

# Investigation of Thermoplasticity of Zein and Kafirin Proteins: Mixing Process and Mechanical Properties

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**Abstract** Conventional direct melt mixing technology was investigated on zein and kafirin, two vegetable proteins extracted, respectively, from maize and sorghum. A lab scale internal mixer has been used to thoroughly study the thermo-plasticization process of the proteins with several plasticizers. Different compositions were investigated under different processing conditions. In particular, the lengthy procedures of forming the protein/solvent/plasticizer solution followed by drying or the protein/plasticizer emulsion followed by the precipitation of the extrudable resin, reported in the literature for these systems, were avoided and the protein and plasticizer were directly fed into the mixer to obtain a plastic-like material. The effect of plasticizer type and content and mixing process variables on the mechanical properties was analyzed. Compression molded slabs were transparent, strong and flexible, with properties similar to the cast films reported in the literature, prepared with the same type of plasticizers. However, lower plasticizer content was sufficient to produce equally flexible films, proving an enhanced

plasticization efficiency of the mixing process, as compared to casting.

**Keywords** Protein · Zein · Kafirin · Plasticizer · Thermoplasticization · Melt mixing · Mechanical properties

## Introduction

Plastic materials made out of plant and animal proteins are gaining attention due to their peculiar bio-properties: they are biodegradable, biocompatible and, often, even edible. Furthermore, they can be readily available and extraction techniques are improving to reduce materials costs [1–4].

The solvent process is the most common technique used to prepare films based on proteins [5, 6]. This process is based on the solubilization of the proteins in a suitable solvent medium. The plastic film is then obtained by a number of different separation techniques, including solvent removal (drying). However, films prepared with this technique are usually brittle at room conditions and plasticizers such as poly(ethylene glycol) (PEG), glycerol, lactic acid (LA), and fatty acids have been added to induce softness and permanent flexibility [6–10].

An increasing interest is today devoted to the possibility of using manufacturing technologies employed for conventional thermoplastic polymers. Thermoplastic synthetic polymers can be melt-processed by simply applying heat and shear; unfortunately, the high crystallinity (the long-range order of the helical structure of the proteins may be referred to as a crystalline structure, despite being quite different from that of synthetic polymers [11]) and the strong intermolecular and intramolecular interactions present in the proteins and polysaccharides lead to thermal

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degradation of the material before achieving melt flow. The latter is however possible if a suitable plasticizer is used in combination with heat and shear (thermoplasticization). The plasticizer acts as an internal lubricant, leading to an increase of molecular mobility, necessary for the melt flow. In this way, several products can be formed by using traditional shaping methods, such as extrusion, compression molding, thermoforming, calendering, injection molding and film blowing [12, 13].

In 1997, Lai and Padua [14] introduced a method for applying the extrusion technology to zein that involved the plasticization of zein by forming emulsions with fatty acids, followed by precipitation of the protein-lipid mixture to form a moldable resin. The plastic resin could be subsequently ground to obtain granular material to be, finally, fed to the extruder.

More recently, Corradini et al. [15] thermoplasticized zein with glycerol via melt mixing, in a work on poly( $\epsilon$ -caprolactone)/zein blends. They analyzed mechanical properties but gave few details on the thermoplasticization process itself. Selling et al. [16], conversely, extensively studied thermoplasticization of zein with triethylene glycol and water, by using an internal mixer and obtained interesting correlations between process and compositions. Their study allowed the scaling up to extrusion [17].

Literature data on mechanical properties of plasticized zein mostly deal with materials prepared by solution casting [18, 19], while the effect of the different film formation techniques has been reported by Lai and Padua [14]. In general, as it could be expected, zein films (like other plasticized proteins or polysaccharides) produced both by casting and by stretched resin technologies showed both tensile strength and moduli decreasing with the increase of plasticizer content, with typical values in the range 1–30 MPa for the tensile strength, and 10–2200 MPa for the tensile elastic modulus and elongation at break up to 240%, depending on the plasticizer type and content (generally, in the range 10–50 wt%) and water content.

In this work, a thorough study on the direct melt processing of zein with different plasticizers was conducted to extend the “thermoplastic” processing window of this protein. Furthermore, kafirin, a novel vegetable protein from sorghum, a indigenous cereal in Africa, was thermoplasticized via melt mixing for the first time. The effect of the mixing conditions (mixing temperature and speed of rotation), as well as the blend composition on the thermoplasticization process was investigated in terms of the evolution of torque and melt temperature during mixing. The effect of processing conditions and of blend composition on the mechanical properties of thermoplasticized zein and kafirin are reported, evidencing the plastic like properties of these materials and, in turn, the feasibility of using melt mixing technologies for their processing.

