CHEMICAL ENGINEERING TRANSACTIONS
VOL. 100, 2023
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Sauro Pierucci, Flavio Manenti

ISBN 979-12-81206-03-8; **ISSN** 2283-9216
DOI: 10.3303/CET23100049

Effect of 3D-printing Conditions of Polylactic Acid Filaments Impregnated by Supercritical Fluids with a Natural Extract

Lidia Verano-Naranjo*, Abraham Galindo-Priego, Cristina Cejudo-Bastante, Lourdes Casas, Casimiro Mantell, Enrique Martínez de la Ossa

Chemical Engineering and Food Technology Department, Wine and Agrifood Research Institute (IVAGRO), University of Cadiz, Avda. República Saharaui, s/n, 11510, Puerto Real, Cádiz, SPAIN
lidia.verano@uca.es

Three-dimensional printing is a type of additive manufacturing of great interest in biomedical engineering. The production in situ of customized structures, tissues, implants, or other devices represents a great advance in this field. In addition, the use of polymers functionalized with bioactive substances that improve treatment represents even greater progress. One of these substances could be olive leaf extract (OLE), which has a wide variety of antioxidant compounds of interest for the treatment of heart, neurodegenerative, or oncological diseases.
This work analyzes the use of supercritical impregnation of polylactic acid (PLA) filaments with an OLE for later use in a 3D printer. The effect of printing temperature (200, 210, and 220 ºC) and printing speed (40, 50, and 60 mm/s) was studied, using the antioxidant activity of the generated devices as a response variable. The results showed that both parameters were statistically significant and negative, an increase in these factors led to a decrease in antioxidant activity.

1. Introduction

Three-dimensional (3D) printing is an additive manufacturing technique used to manufacture a wide range of structures with complex geometries. Since its invention in the 1980s, different 3D printing methods have been developed to meet the demand for specific high-resolution products (Ngo et al., 2018). Among them, fused deposition modeling (FDM) uses thermoplastic polymeric filaments that are heated as they pass through a die and are deposited by extrusion layer by layer on a platform. FDM is one of the most widely used additive manufacturing processes today with the commonly used polymers polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS). The main advantage of the use of PLA over ABS, from a biomedical point of view, lies in its biodegradability and biocompatibility, it is non-petrochemical origin, it is non-toxic, and it needs low operating temperature (which reduces manufacturing costs). That is why FDM with the use of PLA as a polymer filament is the main technology used in biomedical engineering. The biomedical sector accounts for 11% of the additive manufacturing market share (Rajan et al., 2022).
Some examples of the application of FDM in biomedicine are the generation of biomedical implants with different purposes such as tissue regeneration (Liu et al., 2016; Szojka et al., 2017) or the manufacture of devices for the administration and release of drugs (Khosraviboroujeni et al., 2022; Cui et al., 2022). In general, for these applications, there are numerous studies regarding the functionalization of the final devices but the pre-functionalization of printing filaments is becoming a focus of investigation (Viidik et al, 2021).
Medicalized polymeric filaments are generally obtained by mixing the drugs or active substances with the polymeric pellets before their processing by hot melt extrusion (Auriemma et al., 2022). The drug load depends, in addition to the physicochemical properties of the active substance and polymers, on parameters such as the extrusion temperature, the way of mixing, which in turn depends on the type and shape of the extruder, the mixing speed or the feeding rate (Viidik et al., 2021). In the present work, the addition of the active substance was carried out by supercritical solvent impregnation (SSI) over the commercial filament. This technique uses the capacity of CO_2 in supercritical conditions (up to 31 ºC and 73 bar) to penetrate polymeric matrices, driving

Paper Received: 18 January 2023; Revised: 28 March 2023; Accepted: 7 June 2023
Please cite this article as: Verano-Naranjo L., Galindo-Priego A., Cejudo-Bastante C., Casas L., Mantell C., Martinez De La Ossa E., 2023, Effect of 3D-printing Conditions of Polylactic Acid Filaments Impregnated by Supercritical Fluids with a Natural Extract, Chemical Engineering Transactions, 100, 289-294 DOI:10.3303/CET23100049

the active substances solubilized in it toward the interior of the polymer. By bringing the system to atmospheric conditions, CO_2 in a gaseous state can escape from the interior of the polymer, while the active substances remain trapped (Cejudo et al., 2021). SSI has previously been successfully applied for the impregnation of mango leaf extract into PLA filaments before 3D printing (Rosales et al, 2021).

