3D printing of maize protein by fused deposition modeling

Laurent Chaunier, Eric Leroy, Guy Della Valle, Michèle Dalgalarrondo, Benedicte Bakan, Didier Marion, Baptiste Madec, Denis Lourdin

3D printing of maize protein by fused deposition modeling

Laurent Chaunier, Eric Leroy, Guy Della Valle, Michèle Dalgalarrondo, Bénédicte Bakan, Didier Marion, Baptiste Madec, and Denis Lourdin

Citation: AIP Conference Proceedings **1914**, 190003 (2017); doi: 10.1063/1.5016792

View online: https://doi.org/10.1063/1.5016792

View Table of Contents: http://aip.scitation.org/toc/apc/1914/1

Published by the American Institute of Physics

3D Printing Of Maize Protein By Fused Deposition Modeling

Laurent Chaunier[1, 2*], Eric Leroy[2], Guy Della Valle[1], Michèle Dalgalarrondo[1], Bénédicte Bakan[1], Didier Marion[1], Baptiste Madec[3] and Denis Lourdin[1]

[1]*BIA INRA-UR-1268, La Géraudière. 44316 Nantes Cedex 3, France*
[2]*GEPEA UMR-CNRS-6144, CRTT, 37 av. de l'université. 44606 St-Nazaire Cedex, France*
[3]*Ecole Centrale, Plateforme RFI, 1 rue de la Noë. 44321 Nantes Cedex 3, France*

[*]*Corresponding author: laurent.chaunier@nantes.inra.fr*

Abstract. Additive Manufacturing (AM) opens new perspectives for biopolymers to obtain functional parts, like biomedical devices, by exploiting their biocompatibility and resorbability. Plasticized materials from zein, a storage protein from maize seed endosperm, display thermomechanical properties that could match with Fused Deposition Modeling (FDM). The objective of this work was to evaluate their thermo-rheological behavior and their structural modifications during processing. 20% glycerol was added to commercial zein, containing naturally about 4% lipids and 5% water. After storage at intermediate relative humidity (RH=59%), its glass transition temperature, measured by DSC, was T_g=42°C. The main mechanical relaxation, measured by DMA, was found at Tα=50°C, leading to a drop of the elastic modulus from E'=1.1GPa, at ambient temperature, to E'=0.6MPa at Tα+100°C. These values are in a similar range as those of standard polymers used for AM-FDM processing, such as PLA and ABS. The structure of zein was characterized at different scales by SDS-PAGE, reversed-phase HPLC, FTIR and WAXS, at each processing stage: (i) the initial formulation of the plasticized powdery material, (ii) after extrusion at 130°C for shaping printable filaments, and (iii) after deposition through the 3D printer nozzle ($Ø_{nozzle}$=0.5mm, $T_{printing}$=130°C, $v_{printing}$≈10mm/s). The presence of disulfide bond cross-links was evidenced in extruded filaments and remains at the same level after printing. WAXS showed that, in these conditions, no molecular orientation was obtained in the deposited material. By tuning zein-based printable compositions, these results open the field of their processing as resorbable printed parts, with a controlled geometry and a designed tridimensional structure.

Keywords: 3D Printing, Additive Manufacturing, Biopolymer, Corn, FDM, Protein, Zein.

PACS: 81, 62, 65.

INTRODUCTION

Additive Manufacturing (AM) is a family of processes allowing the layer by layer production of functional finished plastic parts, from digital three-dimensional models [1, 2]. This allows now the production of functional finished parts. Applications can be found in various fields as for the production of prostheses or matrices for cells growth in the biomedical domain, complex mechanical parts for industry, or even edible printed foods [3-5]. The main advantage of AM is the fast transition from design to obtaining parts from a digital model providing desired properties [6]. For polymers, one of the common techniques is the Fused Deposition Modeling (FDM). It is based on the deposition layer-by-layer at high temperature of a molten thermoplastic polymer through a nozzle, on a solid substrate. Initially, the thermoplastic material is shaped as a filament, obtained by a previous extrusion step and which allows the feeding of the 3D printer. The deposited material must solidify quickly, by cooling below its glass transition temperature (T_g). The upper layers of the deposited material have to adhere to the previously deposed layer so as to obtain a finished solid part. In the case of FDM applied to the pharmaceutical field, the manufacture of materials with specific porosity (*e.g.* variable deposition patterns for filling) could allow the production of innovative 3D printed tablets, for the controlled release of active constituents. As an example, in a printed part made from PVA, 100% fluorescein, taken as a model molecule, can be released under simulated physiological conditions in less than 6 hours with 10% printed density, while more than 10 hours are required for 50% or more [7].