**Table 1** Plasticizers selected for this study and their properties

Plasticizer type	MW, code	Formula	No. of O atoms
Polyethylene glycol	400, PEG400	H(OCH <sub>2</sub> CH <sub>2</sub> ) <sub>8</sub> OH	9
Lactic acid	90, LA	CH <sub>3</sub> CH(OH)COOH	3
Lauric acid	200, LAU	H(CH <sub>2</sub> ) <sub>11</sub> COOH	2
Stearic acid	284, ST	H(CH <sub>2</sub> ) <sub>17</sub> COOH	2

## Experimental Part

### Materials

Zein protein (code Z3625) was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA); Kafirin was supplied by CSIR, Pretoria, RSA. Source (condensed tannin-free white cultivars) and extraction technique (solution in a 70 wt% ethanol in distilled water, followed by centrifugation, evaporation and freeze drying) have been described by Emmambux and Taylor [20]. Plasticizers investigated in this study were LA, PEG400, lauric acid (LAU), and stearic acid (ST) and were purchased from Sigma–Aldrich Co. Table 1 reports the Mw, formula and number of oxygen atoms for the plasticizers selected for this study.

### Mixing procedures and Compositions

Zein powder (92% purity) or kafirin powder (88.6% purity), as received (moisture content, as determined by thermogravimetric analysis, equal to 7 and 6 wt%, respectively), were mixed with the plasticizer in a beaker using a spatula to provide a crude blend. The blend was then fed into a twin counter rotating internal mixer (Rheomix<sup>®</sup> 600 Haake, Karlsruhe, Germany) equipped with roller rotors and connected to a control unit (Rheocord<sup>®</sup> 9000, Haake). The mixing chamber (volume of 50 cm<sup>3</sup>) was first filled with 50 g total mass for all experiments in approximately 3 min at 5 rpm at different mixing temperatures,  $T_{\text{mix}}$ . Subsequently, the speed of rotation,  $N$ , was raised to and kept at different rpm, for 10 min. Details of the test are reported in Table 2. Torque and melt temperature were recorded during the mixing process to provide the mixing process history.

### Slab Preparation

A hot press (P300P Collin, Germany) was used to prepare slabs for mechanical testing. Samples were heated to and kept at the same temperature employed during mixing of the different systems and pressed at 3.0 MPa for 3 min, then cooled to 30 °C under pressure. Slab thicknesses from 0.3 to 0.5 mm were obtained.

**Table 2** Melt mixing parameters and tensile properties of thermoplasticized zein and kafrin

Protein	Plasticizer	wt% plasticizer	Speed of rotation (min <sup>-1</sup> )	Temperature (°C)	Tensile strength (MPa)	Tensile modulus (MPa)	Strain at break (mm/mm)
Zein	LA	25	50	80	16.4	897	0.043
Zein	PEG400	20	50	60	Brittle		
Zein	PEG400	25	50	60	2.7	144	0.120
Zein	PEG400	30	50	60	0.7	23.9	0.752
Zein	PEG400	25	50	70	16.0	866	0.029
Zein	PEG400	25	50	80	18.0	843	0.028
Zein	PEG400	25	50	100	11.8	626	0.032
Zein	PEG400	25	50	140	10.5	421	0.027
Zein	PEG400	25	100	60	11.9	665.0	0.034
Zein	PEG400	25	20	60	1.9	144.6	0.160
Zein	LAU	25	50	80	13.8	820	0.026
Zein	ST	25	50	80	Brittle		
Kafrin	PEG400	25	50	60	9.0	297	0.089
Kafrin	LA	25	50	60	6.3	126	0.504
Kafrin	LAU	25	50	60	9.9	752	0.023

## Tensile Testing

Tensile testing of the plasticized zein films was carried out by using a 4204 Universal Testing Machine (Instron, USA), according to ASTM D1708. Samples were conditioned at room temperature, 50% RH prior to testing.

