.[27] DMA and calorimetric measurements of T_g of the zein from batch A, B, C, and D are reported in Table I. From both DMA and DSC data, a significant depression of T_g (more than 100°C) was measured for all samples in presence of the plasticizer, suggesting a good compatibility of the PEG400 with

TABLE I
T_g of Powder and TPZm from Different Batches

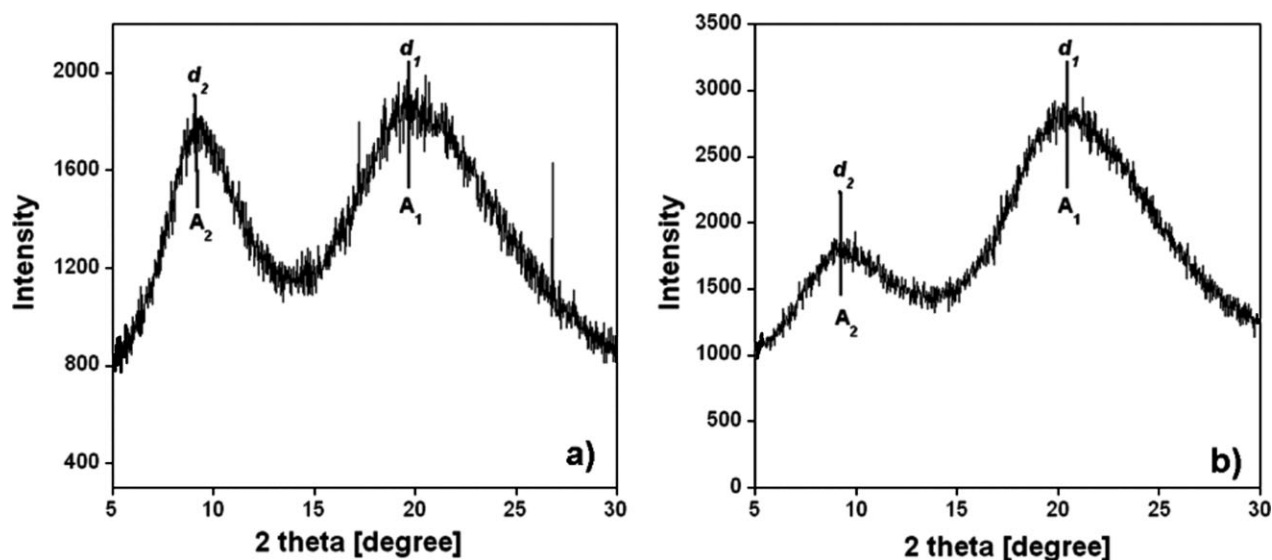| Powder	T_g (DSC) [°C]	TPZm	T_g (DSC) [°C]	T_{g1} (DMA) [°C]	T_{g2} (DMA) [°C]
A	168	A	47	49	58
B	165	B	50	44	58
C	163, 170	C	48	–	58
D	163	D	54	47	58

Figure 4   X-ray patterns of powder (a) and TPZm (b) from batch B.

zein and the effectiveness of plasticization process for all the batches. Beck et al.[28] measured a T_g of 90°C for zein-20 wt % PEG300 prepared via water-plasticization, compression molding, and drying. In view of the natural variability of the material source and of the different plasticization methods used, this result can be considered in agreement with ours.

X-ray diffraction patterns

The X-ray diffraction patterns of zein powder and TPZm from batch B are shown in Figure 4(a,b). Curves for the other batches were similar and are not reported for simplicity. Both patterns show two characteristic peaks at $2\theta = 9.5$ and 20. These peaks correspond to two d-spacings at 9.5 (d_2) and 4.5 Å (d_1), which were attributed to interhelix packing and zein α-helix backbone, respectively, according to Arndt and Riley,[29] who measured the X-ray diffraction of a series of natural α-proteins. They found that the d-spacing of α-helices varied from 4 to 5 Å and those of interhelix packing ranged from 9.5 to 11.5 Å.