In this work, the chosen active substance was olive leaf extract (OLE). Olive leaves are considered a potential source of bioactive compounds that can be used in biomedical applications, mainly due to their high content of phenolic compounds with high antioxidant capacity. In previous works, OLE has been characterized, finding oleuropein, luteolin-3-glucoside, and hydroxytyrosol as the main compounds (Machado et al., 2022). These polyphenols can slow down the progression of cardiovascular, neurodegenerative, and cancer diseases due to their ability to mediate oxidative reactions and can modulate the immune response by affecting leukocyte proliferation and cytokine production (Roleira et al., 2015; Gorzynik-Debicka et al., 2018). In addition, extracts rich in oleuropein have demonstrated their antimicrobial capacity against bacteria, viruses, and fungi (Omar, 2010). Due to all these properties, the functionalization of biomedical polymers with olive leaf extract could be a good option for the creation of release devices that minimize infectious, inflammatory, or rejection risks.

The main objective of this work was to study the effect that 3D printing had on filaments that were previously loaded with an extract of olive leaves using supercritical solvent impregnation. For this, the antioxidant capacity of the devices generated in a conventional desktop 3D printer was studied. The effect of printing speed and temperature was studied. Most of the investigations on the parameters that affect FDM have focused on the improvement of the final surface, the dimensional accuracy, and the mechanical properties of the printed devices (Mohamed, et al., 2015; Rajan, et al., 2022). The approach presented in the current work implies an innovative effect by using the bioactivity of the printed devices as a response variable for the evaluation of the 3D printing parameters.

2. Materials and methods

2.1 Olive leaf extract obtaining and characterization

The raw material to obtain the extract was *Olea europaea* leaves provided by Olivarera San José Lora de Estepa, S. Coop. And. (Seville, Spain). The leaves were dried at atmospheric conditions and then crushed previous their use.

The enhanced solvent extraction (ESE) was carried out in high-pressure extraction equipment provided by Thar Technologies Inc. (Pittsburgh, PA, USA) model SF1000 mainly equipped with an electrical thermal jacketed extraction vessel 1 L capacity, a high-pressure pump for CO_2 and a back pressure regulator to control the system pressure. A diagram of the system was provided in previous work (Verano-Naranjo et al., 2021).

Approximately 200 g of olive leaves was introduced into the extractor vessel with 500 mL of ethanol. The system was then heated to 80 ºC and CO_2 was pumped until reaching a pressure of 120 bar. These conditions were maintained for two hours, subsequently proceeding to cool and depressurize the system and obtain the olive leaf extract (OLE). This method and conditions were used by other authors (Machado et al, 2022; Chinnarasu et al., 2016) to obtain an OLE with high antioxidant power.

The OLE was characterized based on its concentration and its antioxidant capacity. The last one was determined according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical reduction method. For this, 0.1 mL of the extract at different concentrations was mixed with 3.9 mL of $6 \cdot 10^{-5}$ M DPPH ethanolic solution and left it to react for 3 hours. Then the absorbance at 515 nm of the resultant solution was measured in a Cary 60 UV-Vis spectrophotometer by Agilent Technologies (Santa Clara, CA, USA). The same reaction was carried out with ethanol instead the extract as a control. The inhibition of oxidation was calculated through Equation 1.

$$\%\ oxidation\ inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ solution\ test}{Absorbance\ of\ control} \cdot 100 \quad (1)$$

