Journal of Cellular Plastics

Effect of two kinds of lignins, alkaline lignin and sodium lignosulfonate, on the foamability of thermoplastic zein-based bionanocomposites

Maria Oliviero, Letizia Verdolotti, Irma Nedi, Fabio Docimo, Ernesto Di Maio and Salvatore Iannace

Published by:

$SAGE

http://www.sagepublications.com

>> Version of Record - Nov 20, 2012

What is This?

Effect of two kinds of lignins, alkaline lignin and sodium lignosulfonate, on the foamability of thermoplastic zein-based bionanocomposites

Maria Oliviero[1], Letizia Verdolotti[1], Irma Nedi[2], Fabio Docimo[1], Ernesto Di Maio[2] and Salvatore Iannace[1]

Abstract

The aim of this study was to utilize zein, a protein from corn, to develop bioplastic formulations in combination with reactive additives based on ligninic compounds and to investigate the effects of these highly interactive additives on the foamability of zein. In particular, different amounts of alkaline lignin and sodium lignosulfonate were added to zein powder and poly(ethylene glycol) through melt mixing to achieve thermoplastic bio-polymers, which were subsequently foamed in a batch process, with a mixture of CO_2 and N_2 as blowing agent, in the temperature range 50–60°C. The materials before foaming were characterized by X-ray and Fourier transform infrared analysis to highlight the physico-chemical interactions and the eventual destructuration of the protein secondary structure. After foaming, density measurements, scanning electron microscopy and image analysis have been used in order to evaluate the porosity and the pore size distribution of the microstructure of the foams and to determine the effect of the ligninic compounds on the foamability of the bioplastic.

Keywords

Zein, lignins, X-ray, Fourier transform infrared, scanning electron microscopy, foam

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

Corresponding author:
Letizia Verdolotti, Institute of Composite and Biomedical Materials, National Research Council, Naples, Italy.
Email: lverdolo@unina.it

Introduction

Environmental concern about 'plastics' is leading to an increasing interest towards the development of greener materials. In particular, it is noted that synthetic non-biodegradable polymers are a significant source of environmental pollution, harming wildlife when dispersed in nature. The potential of biodegradable polymers and, in particular, of polymers obtained from renewable-resources such as the polysaccharides (e.g. starch) and proteins (e.g. wheat gluten, zein), has long been recognized as one of the most promising approach for substituting petrochemical-based polymers.[1]

Cereal proteins, especially, are available in large amounts as by-products of agricultural and biofuel processing activities. These protein-rich products include spent grain from the brewing and distilling industries, cereal bran streams from milling and protein residues from starch extraction activities. Such by-products are considered of low value, and, in many cases, are disposed of in landfill. However, with proper extraction procedures, specific fractions may be recovered.[2] In particular, the good hydrophobicity of the prolamine and glutelin fractions of cereal storage proteins makes them good candidates for packaging applications.[3] Furthermore, the chemistry of protein components allows targeted functionalization of these biopolymers, for example through crosslinking by chemical and physical means, so that improved functional properties of the packaging material can be obtained.[4]

Zein is found in corn endosperm and has been the object of research as well as industrial interest (being commercially available since 1938)[5] for its film-forming ability and its unique hydrophobicity, which is due to its high content of non-polar amino acids. Like any other protein, upon isolation from the native state, zein shows high density and brittle behavior, with high modulus and stress to break and low strain to break. However, zein is difficult to process. In particular, processing problems are mainly due to reduced plastic flow properties of these polymers and the intrinsically difficult reproducibility and control over the molecular architecture and spatial conformation, typical of natural macromolecules.[6,7] These problems are related to the hierarchical structure of proteins, for instance the strong inter and intra-molecular interactions existing among the amino acids of the protein (secondary structure arrangement), that, upon heating, eventually lead to thermal degradation of the material before achieving melt flow. For this reason, the secondary structure of the protein has to be modified by using specific compounds (such as a plasticizer and/or a reactive additive) that act as an internal lubricant, thereby increasing the molecular mobility due to the substitution of the protein–protein with protein–compound interactions.[8]

Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation. It is an abundant and renewable biomaterial widespread in plants. Industrial lignin, which is a byproduct of the paper industry, exists in the black liquor of the pulping industry. Chemically, lignin is a very reactive macromolecule due to its functional groups such as phenolic, aromatic rings, methoxy and alcohol groups that are sites for chemical modification and/or reaction. Addition of lignin in polymers not only is an effective method in using

renewable biomass, but also provides opportunities to improve mechanical properties, heat and fire resistance, wettability and, for thermoplasticized natural polymers to hinder the plasticizer migration.[9–12]

However, understanding the complexity and the variety of the possible chemical interactions between lignin and polymers (in particular natural polymers) remains, nowadays, an important challenge. In particular, several authors investigated the effect of lignin-natural polymers interactions on functional and mechanical properties of the natural polymers.[10–13]

In these studies, the variation/improvement of physical, functional and mechanical characteristics is strongly dependent upon chemical functional groups of both lignin (alkaline lignin, sodium lignosulfonate) and natural polymers (soy protein, starch and others).

Instead few papers on the use of lignin in foams based on natural polymers are known, i.e. Stevens et al.[14] have examined the use of lignin in starch-kraft lignin foams (for packaging application) to increase the water resistance and mechanical properties with respect to the starch foams. While, in our knowledge the zein-lignin foams have not previously been examined, in particular the effect of interactive filler (as lignin) on foamability of protein polymers.

Foaming of natural polymers has been mostly studied in the context of food engineering and in particular in the preparation of bread and cooked snacks.[15,16] The utilized technologies are different from the gas foaming technologies used for thermoplastic polymers. In fact, bread and cooked snacks are produced via mixing (aeration) and fermentation with gas evolution processes. Recently, however, plastic processing technologies (stream-based extrusion) have been used to produce foams with different formulations including starch, for different application such as packaging and insulation,[17] while the gas foaming technologies to produces foams from thermoplasticized proteins has been reported by Salerno et al.[18]

In this article, the effect of alkaline lignin and sodium lignosulfonate on the hierarchical structure and thermomechanical properties of thermoplastic zein (TPZ) and, consequently, on the foamability of TPZ-based bio-nanocomposites (TPZ-BNCs) were examined.

Materials and methods

Materials

Corn zein powder (code Z3625, lot number: 065K0110) was purchased from Sigma Aldrich, Italy. Poly(ethylene glycol), PEG (Mw = 400, code 81170, Sigma Aldrich, Italy) was used as plasticizer for zein to prepare TPZ. In order to investigate the effect of lignin on hierarchical structure and on foamability of TPZ, two different ligninic systems were used, namely alkali lignin (average Mw = 28,000 approx., code 370959, Sigma Aldrich, Italy), hereafter denoted as AL and lignosulfonic acid–sodium salt (average Mw = 52,000 approx., code 471038, Sigma Aldrich, Italy), hereafter denoted as LSS, with two different functional groups (SH for AL and OH for LSS).

TPZ-BNCs preparation and foaming

TPZ-AL-BNCs were prepared by using a melt mixing method. AL was first added to PEG in amount such that the final concentrations of AL were 1 and 10 wt% of the zein + PEG system. The PEG + AL mixture was stirred in a beaker at room temperature for 3 min in order to allow a good dispersion of AL into the PEG. Subsequently, zein powder was added into the PEG + AL system and mixed in a beaker using a spatula to provide a crude blend. The amount of PEG was 25 wt% of the zein + PEG system. The crude blend was then subjected to temperature and shear stresses in a twin counter-rotating internal mixer (Rheomix 600) connected to a control unit (Haake PolyLab QC, Haake, Germany) for thermoplasticization. Mixing temperature, speed of rotation, and mixing time were 70°C, 50 r/min, and 10 min, respectively.[6,7] The same procedure was used to produce the samples with LSS in place of AL and to produce Neat TPZ for proper comparison. The compositions of TPZ-BNCs are reported in Table 1.

A P300P hot press (Collin, Germany) was then used to prepare slabs with thickness of 1–2 mm. Materials were heated to the same temperature of mixing and pressed at 50 bar for 10 min and subsequently cooled to 30°C under pressure.