The main difference between X-ray patterns of pure [Fig. 4(a)] and thermoplastic zein [Fig. 4(b)] was the relative intensity of the peak at $2\theta = 9.5$. The parameter used to evaluate the relative intensity was the area ratio of the peaks at $2\theta = 9.5$ and 20 (A_2/A_1). Peak areas were measured with Kaleida Graph software. Table II reports the d-spacings and the area ratio (A_2/A_1) for all zein batches, both powders and thermoplastics. The decrease of the area ratio with thermoplasticization suggested that, although zein helical structure (α-helix) resisted during the thermoplasticization process, the molecular aggregates (interhelix packing) were disrupted. Similar conclusions were reported by Wang et al.,[30] who

studied the effect of different film-forming methods on the structures of zein/oleic acid films. In our case, however, the reduction of A_2/A_1 was minor, suggesting that, during the mixing process, the protein destructuration was limited. Moreover, the values of A_2/A_1 after thermoplasticization are similar for the different batches analyzed; therefore, no differences in thermoplasticization can be observed for different batches as observed from thermal data.

FTIR spectroscopy

As described in the Experimental section, the measured spectra were normalized and deconvoluted in the region (1700–1600 cm^{-1}) called amide I region,[31] for the secondary structure analysis of the different zein batches before and after the thermoplasticization. The assignment of the peak around 1650 to α-helices and of the peak around 1620 to β-sheets was done according to Refs. 31 and 32.

The normalized spectrum and Fourier-deconvoluted bands of the amide I region of zein pure and TPZm from batch A are shown in Figure 5(a,b), respectively.

Results show that there were differences in the amide I region between the powder and TPZm samples. The relative intensities of the peaks contained

TABLE II
X-Ray d-Spacings and Peak Area Ratio for Different Batches of Zein (Powder and TPZm)

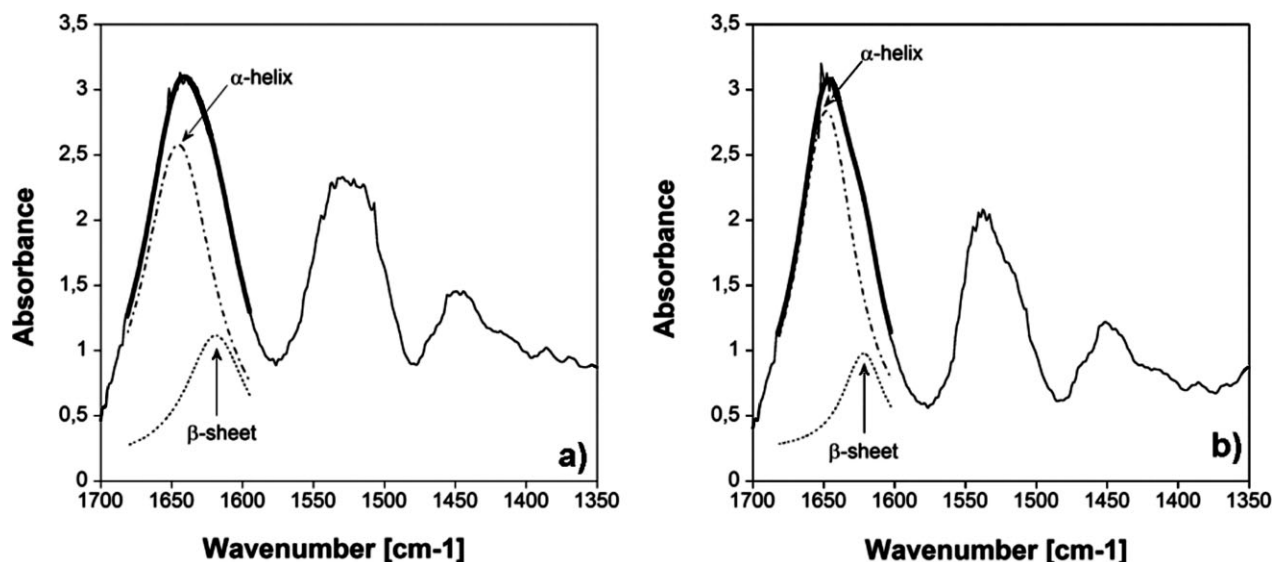| Powder	d_1	d_2	A_2/A_1	TPZm	d_1	d_2	A_2/A_1
A	4.43	9.49	0.55	A	4.36	9.43	0.45
B	4.48	9.49	0.56	B	4.32	9.36	0.42
C	4.45	9.56	0.52	C	4.33	9.56	0.47
D	4.46	9.56	0.55	D	4.33	9.49	0.49