Now, progresses should target the development of new 3D printable materials with adequate behavior for FDM like rheological properties, adhesion ability and mechanical properties [8]. Biopolymers are good candidates to obtain 3D printed parts with novel properties, such as biodegradability, or biocompatibility. Then the present work focuses on a plant protein : zein, a maize storage protein which represents about 5% of the grain dry weight and

Proceedings of PPS-32
AIP Conf. Proc. 1914, 190003-1–190003-5; https://doi.org/10.1063/1.5016792
Published by AIP Publishing. 978-0-7354-1606-2/$30.00

being a by-product of starch and bioethanol production. It is part of the prolamins family [9]. It is used as a water barrier in food or pharmaceutic industries and for obtaining degradable plastics [10, 11].

Zeins are low charge proteins which amino acids sequences display both hydrophobic domains and hydrophilic ones (including glutamine). Accordingly, they constitute amphiphilic polymers that can be thermoplasticized after blending with polar or apolar plasticizers. As an example, in a previous study for a content of 22% glycerol (w/w), it presents a main mechanical relaxation linked to its glass transition temperature at about T_{α}=60°C, and a storage modulus (E') about 1GPa at ambient conditions [12, 13]. Plasticized zein, added with paracetamol as active ingredient, has been recently considered as extruded-molded caplets for controlling drug release [14]. Thus, this protein is a promising compound for processing as a thermoplastic biopolymer, in the case of Fused Deposition Modeling, to obtain tridimensional designed parts presenting a defined porosity. It could be used for the controlled release of bioactive molecules. The present work proposes to investigate 3D printing abilities of plasticized zein and understand structure-properties relationships possibly leading to 3D printed parts with targeted functional properties.

MATERIALS AND METHODS

Raw Materials and Thermomechanical Processing

Zein from maize (Ref.Z3625) and glycerol were purchased from Sigma-Aldrich (F-38, Saint-Quentin Fallavier). The moisture content was determined by thermogravimetry after 2hr at 130°C (TA Instruments, F-78 Guyancourt).

Zein-based plasticized compositions were obtained after blending with glycerol. They were extruded using a SCAMIA single-screw device (SCAMEX, F-91 Crosne), with a 200mm barrel (screw L/D=10) and a cylindrical die ($Ø_{die}$=1.7mm). The screw speed was 20 rpm and temperature was T_{die}=130°C (Fig. 1-a). In these conditions, the average flow rate is Q≈2g/min and the specific mechanical energy about SME≈300J.g^{-1}.

A 3D Printer with an operating volume close to 20^{3}cm^3 (ORDBot-Hadron, $Ø_{die}$=0.5mm) and standard synthetic polymers as feedstock filaments for 3D printing by FDM (*i.e.* Acrylonitrile Butadiene Styrene, ABS, and Polylactic Acid, PLA) were purchased from eMotion Tech (F-31 Toulouse), Figure 1-b. Printing trials with thermoplasticized zein were carried out at T_{nozzle}=130°C. A "ring" geometry model (diameter ∅=1cm) was used.