## Results and Discussion

### Mixing Process

#### *Effect of Mixing Temperature on Zein Thermoplasticization*

In order to evaluate the effect of  $T_{\text{mix}}$  on the plasticization process, five samples from zein/PEG400 at 25 wt% plasticizer content were prepared at 50 rpm and 10 min of mixing, and  $T_{\text{mix}}$  in the range 60–140 °C. Figure 1a and b show the effect of  $T_{\text{mix}}$  on the torque and on the melt temperature developed in the mixer, respectively.

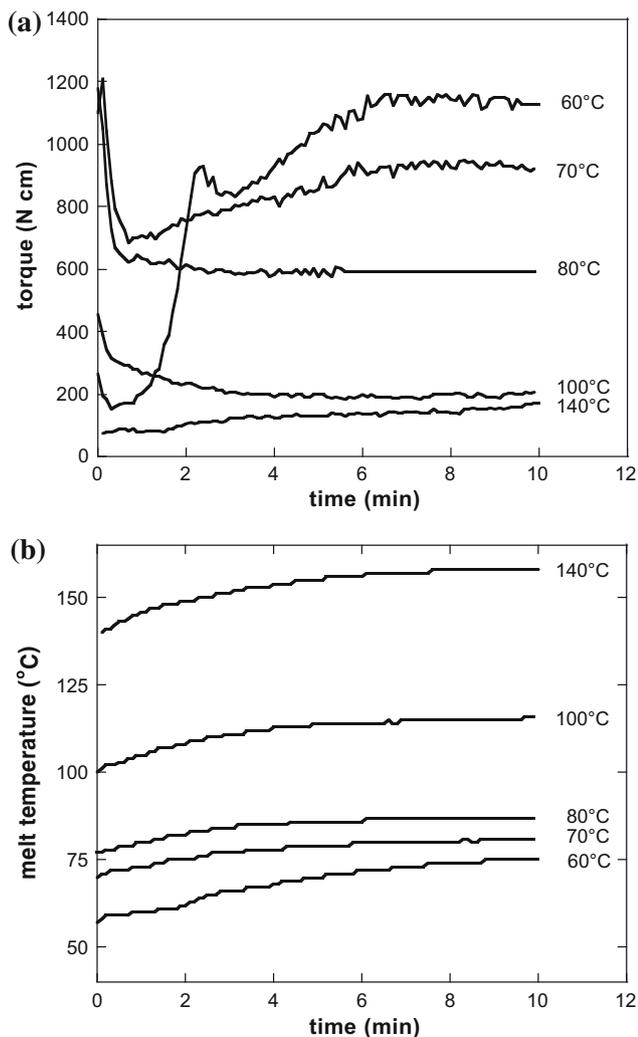
As reported for other protein-plasticizer systems, torque evolution during thermoplasticization can be described as a three-step process [21], characterized by a first horizontal plateau followed, after a certain induction time, by a steep increase of the torque to a maximum and then a continuous decrease to a rather stationary value, when also the melt temperature becomes constant. In our case, this behaviour was not observed for  $T_{\text{mix}}$  equal to or higher than 70 °C, since the torque increase was immediate, with the absence of the induction time. Conversely, at 60 °C the induction time was rather long, with the absence of the decreasing

section of the torque, which continuously increased during the final step of the mixing process.

In general, Fig. 1a shows that the final, stationary value of the torque decreases with the increase of  $T_{\text{mix}}$ , due to its effect on the viscosity of the plasticized zein protein, which behaves as a thermoplastic polymer, in this temperature range. Furthermore,  $T_{\text{mix}}$  was found to profoundly affect the mechanisms of plasticization process; at 100 °C, for example, the torque starts to decrease to reach a minimum value after 3 min, which means that 3 min of mixing are sufficient to plasticize the zein protein. When lower  $T_{\text{mix}}$  were used, longer times to reach stationary values were observed; in particular, at 60 °C the mixing time of 10 min was not even sufficient to complete the plasticization process. Reported data reveal that the  $T_{\text{mix}}$  speeds up the kinetics associated with the plasticization process, as also proved by the delay in the occurrence of the peak value in the torque, almost immediate at 100 °C, while it was observed after 0.1, 0.2 and 2 min for the test performed at 80, 70 and 60 °C, respectively. Finally, it can be observed that, in this  $T_{\text{mix}}$  range, the mixing time, which is the time necessary to fully plasticize the zein protein, can be reduced by increasing the  $T_{\text{mix}}$ .