Thus, it was possible to plot a curve of antioxidant capacity of the extract against its concentration, being estimated the IC_{50} value as the extract concentration at which was inhibited fifty percent of oxidation. The antioxidant activity index (AAI) was determined according to Equation 2, where $[DPPH]_r$ is the concentration of the radical in the reaction medium, this was 23.1 mg/L.

$$AAI = \frac{[DPPH]_r}{EC_{50}} \quad (2)$$

2.2 Supercritical impregnation of 3D-printing polymer filament

The polymeric filament that was functionalized was polylactic acid (PLA) and it was provided by Mundo reader S.L. (Madrid, Spain). Its nominal diameter was 1.75 mm, although experimentally it was found that this was

somewhat smaller, approximately 1.65 mm. The melting temperature of the material indicated by the manufacturer is 145-160 ºC, although a printing temperature higher than 200 ºC is recommended, ideally 220 ºC. The same equipment was used for the impregnation as for the extraction. In this case, 30 mL of OLE was introduced at the bottom of the vessel together with a piece of the filament of approximately 2 meters wound on a metallic support. The system was heated to 35 ºC and 10 g/min of CO_2 was pumped up to 90 bar. These pressure and temperature conditions were maintained for 2 hours and then the system was depressurized and the impregnated filament was obtained.

During the impregnation process, supercritical CO_2 together with the active compounds penetrates the polymeric matrix, where a reorganization of the polymeric chains occurs. Then, during the depressurization process, the CO_2 returns to its gaseous state, escaping from the polymeric matrix and producing pores, cavities, and channels. These two phenomena can cause the polymer to undergo permanent swelling. Therefore, it was necessary to evaluate if the impregnation process produced any deformation of the filament that prevented its subsequent use in the 3D printer. For this, the diameter of the filament was measured before and after the impregnation process, obtaining the percentage of swelling according to Equation 3.

$$\%\ Swelling = \frac{final\ diameter - initial\ diameter}{initial\ diameter} \cdot 100 \quad (3)$$

2.3 Printing process

The printing process with the impregnated filaments was carried out in an Anycubic 3D-printer model Mega S from Shenzhen Anycubic Technology Co., Ltd, (Guangdong, China). The OLE-impregnated filament was charged and discs of 10 mm diameter and 1.75 mm thin were printed modifying the temperature and speed of printing according to the 3^2 experiment design shown in Table 1. Bed temperature (60 ºC) and other printing parameters were maintained constant.

Table 1: Printing conditions

Parameter	Low	Medium	High
Nozzle temperature (ºC)	200	210	220
Print speed (mm/s)	40	50	60

2.4 Antioxidant activity of the printed devices

To evaluate the antioxidant capacity of the printed discs, they were introduced into a mixture of chloroform and ethanol, which allowed the dissolution of the polymer and the release of the OLE contained in it. The solvents were then evaporated and 0.1 mL of ethanol and 3.9 mL of $6 \cdot 10^{-5}$ M DPPH ethanolic solution were added to the dry sample, evaluating the variations in the absorbance and the antioxidant capacity in the same way as for the extract. Using the calibration curve of the antioxidant capacity of the extract against its concentration, it was possible to express this bioactivity of the devices as the mass of antioxidant compounds (AOC) per mass of the polymer. All determinations were conducted in triplicate.

3. Results and discussion

3.1 OLE characterization

It was obtained an extract with a concentration of 75 g/L (dry extract per wet extract) and a global extraction yield (mass of dry extract per mass of dry olive leaves) of 12.4 %. The antioxidant capacity of the extract was assessed with an IC_{50} of 35.70 mg/L and an AAI of 0.65. These results are comparable with those obtained by Chinnarasu and coworkers (Chinnarasu et al., 2016), who make a similar extraction of olive leaves with CO_2 and 50% of ethanol at 100 bar and 55ºC during 24 hours, and obtain a global extraction yield of 13.4% and an AAI of 0.65.