Foaming experiments were carried out by using a batch process on disc-shaped samples (10 mm in diameter and 2 mm in thickness). The samples were placed into the pressure vessel and kept with 80–20 vol% N_2–CO_2 blowing mixture for 3 h at 70°C and at saturation pressure of 170 bar.[19] After solubilization, samples were cooled to the desired foaming temperature (T_f = 50–60°C). The pressure was then quickly released to ambient to allow foaming. To stabilize the cellular structure, foams were immediately removed from the vessel and allowed to cool at room conditions.

Characterization

The densities of the samples, reported in Table 1, were determined as the ratio between the weight (as measured with an analytical balance) and volume

Table 1. Classification, foam density and E' values in the glass transition region of the TPZ-BNCs

TPZ-BNC	AL (wt%)	LSS (wt%)	Film density (g/cm^3)	Foam density (g/cm^3)	E' (30°C)–E'(50°C)*(Pa)
TPZ	0	0	1.27	0.53	4.0×10^8–4.0×10^6
TPZ-1AL	1	–	1.22	0.45	4.0×10^8–6.0×10^5
TPZ-10AL	10	–	1.23	–	1.0×10^9–5.0×10^7
TPZ-1LSS	–	1	1.10	0.75	4.0×10^8–8.0×10^5
TPZ-10LSS	–	10	1.24	–	1.0×10^9–1.0×10^8

*Data from Oliviero et al.[24]
TPZ: thermoplastic zein; BNCs: bio-composites; AL: alkali lignin; LSS: lignosulfonic acid–sodium salt.

(as measured by a high resolution caliper) of cylindrical samples obtained from the films, before and after foaming process.

Foam morphologies were characterized by scanning electron microscopy (SEM). Samples were cross-sectioned, gold-sputtered, and analyzed by SEM (S440, LEICA) at an accelerating voltage of 20 kV, at various magnifications. To evaluate the effect of different lignin (AL or LSS) content on foam microstructures, the pore size distribution was evaluated by image analysis. A minimum of 100 pores for each sample were selected from the micrographs and analysed with IrfanView software and correcting the size of the pores, as evaluate by the software with the factor $4/\pi$, according to the ASTM D3576.[20]

To evaluate the effect of lignins in modifying the *ordered* and *disordered* phases of TPZ secondary structure, X-ray diffraction (XRD) and Fourier transform infrared (FTIR) characterization were performed. The X-ray patterns of the different BNCs were obtained by using a diffractometer PW1710 (Philips, Netherlands) with a nickel-filtered Cu Kα radiation of wavelength 1.54 Å at voltage of 40 kV and a current of 20 mA. Samples were examined in a range of diffraction angle $2\theta = 3$–$60°$ at a scanning rate of $0.6°$/min with $0.01°$/s steps. FTIR measurements were carried out at room temperature by using a Nicolet apparatus (Thermo Scientific, Italy) and selecting a wavenumber resolution of $4 \, cm^{-1}$ for 64 scans from 4000 to $600 \, cm^{-1}$. The FTIR spectra were collected in absorbance mode on transparent pellets obtained by dispersing the sample in the form of powder in KBr (2 wt%). To estimate the content of the various secondary structures, the absorption of the amide I band in the range 1800–$1600 \, cm^{-1}$ was used.[19] The spectral region analyzed was deconvoluted with OriginPro 8.0 software by using the best fits by Lorentzian sum and specifying the positions of the peaks corresponding to the different conformations in secondary structures: *ordered* phases (*α-helix* and *β-sheet*) and *disordered* phase (*β-turns*).[21]

Results and discussion

The foamability of the TPZ-BNCs was studied by analyzing the effect of the different kind of ligninic additives (AL or LSS), in different amounts, on foam morphologies and densities. SEM micrographs of foams obtained from TPZ, TPZ-1AL and TPZ-1LSS systems, with a foaming temperature of 55°C are shown in Figure 1. As it is possible to observe, microcellular structures were obtained both from neat TPZ and TPZ-BNCs with low lignin content. Furthermore, we observed a higher number of cells only in the formulation containing 1 wt% of AL, with a slight reduction of foam density from 0.53 to 0.45 g/cm³ with respect to TPZ. Foams prepared from TPZ-1LSS showed, instead, a lower cell number and higher foam density (0.75 g/cm³) with respect to both TPZ and TPZ-1AL (Table 1). Furthermore, by analyzing the pore-size distribution reported in Figure 2, no significant difference can be observed in the pore size window, suggesting that the lignin, at low concentration, does not act as an efficient nucleating agent for TPZ.