#### *Effect of Speed of Rotation on Zein Thermoplasticization*

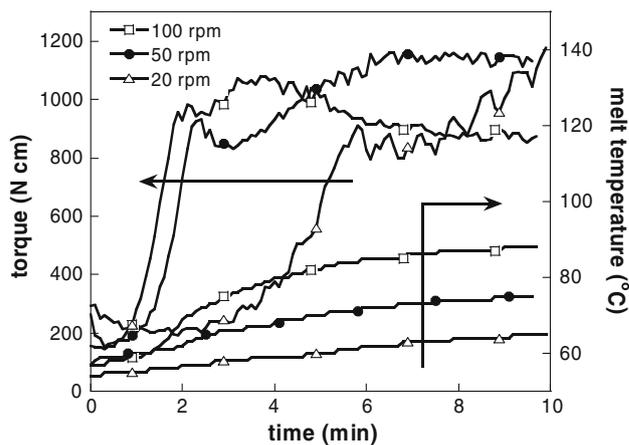
In order to evaluate the effect of  $N$  on the plasticization process, three experiments were performed at 20, 50 and 100 rpm, on the system zein/PEG400 at 25 wt% plasticizer content and at  $T_{\text{mix}}$  of 60 °C. Figure 2 reports the torque



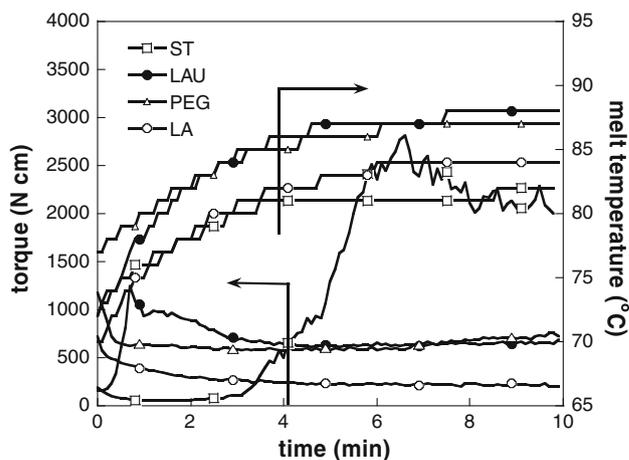
**Fig. 1** Effect of  $T_{mix}$  on the mixing process of zein plasticized by 25 wt% PEG400 at 50 rpm: **a** torque and **b** melt temperature

and melt temperature evolution during the mixing process and shows that the increase of  $N$  speeds up the plasticization process. In effect, the increase of torque is very slow when 20 rpm was used, with respect to the experiments conducted at 50 and 100 rpm. In particular, the steep increase of the torque, which indicates the incipient plasticization process, occurs after 5 min at 20 rpm, while it takes less than 2 min at 50 and 100 rpm. Furthermore, it is evident that neither at 50, nor at 20 rpm the plasticization process was completed, as indicated by the continuous increase of the torque after 10 min, while at 100 rpm a stationary value of the torque was attained within 10 min of mixing.

These results suggested that  $N$  is a very important parameter in the mixing process since it speeds up the plasticization process due to the higher shear stresses (higher torque values) that contribute to a faster thermo-mechanical denaturation of the protein structures.



**Fig. 2** Effect of  $N$  on torque and melt temperature of zein plasticized by 25 wt% PEG400 at 60 °C

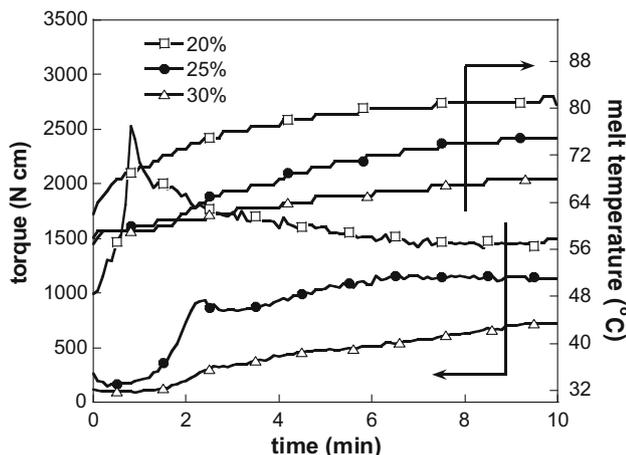


**Fig. 3** Effect of type of plasticizer on torque and melt temperature of zein thermoplasticized with 25 wt% of plasticizer

### Effect of Type of Plasticizer on Zein Thermoplasticization

Figure 3 reports the results of the mixing experiments conducted on zein/plasticizer mixtures prepared with different plasticizers (at plasticizer content of 25 wt%) at 50 rpm and 80 °C.