3.2 Swelling of the polymer after the impregnation

After the impregnation process, it was estimated a small swelling of the filament of 1.81 ± 0.11 %, which allowed the filament to continue to be used in the 3D printer. This result is in concordance with a previous work (Verano-Naranjo et al, 2021) that studied the swelling of PLA in its supercritical impregnation with ketoprofen at different conditions of temperature and pressures; the swelling percentage did not exceed 2% for the conditions of the present work.

3.3 Antioxidant activity of the printed devices

A priori, the effect of printing temperature should be a determining factor in the antioxidant activity of the printed devices, since high temperatures could degrade the major polyphenolic compounds in OLE that are responsible for this activity, as several authors have pointed out (Stamatopoulos et al., 2014; Attya et al., 2010). So, it was decided to study the effect on the reduction of the printing temperature from that indicated as ideal by the manufacturer (220 ºC), always within the optimal range (above 200 ºC). Regarding the printing speed, as a starting hypothesis it was proposed that at a higher printing speed, the polyphenolic compounds would spend less time in the printer nozzle at high temperatures, so their degradation would be less, and the antioxidant capacity would be greater. Thus, it was decided to study the effect of increasing the printing speed, from the optimum (40 mm/s) to the one in which the print quality is seriously compromised (60 mm/s) for the 3D printer used.

To assess whether the different printing conditions studied affected the antioxidant activity of the printed material, the printed samples were subjected to a dissolution and extraction process, the inhibition of oxidation against DPPH was studied, and the ratio of antioxidant compounds is shown in Figure 1. The statistical study of the effect of these parameters on antioxidant capacity is shown in the ANOVA table (Table 2) and the Pareto diagram in Figure 2.

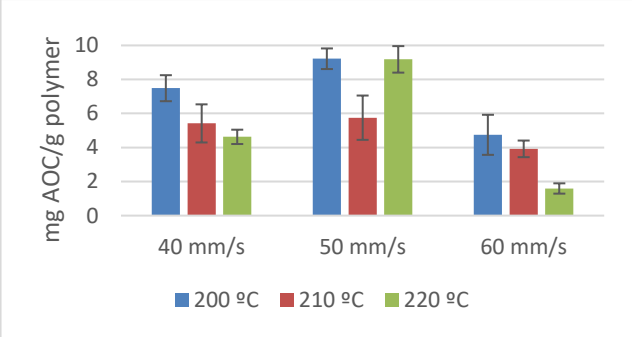

Figure 1: Antioxidant activity of printed devices at different conditions of printing temperature (200, 210, and 220 ºC) and printing speed (40, 50, and 60 mm/s).

Table 2: ANOVA table for effects of printing temperature (A) and printing speed (B) against the antioxidant capacity of printed devices

Effect	Sum sq	Df	Mean Sq	F-value	p-value
A (printing temperature)	26.4186	1	26.4186	13,90	**0,0012**
B (printing speed)	18.3063	1	18.3063	9,63	**0,0054**
AA	70.068	1	70.068	36,88	**0,0000**
AB	0.0651258	1	0.0651258	0,03	0,8549
BB	7.40736	1	7.40736	3,90	0,0616
Error	39.9028	21	1.90013		
Total	162.168	26			

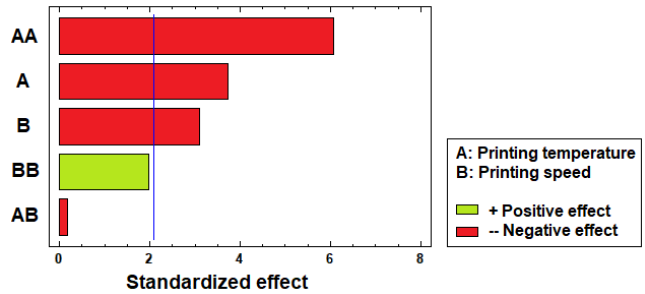

Figure 2: Pareto chart for effects of printing temperature (A) and printing speed (B) against the antioxidant capacity of printed devices.