Figure 5 Normalized FTIR spectra of zein powder (a) and TPZm (b) from batch A. Deconvoluted spectrum (dot dash: α-helix, dot: β-sheet) and reconstituted spectrum after fitting (solid) in amide I region.

in the amide I band and the width of this band changed. In the powder, the band was much broader than the TPZm. This may be used as an indication that the distribution of protein conformations changes during the mixing process.

Table III reports the areas corresponding to the Fourier-deconvoluted bands of α-helices and β-sheets for different batches of zein. The areas were used to evaluate the content of α-helices and β-sheets in the different samples.

These results show that, during the mixing process, the proportion of β-sheets decreased, whereas α-helical structure did not change considerably. This indicates that during mixing, zein underwent a change in its secondary structure favoring unordered structures. The results of Table III show, furthermore, that the various batches of zein have different secondary structures. In effect, the powder

and thermoplastic zein from batch A had a higher content of α-helices with respect to β-sheets than the other batches.

Characterization by SDS-PAGE

The SDS-PAGE was used to evaluate the molecular size of polypeptides present in the different zein batches. It is known, in fact, that biologically zein is a mixture of proteins varying in molecular size[33,34] and solubility and that these proteins can be separated by their differential solubility and related structure into three distinct types: α-($M_r = 18$ and $21–25 \times 10^3$), β-($M_r = 17$ and 18×10^3) and γ-zein ($M_r = 27 \times 10^3$).[35] In Figure 6 is reported the SDS-PAGE of the powders zein A, B, C, and D. Although powders from batch A and C showed only the

TABLE III
Area Corresponding to the Peaks of α-Helices and β-Sheets from FTIR for Different Batches of Zein

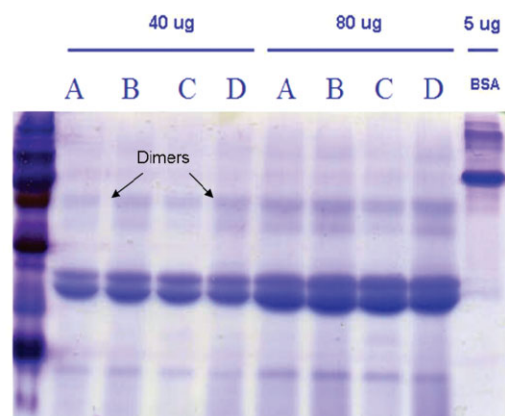| Batch	α-Helix Wavenumber (cm^{-1})	Area	β-Sheet Wavenumber (cm^{-1})	Area
Zein powder				
A	1645	230	1618	80
B	1646	145	1616	62
C	1644	155	1613	72
D	1645	180	1616	69
TPZm				
A	1647	213	1621	45
B	1649	144	1625	53
C	1649	148	1625	49
D	1648	158	1625	58