Figure 3 shows that the time necessary to complete the thermoplasticization is strongly dependent on the type of plasticizer. In particular, ST requires the highest time (10 min) to reach the plateau; for LAU the thermoplasticization process occurred in 5 min, while thermoplasticization with LA or PEG400 was very fast and led to materials of lower viscosity, as evidenced by the very low stationary value of the torque.



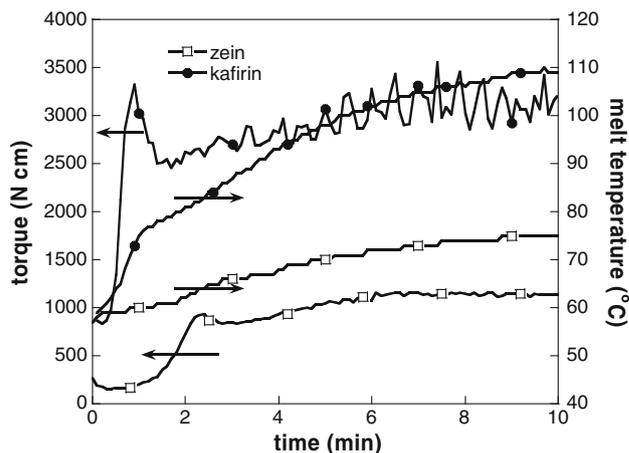
**Fig. 4** Effect of plasticizer concentration on zein systems plasticized with PEG400

#### *Effect of Plasticizer Content on Zein Thermoplasticization*

Figure 4 shows the effect of plasticizer content in experiments conducted at 60 °C, 50 rpm for PEG400. As a general comment, torque decreases with the increase of plasticizer content, due to the enhanced lubricating effect of the plasticizers. As a consequence, due to the reduced dissipation, melt temperature evolution is milder, and final melt temperature decrease with the increase of the plasticizer content. The torque peak becomes less pronounced with the increase of concentration, becoming almost unreadable at 30 wt% plasticizer. For the system containing the 30 wt% of PEG400, 10 min of mixing was not enough for the completion of the thermoplasticization process (at  $T_{\text{mix}}$  equal to 60 °C), while at 20 and 25 wt%, 10 min of mixing was sufficient, as evidenced by the attainment of a stationary value of torque and melt temperature.

#### *Thermoplasticization of Kafirin*

Due to limited material availability, the study of the thermoplasticization process of kafirin via direct melt mixing was investigated in a narrow experimental processing range. This was still possible after the thorough study conducted on zein, a similar system which, however, showed substantial differences, in thermoplasticization. Figure 5 shows the comparison between the two proteins, plasticized with PEG400 at 25 wt%, at 60 °C and 50 rpm, evidencing that the increase of torque and temperature during mixing is much higher in kafirin than in zein. These results suggest that the two proteins have a different compatibility with PEG400, leading to materials of different viscosity. This behavior can be attributed to the