As can be noted in Figure 1, an increase in temperature generally supposed a decrease in antioxidant activity, what is in concordance with the previous hypothesis. In this line, studies on the degradability of polyphenols compounds, although they are usually carried out up to temperatures around 100 ºC, found a decrease in the amount of compounds such as oleuropein as temperature increases (Volf, 2014). In the Pareto diagram and the ANOVA table, it can be seen how the effects of temperature (A) and squared temperature (AA) are negative (as the temperature increases, the antioxidant capacity decreases) and significant (p-value < 0.05).
Regarding the effect of printing speed, the Pareto chart shows a significant and negative effect of printing speed (B) a decrease in antioxidant capacity was noticed as printing speed increased. However, a positive effect of this squared factor (BB) suggests that the effect of this variable on antioxidant capacity was not clear, at least for the values studied.

4. Conclusions

In this project, the use of impregnation techniques with supercritical fluids has been analyzed to obtain filaments preloaded with natural bioactive substances for use in biomedical engineering. Devices printed with these filaments have been found to maintain antioxidant capacity, which is directly related to printing conditions. An increase in printing temperature led to a decrease in the antioxidant activity of the printed devices. However, the effect of printing speed had less influence on the bioactivity of the printed devices. Nevertheless, a deeper study of these printing variables with wider ranges and through the analysis of other bioactive properties of biomedical interest is necessary, as well as the possible application of other polymers and pharmacoactive substances for similar processes.

Acknowledgments

This publication is part of the R+D+i project reference PID2020-116229RB-I00 funded by MCIN/AEI /10.13039/501100011033/ and “ERDF A way of making Europe” and R+D+i project reference TED2021-131822B-I00 funded by MCIN/AEI/10.13039/501100011033/FEDER, UE.
Thanks to Olivarera San José de Lora de Estepa, S. Coop. And. (Seville, Spain) for providing the olive leaves.

References

Attya, M.; Benabdelkamel, H.; Perri, E.; Russo, A.; Sindona, G. 2010. Effects of Conventional Heating on the Stability of Major Olive Oil Phenolic Compounds by Tandem Mass Spectrometry and Isotope Dilution Assay. Molecules, 15, 8734-8746.

Auriemma, G.; Tommasino, C.; Falcone, G.; Esposito, T.; Sardo, C.; Aquino, R.P. 2022. Additive Manufacturing Strategies for Personalized Drug Delivery Systems and Medical Devices: Fused Filament Fabrication and Semi Solid Extrusion. Molecules, 27, 2784

Cejudo Bastante, C., Casas Cardoso, L., Fernández Ponce, M. T., Mantell Serrano, C., Martínez de la Ossa-Fernández, E. J. 2018. Characterization of olive leaf extract polyphenols loaded by supercritical solvent impregnation into PET/PP food packaging films. The Journal of Supercritical Fluids, 140, 196–206.

Cejudo, C.; Gallardo, M.; Fernández-Ponce, M.T.; Casas, L.; Mantell, C.; Martínez de la Ossa, E.J. 2021. Preservation of food with high oil content by supercritical impregnation techniques. Chemical Engineering Transactions, 87, 541-546.

Chinnarasu, C., Montes, A., Pereyra, C., Casas, L., Fernández-Ponce, M. T., Mantell, C., Pattabhi, S., & Martínez de la Ossa, E. 2016. Preparation of polyphenol fine particles potent antioxidants by a supercritical antisolvent process using different extracts of Olea europaea leaves. Korean Journal of Chemical Engineering, 33(2), 594–602.

Cui, M., Hu, N., Fang, D., Sun, H., Pan, H., & Pan, W. (2022). Fabrication and evaluation of customized implantable drug delivery system for orthopedic therapy based on 3D printing technologies. International Journal of Pharmaceutics, 618, 121679.

Gorzynik-Debicka, M., Przychodzen, P., Cappello, F., Kuban-Jankowska, A., Marino Gammazza, A., Knap, N., Wozniak, M., Gorska-Ponikowska, M. 2018. Potential Health Benefits of Olive Oil and Plant Polyphenols. International Journal of Molecular Sciences, 19(3), 686.