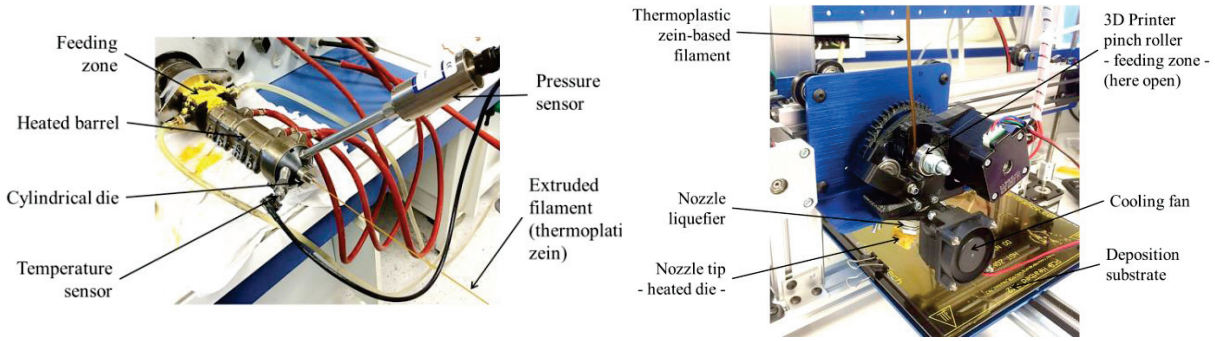


FIGURE 1. Extrusion of thermoplasticized zein as filament, at T_{die}=130°C (a). 3D Printer-FDM, ORDBot Hadron (20x20cm^2 solid deposition substrate, T_{nozzle}=130°C) : feeding zone with zein-based rigid filament with 20% glycerol content (Z20GLY) (b)

Glass Transition and Thermomechanical Properties of Zein-based materials

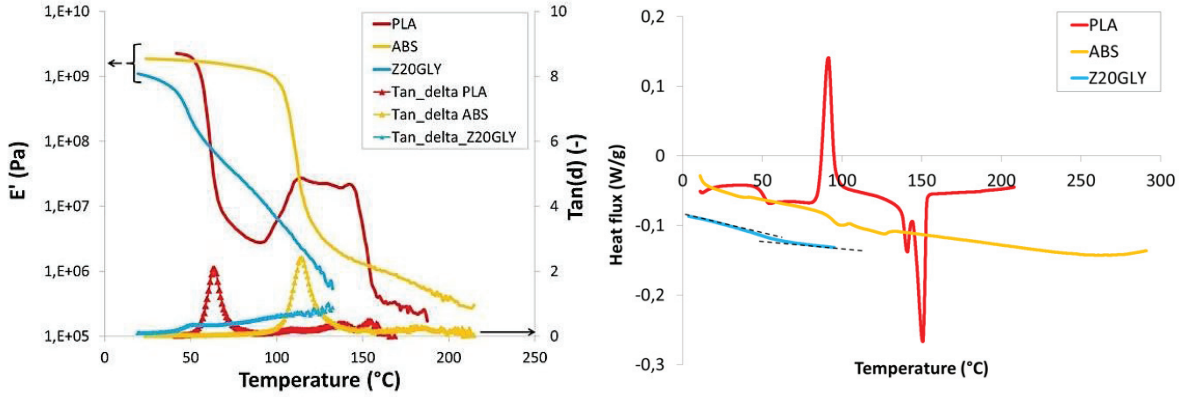
To determine the glass transition temperature, thermograms were recorded with an automated Differential Scanning Calorimeter DSC (Q100, TA Instruments, F-78 Guyancourt). Each specimen (10 mg) was placed into a sealed stainless-steel cell. Measurements were performed at 3 °C/min from 10 to 140 °C during a second scan, to cancel thermal events due to aging during storage. Extruded cylindrical samples (Ø≈1.75mm), cut in 20mm long specimen, were submitted to Dynamic Mechanical Analysis in tensile mode (DMA-50N-O1dB Model, Metravib, F-59 Lyon). The mechanical active length between the grips was 10 mm and extrudates were characterized at 1 Hz by DMA, with a strain set at 0.1% and heating rate of 3 °C/min, up to 160°C.