**Fig. 5** Comparison between zein and kafirin plasticized by 25 wt% PEG400

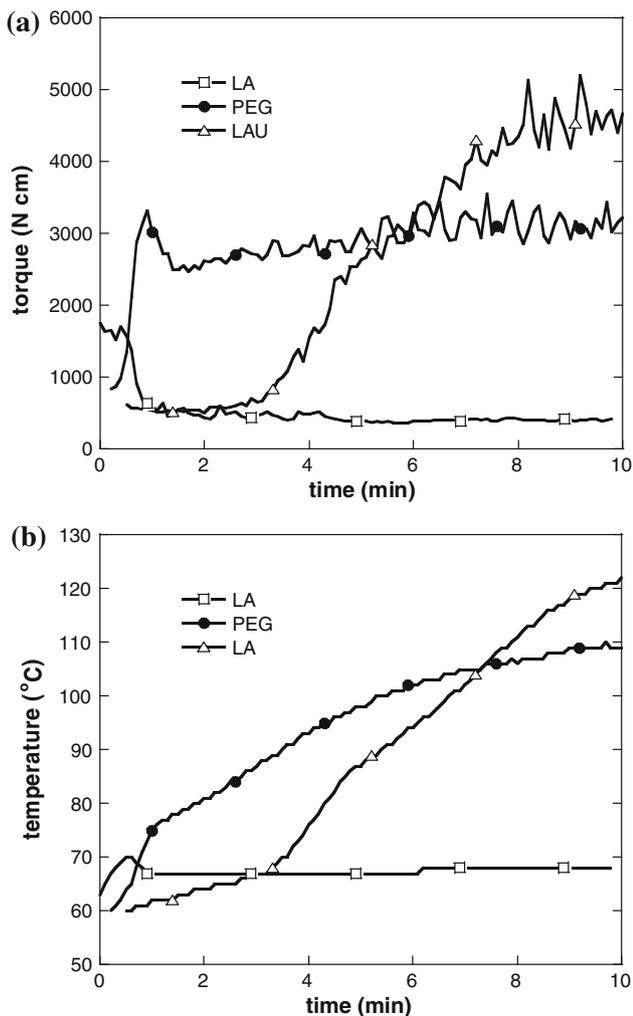
difference in the chemical composition and to the different secondary/tertiary structures of the two proteins. The analysis of the effect of the type of plasticizer (Fig. 6) showed, conversely, a behavior similar to zein. Also in this case, in effect, LA promoted a faster thermoplasticization and led to materials of lower viscosity with respect to LAU and PEG400. It can be concluded, however, that kafirin, as well as zein, could be successfully processed via direct melt mixing. As it could be expected, final properties of the thermoplasticized materials from kafirin and zein, as will be discussed in the following, were substantially different when using the different plasticizers and different processing conditions.

#### *Mechanical Properties*

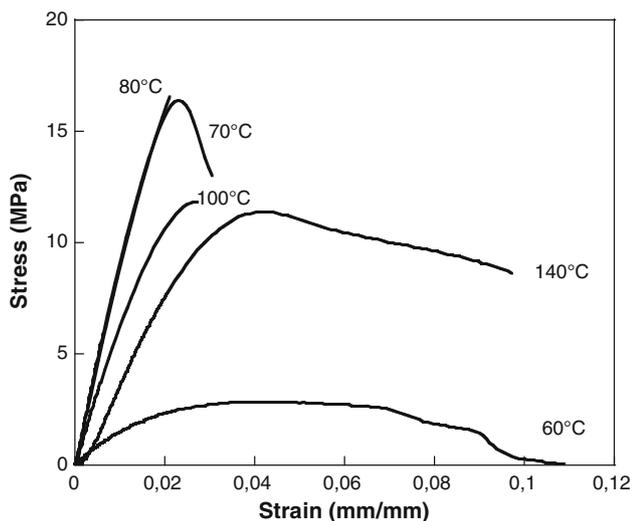
##### *Effect of Mixing Variables and Composition on Mechanical Properties of Thermoplasticized Zein*

Figure 7 shows the effect of mixing temperature on the mechanical properties of thermoplasticized zein plasticized with 25 wt% PEG at 50 rpm. Results are summarized in Table 2. The tensile strength and modulus of the sample prepared at 60 °C are substantially lower than the other samples suggesting that the protein thermoplasticization was not complete, as already observed in the preceding section. The increase of the mixing temperature to 70 or 80 °C led to an increase in both the tensile strength and modulus, but a further increase of the mixing temperatures, to 100 and 140 °C, induced a decrease of the tensile strength and modulus.

These results suggested that 70 °C is the minimum temperature for fully thermoplasticize the zein protein in these operative conditions; at lower temperatures, as already observed, the mechanism of thermoplasticization



**Fig. 6** Effect of type of plasticizer on torque and melt temperature of kafirin thermoplasticized with 25 wt% of plasticizer: **a** torque and **b** melt temperature



**Fig. 7** Effect of mixing temperature on tensile properties of zein protein plasticized with 25 wt% PEG400

takes more than 10 min to go to completion and mechanical properties are inferior. When higher initial mixing temperatures were used (100 and 140 °C, with respect to 70–80 °C), we observed a decrease in the tensile strength and modulus, which could be related to a reduced efficiency of the thermoplasticization process, due to the lower viscosity of the melt at higher temperatures. In effect, in thermoplasticization, the effect of plasticizer, heat and shear concur to the attainment of the entangled structure out of the native structure of the protein. As a matter of fact, when temperature is too high, the viscosity of the protein/plasticizer medium in the mixing chamber is low and the mechanical stresses developed during mixing are not enough for the destructure of the proteins.