Khosraviboroujeni, A., Mirdamadian, S. Z., Minaiyan, M., & Taheri, A. 2022. Preparation and characterization of 3D printed PLA microneedle arrays for prolonged transdermal drug delivery of estradiol valerate. Drug Delivery and Translational Research, 12, 1195–1208.

Liu, A., Xue, G., Sun, M., Shao, H., Ma, C., Gao, Q., Gou, Z., Yan, S., Liu, Y., & He, Y. 2016. 3D Printing Surgical Implants at the clinic: A Experimental Study on Anterior Cruciate Ligament. Reconstruction. Scientific Reports, 6, 21704

Machado, N. D.; Cejudo-Bastante, C.; Goñi, M. L.; Gañán, N. A.; Casas-Cardoso, L.; Mantell-Serrano, C. 2022. Screening of the Supercritical Impregnation of Olea europaea Leaves Extract into Filaments of Thermoplastic Polyurethane (TPU) and Polylactic Acid (PLA) Intended for Biomedical Applications. Antioxidants, 11(6), 1170.

Mohamed, O. A., Masood, S. H., & Bhowmik, J. L. 2015. Optimization of fused deposition modeling process parameters: a review of current research and future prospects. Advances in Manufacturing, 3, 42-53.

Ngo, T. D., Kashani, A., Imbalzano, G., Nguyen, K. T. Q., Hui, D. 2018. Additive manufacturing (3D printing): A review of materials, methods, applications and challenges. Composites Part B: Engineering, 143, 172-196.

Omar S.H. 2010. Oleuropein in olive and its pharmacological effects. Scientia Pharmaceutica, 78(2):133-54.

Rajan, K., Samykano, M., Kadirgama, K., Harun, W. S. W., Rahman, M. M. 2022. Fused deposition modeling: process, materials, parameters, properties, and applications. The International Journal of Advanced Manufacturing Technology, 120, 1531-1570.

Roleira, F. M. F., Tavares-da-Silva, E. J., Varela, C. L., Costa, S. C., Silva, T., Garrido, J., Borges, F. 2015. Plant derived and dietary phenolic antioxidants: Anticancer properties. Food Chemistry, 183, 235-258.

Rosales, J. M., Cejudo, C., Verano, L., Casas, L., Mantell, C., & Martínez de la Ossa, E. J. 2021. Supercritical Impregnation of PLA Filaments with Mango Leaf Extract to Manufacture Functionalized Biomedical Devices by 3D Printing. Polymers, 13(13), 2125.

Stamatopoulos, K.; Katsoyannos, E.; Chatzilazarou, A. 2014. Antioxidant Activity and Thermal Stability of Oleuropein and Related Phenolic Compounds of Olive Leaf Extract after Separation and Concentration by Salting-Out-Assisted Cloud Point Extraction. Antioxidants, 3, 229-244.

Szojka, A., Lalh, K., Andrews, S. H. J., Jomha, N. M., Osswald, M., & Adesida, A. B. 2017. Biomimetic 3D printed scaffolds for meniscus tissue engineering. Bioprinting, 8, 1-7.

Verano-Naranjo, L., Cejudo-Bastante, C., Casas, L., Mantell, C., Martínez De La Ossa, E.J. 2021. In vitro study of the release of drugs impregnated by supercritical technology in polylactic acid for biomedical applications. Chemical Engineering Transactions, 86, 1063-1068.

Viidik, L.; Vesala, J.; Laitinen, R.; Korhonen, O.; Ketolainen, J.; Aruväli, J.; Kirsimäe, K.; Kogermann, K.; Heinämäki, J.; Laidmäe, I.; Ervasti, T. 2021. Preparation and characterization of hot-melt extruded polycaprolactone-based filaments intended for 3D-printing of tablets. Eur. J. Pharm. Sci., 158, 105619

Volf, I., Ignat, I., Neamtu, M., Popa, V.I. 2014. Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols. Chemical Papers volume 68, 121–129.