Figure 6 SDS-PAGE of the zein batches A, B, C, and D. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
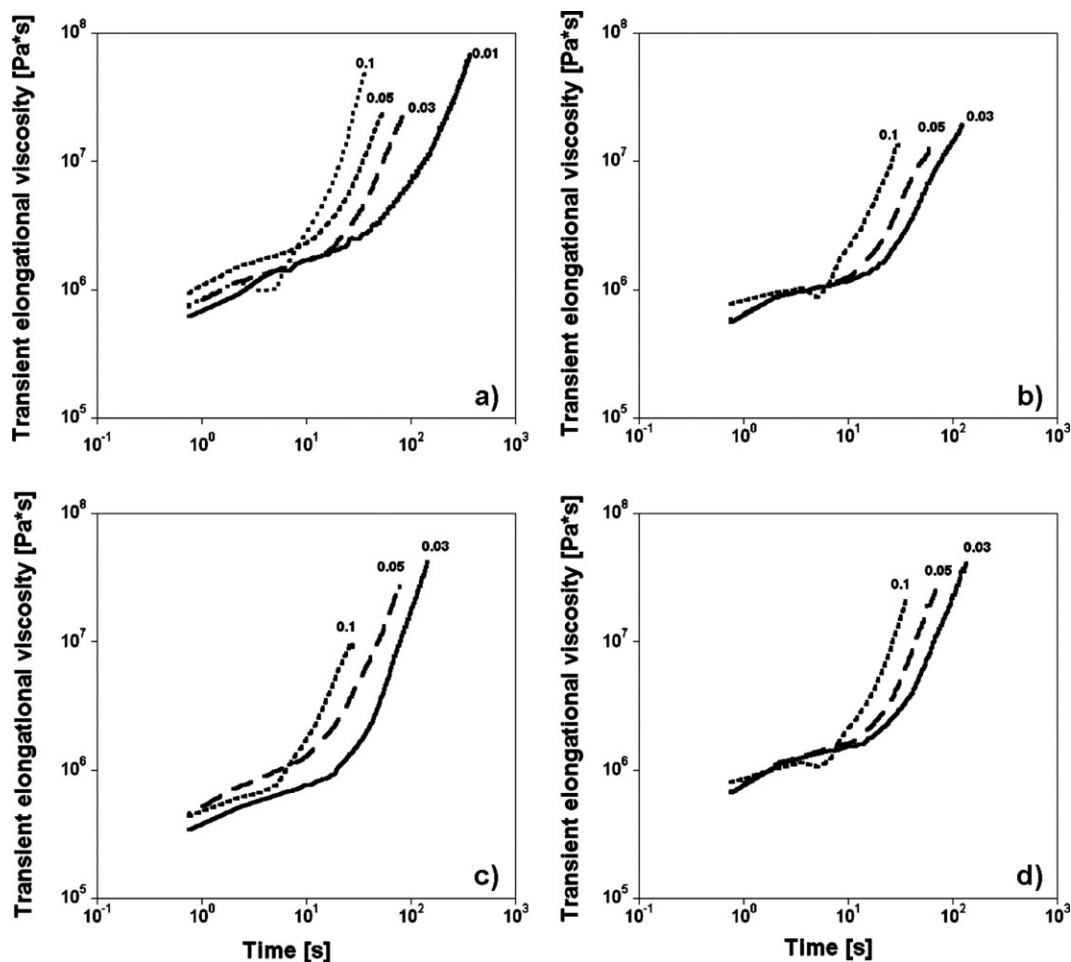
Figure 7 Elongational behavior at different constant strain rates ($\dot{\varepsilon}_0$ = 0.01–0.1 s^{-1}): (a) batch A, (b) batch B, (c) batch C, and (d) batch D.

presence of monomers α_1-($M_r = 23 \times 10^3$) and α_2-zein ($M_r = 25 \times 10^3$), in the powder B and D the presence of few monomers γ-($M_r = 27 \times 10^3$) and

Figure 8 Elongational behavior of various batches of zein at constant strain rate of 0.1 s^{-1}.

bands of high-molecular-weight (dimers) were also evidenced.