In order to modify the thermoplasticity of zein protein, different plasticizers such as LA, PEG400, LAU and ST were used to prepare samples with a plasticizer content of 25 wt%, at 50 rpm, 80 °C. Table 2 reports the mechanical properties of these systems, showing that tensile strength, modulus and strain at break are strongly dependent on the type of plasticizer.

In fact, it is known that proteins and, in general, naturally occurring polymers, have a complex, hierarchical, highly interactive structure which makes them very difficult to process. In particular, proteins are heteropolymers with amino acids as building blocks, arranged in secondary structures of helices and sheets, in turn packed to form globular units. These three dimensional structures are tightly bonded by hydrogen as well as disulfide bonds and have to be (at least partially) disrupted, to obtain separate, entangled macromolecules, in order to achieve plastic-like properties. The role of the plasticizers is, then, to reduce intermolecular and intramolecular interactions and increase the flexibility of the polymer. The efficiency of the plasticizer in this process depends on several factors such as its size (Mw), shape and number and type of interacting atoms. Plasticizer selection is normally based on compatibility between plasticizer and protein, permanence in the formed film and efficiency in terms of amount necessary to plasticize films [22]. In this study we selected four linear molecules, with different composition and size to compare and quantify effects on zein mechanical properties.

Results reported in Table 2 show that LA provided the best mechanical properties to thermoplastic zein, with PEG400 slightly below. However, it has to be evidenced that LA tends to migrate to the surface of zein slabs, resulting in a loss of flexibility as also observed by Tillekeratne and Eastal [18]. For this reason, PEG400 has been selected for more extensive testing (see Table 2 for conditions and properties). LAU and ST proved not to be good plasticizers for zein, due to their large size and, mainly, to the low relative content of oxygen atoms available for

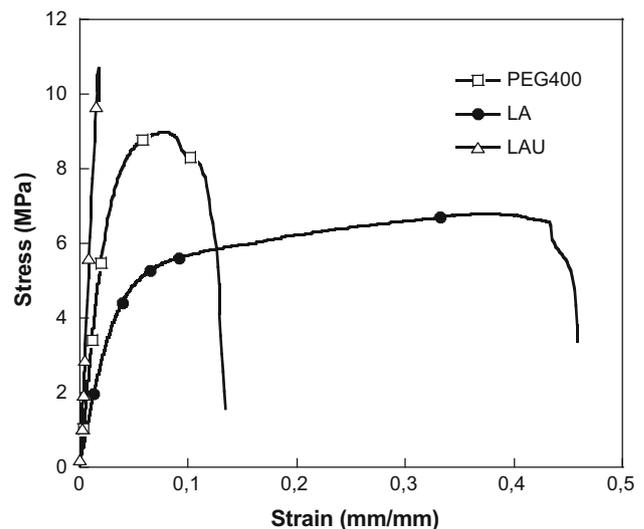
H-bonding. ST, in particular, gave films which were too brittle to characterize [22, 23].

In order to analyze the effect of plasticizer content on tensile properties of zein protein, samples were prepared by using PEG400 at 60 °C, 50 rpm, and 10 min of mixing, with plasticizer concentration varying from 20 to 30 wt%. Table 2 shows the effect of plasticizer content on tensile strength, modulus and strain at break. Results show that the samples containing 20 wt% plasticizer were too brittle and mechanical testing was not feasible. As expected, the increase of the plasticizer content reduces the tensile strength and modulus and increases the strain at break, in agreement with the studies of Masco-Arriola [24] and Jongijareonrak et al. [19].