For high lignin content (AL and LSS at 10 wt%) foaming did not occurred to a visible extent. These results can be related to an extensive modification of materials
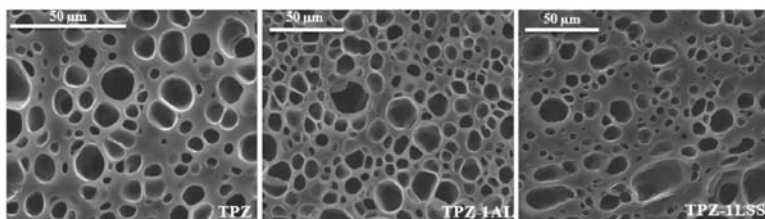
Figure 1. SEM micrographs of TPZ-BNCs foamed with N_2–CO_2 80–20 vol% at $P_{sat} = 170$ bar and $T_f = 55°C$.
SEM: scanning electron microscopy; TPZ: thermoplastic zein;
BNCs: bio-nanocomposites.
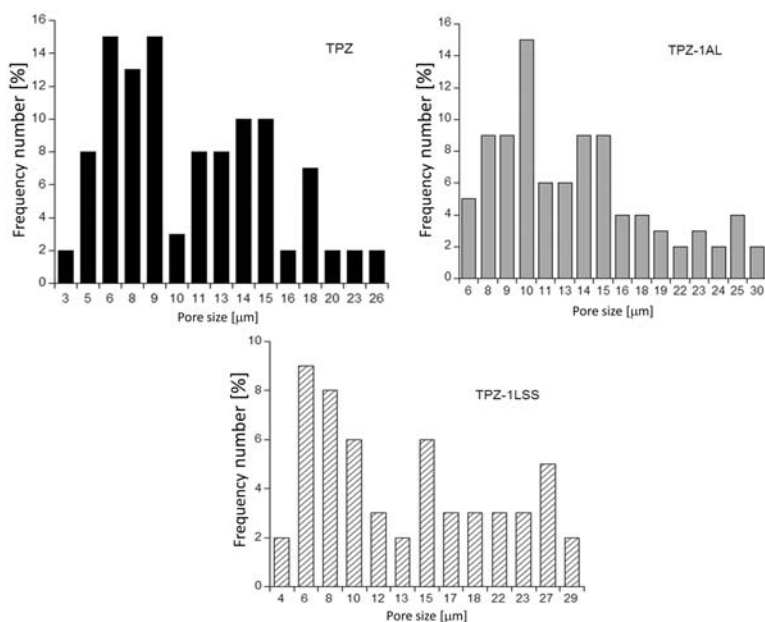

Figure 2. Pore-size distribution of TPZ, TPZ-1AL and TPZ-1LSS foams prepared at
$P_{sat} = 170$ bar and $T_f = 55°C$.
TPZ: thermoplastic zein; AL: alkali lignin; LSS: lignosulfonic acid–sodium salt.

properties as function of lignin type and content, including secondary structure and thermo-mechanical properties of zein, as it will be discussed in the following.

In order to explain the foaming results, the physico-chemical interactions between the lignins and zein protein molecules, and the eventual, partial, destructuration of the protein secondary structure were evaluated by using of XRD and FTIR characterizations.