Extensional rheological properties

The transient elongational viscosity curves for the batches of thermoplastic zein A, B, C, and D at different constant strain rate $\dot{\varepsilon}_0$ are represented in Figure 7(a–d). For all materials investigated and at all applied elongational rates, the transient elongational viscosity, defined as: $\eta(t) = \sigma(t)/\dot{\varepsilon}_0$, increased with time. In particular, this increasing was slight in
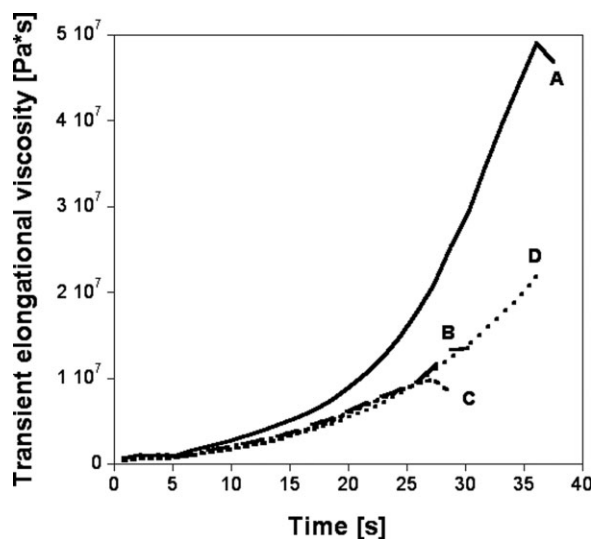
TABLE IV
Strain Hardening Parameter for TPZm from Various Batches at Different Strain Rates

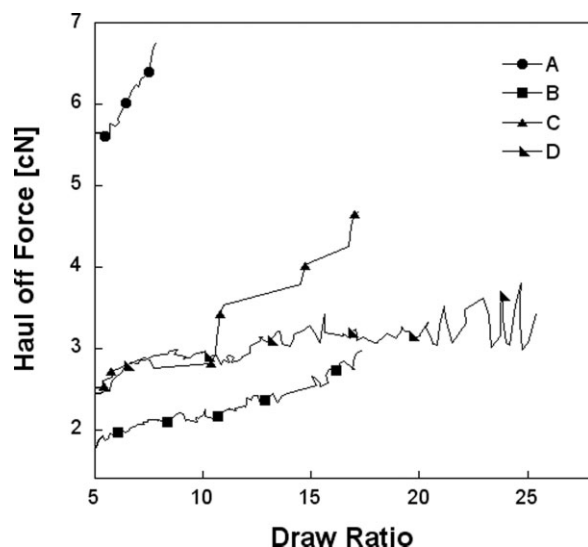| Batch	SHP at 0.01 s^{-1}	SHP at 0.03 s^{-1}	SHP at 0.05 s^{-1}	SHP at 0.1 s^{-1}
	Strain hardening parameter (SHP) *10^5			
A	2.5	4.2	6.8	24
B	–	1.9	2.6	5
C	–	4.0	4.5	6.9
D	–	3.9	4.6	8.8

Figure 9 Haul-off force as a function of draw ratio for different batches of zein.

the so-called "linear" region and became more rapid in the "strain hardening" region of deformation. Similar behavior has also been observed by Oom et al.[36] on kafirin and zein resins plasticized with oleic acid. They observed, in fact, that these resins had a longer start up period compared with gluten-resin with a gradual increase in strain hardening during the extension. The authors hypothesized that the reason of this behavior might be the differences in molecular weight, with gluten polymers having higher molecular weight than kafirin and zein