Literature data on mechanical properties of plasticized zein mostly deal with materials prepared by solution casting, revealing that samples obtained by casting show, generally, inferior mechanical properties with respect to melt mixed materials, mostly in terms of deformability. Tillekeratne and Easteal [18], for example, reported a value of the strain at break of 1.5% for zein plasticized with PEG400 at 30 wt%, while we measured a strain at break of 75%, with the same composition of PEG400. Unfortunately data on elastic modulus and strength were not reported by the cited reference. The effect of the different film formation technologies has been reported by Lai and Padua [14] which investigated the mechanical properties of zein films plasticized with 33 wt% of oleic acid and prepared from casting solutions and stretched resins. They observed that, in general, stretched resin films showed better mechanical properties with respect to cast films in terms of deformability (fourfold increases in the strain at break) at comparable modulus (18% lower in the case of stretched resin) and strength (20% higher in the case of stretched resin), proving a lower plasticizing efficiency of the casting technique. Few literature data on thermoplasticized zein have been reported, with different plasticizer type and content. In particular, Corradini et al. [15], on zein/glycerol system at 5 wt% plasticizer (and water content of 10 wt% ca., as it could be evaluated from reported thermogravimetric curves) reported tensile strength of 18 MPa and elastic modulus of 800 MPa ca., while Selling et al. [17], on extruded zein/tri(ethylene)glycol/water, reported tensile strength in the range 14–40 MPa, which are in good agreement with our results.

#### *Effect of Mixing Variables and Composition on Mechanical Properties of Thermoplasticized Kafirin*

Based on the results obtained by studying the effect of mixing variables on the properties of zein protein, LA, PEG400 and LAU were used to prepare thermoplastic materials from kafirin protein at selected processing



**Fig. 8** Effect of plasticizers on tensile properties of plasticized kafirin protein thermoplasticized with 25 wt% of plasticizer

conditions. All the samples, containing 25 wt% of plasticizer, were prepared at 60 °C, 50 rpm and 10 min of mixing. Figure 8 reports the stress versus strain curves of thermoplasticized kafirin, showing that the use of LA led to films of lowest tensile modulus and highest strain at break, while LAU led to the highest tensile modulus and the lowest strain at break. Results are summarized in Table 2. Literature data on mechanical properties of plasticized kafirin again deal with materials produced by solution casting [6, 25]. In particular, Gillgren and Stading [25] reported, on films with 20–40 wt% PEG/glycerol/LA mixture (1:1:1) plasticizer, mechanical properties comparable with our results.

By comparing results of our mechanical testing on kafirin and zein (from Table 2) it is possible to observe that the effect of plasticizer is very similar and, in particular, LA and PEG400 are best plasticizers for both proteins.

From these results, it can be concluded that the tensile properties of plasticized zein and kafirin proteins are strongly dependent not only on the plasticizer type and its content, but in a significant manner on the mixing processing conditions. LA, PEG400 and LAU were found to be good plasticizers for both proteins while ST did not lead to good and flexible films. Finally, it is possible to evidence that, despite of natural origin, our polymers share some good mechanical performance with common synthetic polymers. In fact, for example, high density polyethylenes have tensile moduli in the range 413–1034 MPa, tensile strength in the range 17.9–33.1 MPa, being, however more deformable (12–700%). Isotactic polypropylenes are stiffer (tensile moduli in the range 1032–1770 MPa, almost twice our stiffest material) but as deformable as our systems [26]. It is worth of note, however, that stability (in particular in

humid environments) of mechanical properties as well as, among others, barrier properties of materials from natural polymers is still the main limitation (together with cost) for their usage for commodity applications.

## Conclusions

Several plasticizers, with different hydrophilic/hydrophobic characteristics and molecular weight were used to investigate the thermoplasticity of zein and kafirin proteins. Thermoplasticization was performed successfully for the two proteins. In particular, the processing window for zein included temperatures between 60 and 100 °C, screw speed in the range 20–100 rpm and was possible with PEG400, LA, LAU and ST as plasticizers, typically at 25–30 wt%. In the case of kafirin, destructurezation required higher mechanical shear, and was conducted with PEG400, LA and LAU at 60 °C, and 50 rpm, 25 wt% plasticizer.

Tensile mechanical properties proved that the different plasticizer and processing conditions resulted in different efficiencies of the thermoplasticization process. Tensile moduli as high as 897 MPa were observed with zein plasticized with 25 wt% of LA, melt mixed for 10 min at 50 rpm and 80 °C, and as low as 23 MPa for the system with 30 wt% PEG, melt mixed for 10 min at 50 rpm and 60 °C, with elongation at break and tensile strength changing, accordingly, from 4% to 75% and from 16.4 to 0.7 MPa, respectively. Thermoplasticized kafirin resulted in comparable tensile properties, with moduli as high as 750 MPa, strength of 10 MPa and strain at break of 2%, at 25 wt% of LAU, while the use of LA led to films more deformable, with strain at break as high as 50%.

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