Structural Characterization of Zein during Processing

The structure of the plasticized zein was evaluated at the different steps of the FDM processing, from the initial powdery composition, the extruded and the 3D-printed filament out of the nozzle of the 3D-printer. Each specimen was diluted to obtain a 2mg/ml solution in a Tris/HCl 50mM containing Sodium Dodecyl Sulfate 2% (SDS) buffer at pH=6.8 and submitted to Electrophoresis on a 12% Polyacrylamide Gels (PAGE). Samples were characterized by SDS-PAGE without and with 3% β-mercaptoethanol as reducing agent. After staining, migration bands were seen, and molecular weight was calculated by comparison with standard molecular weight markers (Euromedex, F-67 Souffelweyersheim) submitted to SDS-PAGE in the same conditions. The molecular orientation was assessed by direct beam X-ray scattering (Cu-$K_{\alpha 1}$=1.5405Å) on a Bruker D8 diffractometer (Madison, WI, US), after integration of the azimuthal angle (Φ from 0 to 360°) on diffractograms obtained for 2ϑ varying from 3° to 30°. FTIR was carried out on molded 120mg KBr pills, with 2mg grinded zeins at their different processing steps, on a Nicolet iS50 (Thermo Fisher Scientific, Madison, WI, US) for the acquisition of spectra in the [400; 4000cm^{-1}] range.

RESULTS AND DISCUSSIONS

Commercial zein presents a moisture content at MC=5% (total wet basis) and T_g=88°C. When plasticized with 20% glycerol, T_g decreases to 42°C which leads to decrease the storage modulus from E'=1.1 GPa at 20°C, to less than 0.6 MPa at 130°C, above the principal mechanical relaxation linked to its glass transition temperature (Fig. 2-a). Such results are in the same range as those previously published in the literature [12]. Compositions containing 10% glycerol added, or without plasticizer, do not present such an important decrease of storage modulus, limiting their flowing abilities in the molten state above T_g, and then avoiding their application in 3D printing by FDM. In the case of glycerol content above 30%, lower mechanical properties are obtained at ambient conditions than with 20% (E'< 1GPa), but similar values at high temperature. Thus, extrudates based on zein with 20% glycerol, namely "Z20GLY", could be the most pertinent to carry out trials of 3D printing by FDM. Furthermore, despite different structures and relaxation mechanisms, the loss of the mechanical strength at high temperature allows to evaluate the printing temperature of Z20GLY at $T_{printing}$>120°C, whereas $T_{printing}$>180°C for PLA and $T_{printing}$>220°C for ABS.

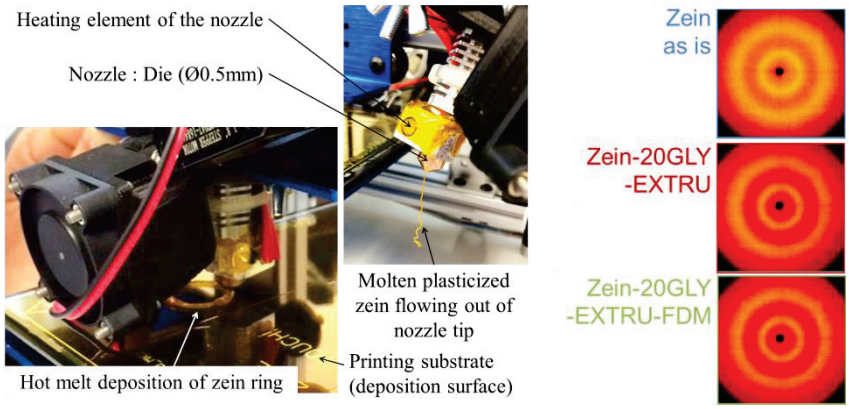


(a) (b)

FIGURE 2. Thermomechanical behavior of the plasticized zein with 20% glycerol added (Z20GLY), PLA and ABS filaments : evolution of the elastic modulus and the damping factor -tanδ- (a); characterization by DSC (b)