polymers. In analogy with many homogeneous polymers, for all materials investigated, the phenomenon of strain hardening was more pronounced at higher elongational rates. This behavior has also been previously observed by Munstedt et al.[37] in their work on polyethylene with different molecular weights. Transient elongational viscosity curves for the different batches, at $\dot{\varepsilon}_0 = 0.1$ s^{-1}, are reported in Figure 8 for comparison. Values of transient elongational viscosity and of breaking time of batch A were significantly higher than the other batches, which, instead, showed similar values but with different breaking times (the batch D had higher breaking time with respect to batch B and C). This different behavior might be due to the highlighted differences in the secondary structures (α-helix and β-sheet content). The TPZm from batch A, with higher α-helix content and lower β-sheet content has, in fact, a less aggregated and then more extensible secondary structure. To evaluate the strain hardening characteristics of various batches at different strain rates, the strain hardening parameter (SHP) defined as slope of the elongational viscosity versus time curve[38] in the region of rapid deformation was calculated and presented in Table IV. The value of SHP revealed a slight increase in the strain hardening characteristic with increasing strain rate up to a value of $\dot{\varepsilon}_0 = 0.05$ s^{-1}. From 0.05 to 0.1 s^{-1} the SHP undergoes instead a step increase. Another interesting feature is that the batch A always showed an higher SHP than the other batches. This behavior was more evident at 0.1 s^{-1}.
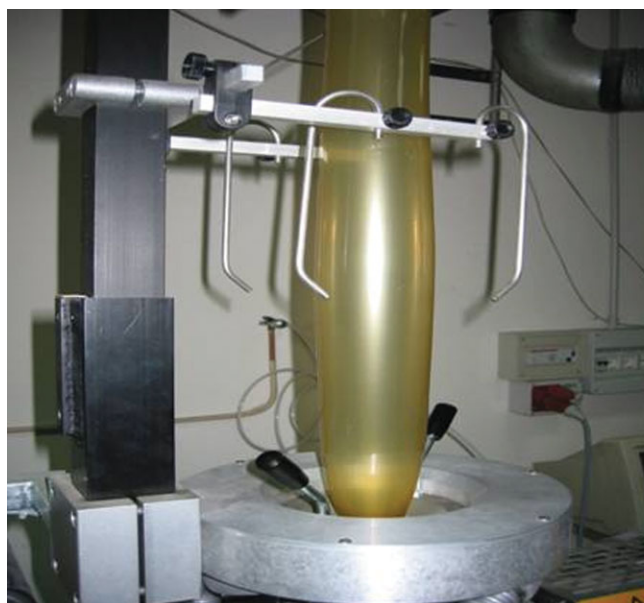

Figure 10 Picture of lab-scale film blowing of TPZe (batch A). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE V
Processing Parameters, Final Thickness, and Result of Film Blowing Process

Batch	Temperature profile zones 1/2/3/4/head (°C)	Thickness (μm)	Notes
A	70/80/85/95/100	80	Excellent
B	85/100/120/115/115	150	Not good
C	85/100/120/115/115	150	Not good
D	85/100/120/115/115	90	Good

Haul-off force

The haul-off forces for different zein batches are given in Figure 9 as a function of draw ratio. All samples showed an increase of haul-off force with draw ratio except batch D, for which almost constant value of haul-off force was measured. In the draw ratio range from 5 to 8 (typical range of film blowing process), the value of haul-off force was always higher for batch A. Data were in agreement with elongational measurements that gave elongational viscosity values higher for batch A and suggested that the batch A will be resistant to gravity force during the start up step of the process and produce stable bubbles with lower final thicknesses.