The XRD patterns of TPZ and TPZ-BNCs, reported in Figure 3(a) and (b), highlighted two peaks, at $2\theta = 9.5°$ and $20°$, that correspond to two characteristic
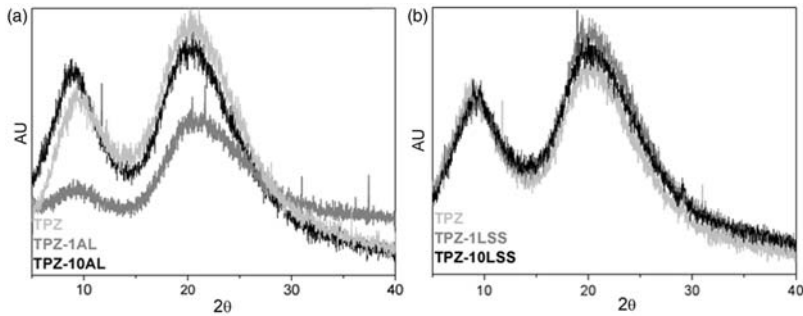
Figure 3. X-ray patterns of TPZ-BNCs: (a) TPZ-AL, (b) TPZ-LSS.
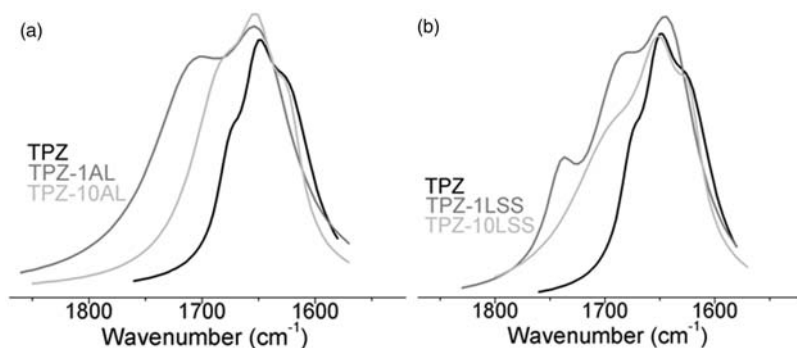TPZ: thermoplastic zein; BNCs: bio-composites; AL: alkali lignin; LSS: lignosulfonic acid–sodium salt.

d-spacings, around 4.6 Å (d_0) and 8.96 Å (d_i), attributed to the average backbone distance within α-*helix* structure of TPZ and the d-spacing of α-*inter-helix* packing, respectively.[22] TPZ-BNCs containing LSS (Figure 3(b)) show similar peaks as TPZ, while BNCs containing AL (Figure 3(a)) highlighted changes in both the peak area and the d-spacing. These results suggest that the AL is able to modify the hierarchical organization of TPZ. For instance, the area ratio of both peaks (A_i/A_o), (corresponding to the area of d_0 and d_i d-spacing obtained by Lorentian fit procedure as input data) for TPZ-1AL decreases with respect to TPZ. This decrease may be attributable to the disruption of α-*inter helix* packing (d_i). In particular, low AL percentages induced a new arrangement of *inter helix* packing of zein, with an insertion of the lignin macromolecule within the layers, and the formation of the 'novel' hydrogen bonding between the amino acids of zein (C=O, OH, RNH) and the functional groups (OH, SH) of lignin fragments. At higher lignin content (10 wt%), the A_i/A_0 values increased, suggesting the existence of a certain degree of phase separation between lignin-rich phase and zein-rich phase.

These aforementioned results were confirmed by FTIR characterization, where the effect induced by different content of lignin (AL and LSS) on zein secondary structure (β-*sheet*, α-*helix* and β-*turns*) was evaluated. The measured FT-IR spectra were deconvoluted in the region (800–1600 cm^{-1}) related to the 'Amide I' frequency range.[23,24] In this region, the assignment of the *ordered* phases (β-*sheet* and α-*helix*) and *disordered* phase (β-*turns*) stretching vibrations at 1623, 1651 and 1676 cm^{-1}, respectively, was done according to Rahmelow et al.[21] and Singh et al.[23]

Results showed that the presence of both AL and LSS induced shifting in frequencies of band corresponding to different secondary conformations (Figure 4(a) and (b)). In particular, for samples with low lignin concentration, it is possible to observe an overall shift towards lower vibration frequency for α-*helix*, the disappearance of absorption peaks for β-*sheet* conformations and a corresponding shift towards high frequency for β-*turns*. The shift to lower frequencies, or the disappearance of peak, could be attributed to the insertion of lignin component in the both secondary structure conformations and the corresponding

Figure 4. FTIR spectra of TPZ-BNCs: (a) TPZ-AL, (b) TPZ-LSS.
FTIR: Fourier transform infrared; TPZ: thermoplastic zein; BNCs: bio-composites; AL: alkali lignin; LSS: lignosulfonic acid–sodium salt.

formation hydrogen bondings between amino-acid-zein and functional groups of lignin.[24] As reported by Oliviero et al.,[24] the reduction of aminoacid–aminoacid interactions in the zein secondary structure, induced by insertion of small molecules, was already observed by several authors.[25,26]

Conversely, at high lignin content an increase of peak wavenumber for *ordered* conformations and a decrease of peak wavenumber for *disordered* conformations was observed, due to higher number of interaction between zein structure and lignin functional groups.