In these conditions, each polymer is beyond its relaxation temperature, *i.e.* above T_g for amorphous Z20GLY (at the half-height in heat flux change at T_g=42°C, Fig. 2-b) and ABS (T_g=100-120°C), or above its melting temperature as for PLA (endotherm at $T_{melting}$=140-160°C, above the exothermic crystallization at about 90°C and T_g at 55°C). Even if the mechanical strength is lower than the one of standard polymers, like PLA and ABS which present moduli above 2GPa at 20°C, it is large enough to perform feasibility trials with plasticized zein on a FDM 3D printer at 130°C. Rings of diameter ∅=1cm with 2-3 layers of deposited zein were successfully superimposed (Fig. 3-a). Samples of extruded thermoplastic filaments were also collected at the outlet of the nozzle for molecular characterization.

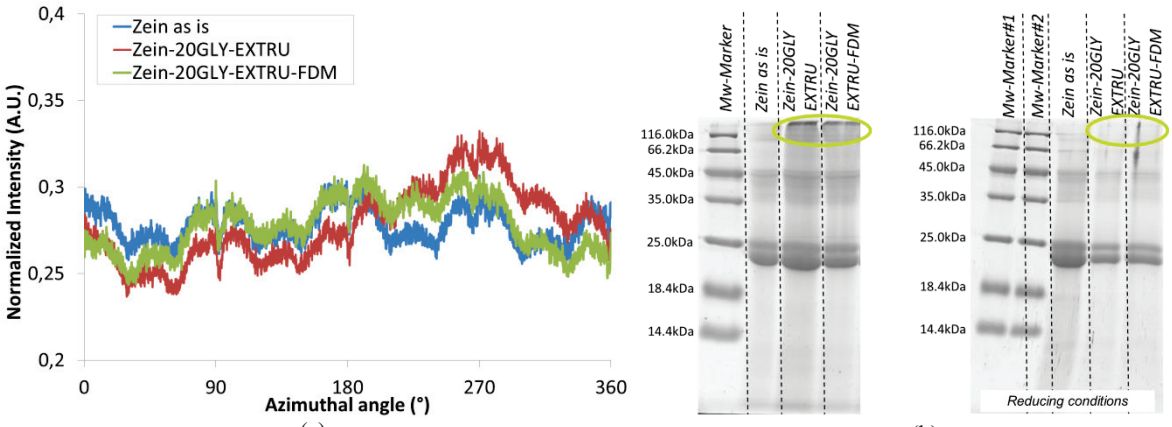
By direct beam X-ray scattering (WAXS), similar patterns were obtained, whatever the step considered: from the pulverulent initial material, then after being plasticized and extruded with 20% glycerol added as polar plasticizer and finally after flowing through the nozzle of the FDM printer (Fig. 3-b). No-significant differences were found in IR absorbances, at main amide bands wavenumber ranges from 1200 to 1700cm^{-1}. Furthermore, the structure of zein remains amorphous and once applying the Bragg's law to diffractogramms, the maximum values can be assigned to typical distances of α-helixes (4.5Å) and their packing (9.5Å) as depicted for thermoplastic zein [15].



(a) (b)

FIGURE 3. 3D Printing trials of zein-based composition with 20% glycerol added: molten material flowing through the die at 130°C and its deposition (a). Direct X-ray beam scattering (WAXS) patterns on 2D detector at different steps: powder raw material, after adding 20% glycerol and extrusion "Zein-20GLY EXTRU" and after FDM "Zein-20GLY EXTRU-FDM" (b)

No molecular orientation was obtained for the plasticized zein in these printing conditions ($Ø_{nozzle}$=0.5mm, $T_{printing}$=130°C, $v_{printing}$≈10mm/s), as shown by flat profiles assessed whatever the azimuthal angle and similar to the one of the initial zein powder (Fig. 4-a). Thus, a non-oriented amorphous material, corresponding to domains of packed repeated α-helixes is maintained in the thermoplasticized zein at the different steps of its thermomechanical processing, until the flowing at the outlet of the nozzle of the printer in the molten state.