Film blowing (TPZe)

Pictures in Figure 10 show the film bubble during film blowing process of thermoplastic zein. In Table V are summarized, for the different zein batches, some of the processing conditions used, final thickness with a general comment on the film blowing process. The film blowing characteristics are difficult to report because of the nature of the experiment. For this reason, much of the information gathered (e.g., bubble stability, film quality) relies heavily on the observations and opinion of the operator. The temperature profile was adjusted for the different TPZs to ensure an optimal extrudate viscosity to blow the best film. In particular, for the batch A, which showed a lower viscosity, lower processing temperatures were used with respect to the other batches. As shown in the table, not all of the thermoplasticized zein-based materials were suitable to be filmable, and only the film blowing process of batch A, containing a large proportion of α-helical structure and having the highest elongational viscosity and haul-off force, was continuous, the bubble was stable and the final thickness of the film was as low as 80 μm. These results are in agreement with the studies reported in literature on natural and synthetic polymers. Gao et al.[17] reported, in fact, in their study on effect of preparation conditions on protein secondary structure and biofilm formation of kafirin, that the best films were made with kafirin containing a large

proportion of nativelike α-helical structures as indicated by the Fourier transform infrared reflectance peak intensity ratios associated with these secondary structures. Minoshima and White[18,19] correlated rheological behavior in uniaxial elongation, film blowing properties, and molecular structure for a variety of LDPE, linear low-density polyethylene (LLDPE), and high-density polyethylene (HDPE) resins. They found LDPEs as the most stable resins in the film blowing process for their distinct strain hardening behavior, whereas the HDPEs and LLDPEs were unstable for their narrower molecular weight distribution. Later, Mekhilef et al.[22] studied the effect of rheological strain hardening on extrusion blown film of polyvinylidene fluoride (PVDF). He observed that the strain hardening of the branched PVDF samples increases the processing window with respect to reference samples (not branched) and allows for the formation of a large and stable bubble, thus producing films with reduced thickness and higher clarity.

Tensile properties of zein blown films (TPZe)

The tensile data for the different films (TPZe) are summarized in Table VI. The modulus of elasticity and stress at break were higher for films from batches A and D that have higher α-helices content. Similar correlation has been determinate by Gao et al. in 2005 for kafirin films.[17] Wang and Padua[12] reported values of modulus of elasticity, stress and strain at break of 81.3 MPa, 3.3 MPa, and 79.2%, respectively, for films produced by a film blowing process of a zein-based resin. By comparison of these values with our results, we can conclude that our best film (batch A) showed higher modulus than films by Wang and Padua and comparable stress and strain at break. This behavior, possibly, depends on the plasticization effect related to the higher water content in the films by Wang and Padua.

CONCLUSIONS

Natural polymers have much more complex molecular structure and lower thermal stability than common synthetic polymers and, for these reasons, they are mainly processed by using solvent-based

TABLE VI
Tensile Properties of Zein Blown Films

Material	Young Modulus (MPa)	Stress at break (MPa)	Strain at break (%)
A	383 ± 39	3.57 ± 0.22	42 ± 12
B	5.7 ± 1.2	0.04 ± 0.003	270 ± 20
C	4.1 ± 0.6	0.13 ± 0.02	242 ± 50
D	56.1 ± 6	0.73 ± 0.08	79 ± 2

technologies. However, processing of biobased materials by conventional thermoplastic polymer processing technologies is possible if suitable plasticizer and processing conditions are used. Throughout this work, it has been shown, in effect, that zein/PEG 400 mixtures can be processed via film blowing technologies, like common synthetic polymers, giving films with low-enough thicknesses and suitable mechanical properties for packaging applications. However, this was not possible for all the batches of zein considered. Only thermoplasticized zein-based material that showed a pronounced strain hardening behavior was filmable and film blowing processes were continuous, bubbles were stable and the final thickness of the film was about 80 μm. The different rheological behavior was assessed not to be due to different thermoplasticization, as confirmed by DSC, TGA, DMA, and X-ray analysis, or to different molecular size of the polypeptides (SDS-PAGE) but to different secondary structures (content of α-helices with respect to β-sheets). In conclusions, this work indicated that the best films were made with thermoplastic zein with a large content of α-helices with respect to β-sheets, as proved by extensional rheological properties. An industrial-scale extraction should therefore minimize protein aggregation and maximize native α-helical structures to achieve materials suitable for giving optimal film quality.

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