The addition of lignin in different amounts (both for AL and LSS) induced also significant changes in the thermo-mechanical properties of TPZ, and in particular of the storage modulus E' around the glass transition region, that we observed in the range 30–50°C. In particular, for both lignin systems at low concentration, a drop of the storage modulus of about 3 order of magnitudes in the transition region was measured (Table 1), analogous to amorphous polymers. As observed by FTIR analysis, this effect could be associated to the increasing of *disordered* phase in zein secondary structure as a consequence of modification/disruption of the *α-helix* structure and the *β*-conformations induced by the insertion of the lignin (see Figure 3 by Oliviero et al.[24]).

At higher lignin content, the drop of the storage modulus was, less than 2 order of magnitude, as reported in Table 1. These results, usually occurring in polymeric materials with high crosslinking density or high degree of crystallinity, can be attributed to the high number of interactions between functional groups of zein and lignin (see, for instance, the scheme in Figure 5).

In view of these results, it is possible to conclude that the presence of lignin extensively affects the foamability of the different BNCs. In particular, the high lignin content (both for AL and LSS) hinders expansion, in view of the strong interactions building up between the amino acids of zein and the functional groups of lignin fragments (as in crosslinked polymeric materials), as verified with FTIR and
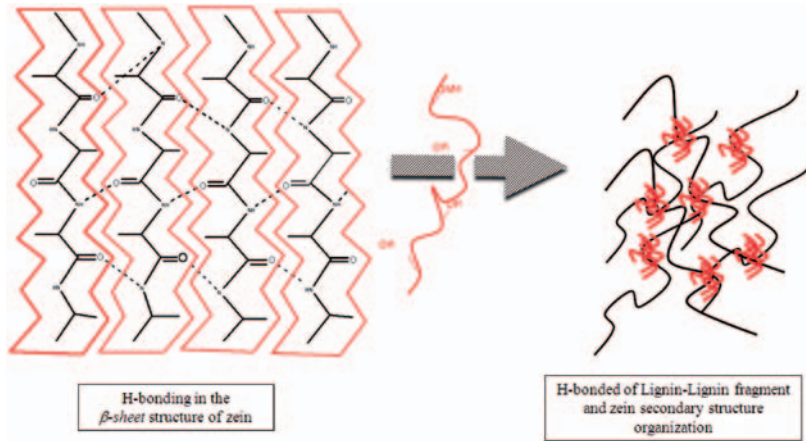
Figure 5. Proposed mechanism of interaction between lignin and zein.

thermo-mechanical analyses. Conversely, at low lignin content, the new arrangement of the protein structure allowed foaming of the corresponding BNCs, in particular in the case of AL, where a density reduction was also observed with respect to TPZ.

Conclusions

TPZ-based BNCs with two different ligninic systems, AL and LSS were developed through melt mixing and subsequently foamed with N_2–CO_2 mixtures in the temperature range 50-60°C by using the batch foaming technique. Results of FTIR and XRD proved the effect of lignin in modifying the protein secondary structure and, consequently, the thermo-mechanical properties. Microcellular foams with fine cellular structures, characterized by high number of cells, were obtained only in presence of low percentage (1 wt%) of AL, while at higher concentration of lignin (10 wt% both for AL and LSS) and at 1 wt% concentration of LSS, the interactions between zein and lignin, reduce the overall foamability of the TPZ-BNC.

Funding

References

1. Mohanty AK, Misra M and Drzal LT. Sustainable bio-composites from renewable resources: opportunities and challenges in the green materials world. *J Polym Environ* 2002; 10: 19–26.
2. Belton P, Taylor J, Duodu G, et al.. Out of Africa: Sorghum, the sustainable cereal. In: *Royal Society Summer Science Exhibition*, 2003.