(a) (b)

FIGURE 4. Assessment of materials orientation at the different processing steps by direct X-ray beam scattering (WAXS) for varying azimuthal angles (a). SDS-PAGE characterization of zein at the different steps of 3D printing (normal conditions on left hand and with reducing agent on right hand; molecular weight markers as "Mw-Marker") (b)

Finally, like the raw material before extrusion, zein is mainly composed of α-zein (with an average molecular weight being from 20 to 25kDa, Fig.4-b). High molecular weight molecules (>120kDa) are created by crosslinkings during thermomechanical treatment (SME≈300J/g, 130°C). The addition of β-mercaptoethanol leads to cancel such crosslinkings by a chemical reduction reaction (Fig.4-b, on right hand). Indeed, the crosslinked structure of the material remains in the 3D printed part.

CONCLUSIONS AND MAIN PROSPECTS

Our present data demonstrate that plasticized zein can be processed by 3D printing with Fused Deposition Modeling. Indeed, the thermomechanical properties of this plasticized biopolymer, as well as its hot melt flowing, match with process specifications. No molecular orientation was checked in the deposited filament at the outlet of the nozzle of the 3D printer and the amorphous structure of zein was shown as crosslinked after extrusion and remaining in the 3D printed part.

Such results will be used to study more accurately the rheological behavior of zein-based compositions in the molten state and their hot welding properties. Furthermore, the possible interactions of zein with different plasticizing compounds (*e.g.* polar, apolar, or ionic liquids) could lead to enhance their 3D printing abilities and to open the field of novel applications in the biomedical or pharmaceutical domains, for instance.

ACKNOWLEDGMENTS

The authors would like to thank Roselyne Désirest, André Lelion, Patrice Papineau and Bruno Pontoire (INRA, Nantes) for their helpful contributions to samples extrusion and characterization.

REFERENCES

1. ASTM. F2792-12a, Standard Terminology for Additive Manufacturing Technologies. *ASTM International,* West Conshohocken, PA (US), 2012.
2. D.T. Pham and R.S. Gault. *International Journal of Machine Tools & Manufacture* **38(10-11)**, 1257-1287 (1998).
3. A. Pfister, R. Landers, A. Laib, U. Hubner, R. Schmelzeisen and R. Mulhaupt. *Journal of Polymer Science Part a-Polymer Chemistry* **42(3)**, 624-638 (2004).
4. J.T. Belter and A.M. Dollar. *Plos One* **10(4)** (2015).
5. T.F. Wegrzyn, M. Golding and R.H. Archer. *Trends in Food Science & Technology* **27(2)**, 66-72 (2012).
6. A.W.L. Yao. *International Journal of Advanced Manufacturing Technology* **26(11-12)**, 1284-1288 (2005).
7. A. Goyanes, A.B.M. Buanz, A.W. Basit and S. Gaisford. *International Journal of Pharmaceutics* **476(1-2)**, 88-92 (2014).
8. B.N. Turner, R. Strong, and S. Gold. A. *Rapid Prototyping Journal* **20(3)**, 192-204 (2014).
9. T.J. Anderson and B.P. Lamsal. *Cereal Chemistry* **88(2)**, 159-173 (2011).
10. J.W. Lawton. *Cereal Chemistry* **79(1)**, 1-18 (2002).
11. E. Corradini, P.S. Curti, A.B. Meniqueti, A.F. Martins, A.F. Rubira and E.C. Muniz. *International Journal of Molecular Sciences* **15(12)**, 22438-22470 (2014).
12. E. Corradini, E.S. de Medeiros, A.J.F. Carvalho, A.A.S. Curvelo and L.H.C. Mattoso. *Journal of Applied Polymer Science* **101(6)**, 4133-4139 (2006).
13. G.W. Selling and K..D. Utt. *Polymer Degradation and Stability* **98(1)**, 184-189 (2013).
14. J. Bouman, P. Belton, P. Venema, E. van der Linden, R. de Vries and S. Qi. *Pharmaceutical Research* **32(8)**, 2775-2786 (2015).
15. M. Oliviero, E. Di Maio and S. Iannace. *Journal of Applied Polymer Science,* **115(1),** 277-287 (2010).