3. Taylor JRN and Silva LS. Sorghum bran as a potential source of kafirin. *Cereal Chem* 2004; 81: 322–327.

4. Bourtoom T. Edible protein films: properties enhancement. *Inter Food Res J* 2009; 16: 1–9.

5. Lawton JW. Zein: a history of processing and use. *Cereal Chem* 2002; 79(1): 1–18.

6. Di Maio E, Mali R and Iannace S. Investigation of thermoplasticity of Zein and Kafirin proteins: mixing process and mechanical properties. *J Appl Polym Sci* 2010; 18: 626–633.

7. Oliviero M, Di Maio E and Iannace S. Effect of molecular structure on film blowing ability of thermoplastic zein. *J Appl Polym Sci* 2010; 115: 277–287.

8. Pommet M, Redl A, Guilbert J, et al. Intrinsic influence of various plasticizers on functional properties and reactivity of wheat gluten thermoplastic materials. *J Cereal Sci* 2005; 42: 81–91.

9. Doerthy WOS, Mousaviom P and Fellows CM. Value-adding to cellulosic ethanol: lignin polymers. *Ind Crops Prod* 2010; 33: 259–276.

10. Huang J, Zhang L and Chen F. Effects of lignin as a filler on properties of soy protein plastics. I. Lignosulfonate. *J Appl Polym Sci* 2003; 88: 3284–3290.

11. Huang J, Zhang L and Chen P. Effects of lignin as a filler on properties of soy protein plastics. II. Alkaline lignin. *J Appl Polym Sci* 2003; 88: 3291–3297.

12. Baumberger S, Lapierre C, Monties B, et al. Use of kraft lignin as filler for starch films. *Polym Degrad Stab* 1998; 99: 273–277.

13. Stevens ES, Willett J and Shogren RL. Thermoplastic starch-kraft lignin-glycerol blends. *J Biobased Mater Bioenergy* 2007; 1: 351–359.

14. Stevens ES, Klamczynski A and Glenn GM. Starch-lignin foams. *eXPRESS Poly Letters* 2010; 5: 311–320.

15. Glenn GM. Effect of starch, fiber and CaCO$_3$ on the properties of foams made by a baking process. *Ind Crops Prod* 2001; 14: 201–212.

16. Shogren RL. Starch modifications and additives improve process parameters, structure and properties. *Ind Crops Prod* 2002; 16: 69–79.

17. Willett JL and Shogren RL. Processing and properties of extruded starch/polymer foams. *Polym* 2002; 43: 5935–5947.

18. Salerno A, Oliviero M, Di Maio E, et al. Thermoplastic foams from zein and gelatin. *Int Polym Proc* 2007; 22: 480–488.

19. Marrazzo C, Di Maio E, Iannace S, et al. Foaming of synthetic and natural biodegradable polymers. *J Cel Plast* 2007; 43: 123–133.

20. Kumer N and Suh NP. A process for making microcellular thermoplastic parts. *Polym Eng Sci* 1990; 30: 1323–1329.

21. Rahmelow K and Hubner W. Fourier self-deconvolution: parameter determination and analytical band shapes. *Appl Spectrosc* 1996; 50: 795–804.

22. Wang Y, Filho FL, Geil P, et al. Effects of processing on the structure of zein/oleic acid films investigated by X-ray diffraction. *Macromol Biosci* 2005; 5: 1200–1208.

23. Singh N, Georget DMR, Belton PS, et al. Physical properties of zein films containing salicylic acid and acetyl salicylic acid. *J Cereal Sci* 2005; 2: 282–287.

24. Oliviero M, Verdolotti L, Aurilia M, et al. Effect of supramolecular structures on thermoplastic zein-lignin bionanocomposites. *J Agri Food Chem* 2011; 59(18): 10062–10070.

25. Athamneh AI, Griffin M, Whaley M, et al. Conformational changes and molecular mobility in plasticized proteins. *Biomacromolecules* 2008; 9: 3181–3187.

26. Gao C, Stading M, Wellner N, et al. The plasticization of protein-based film by glycerol: a spectroscopic, mechanical and thermal study. *J Agri Food Chem* 2006; 54: 4611–4616.