

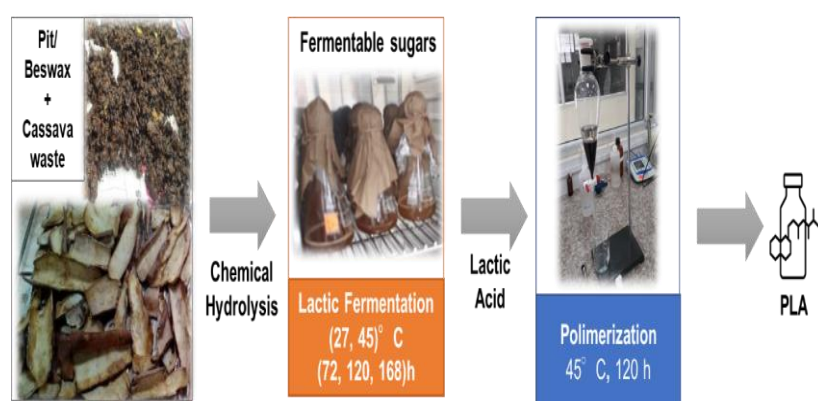
Full Paper | <http://dx.doi.org/10.17807/orbital.v18i1.24723>

Valorization of Cassava and Beeswax Waste to Obtain Bioplastic

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Global agricultural production generates a wide variety of valuable waste. In a world seeking to become increasingly sustainable, it is necessary to take advantage of these resources by establishing processes based on the bioeconomy and the circular economy. Cassava waste and beeswax were used to obtain polylactic acid (PLA). The fermentable sugars were extracted from each residue using a chemical hydrolysis process. Lactic fermentation using a commercial strain and with a multiple factorial design was carried out to evaluate the type of residue (cassava peel, pit, cassava peel-pit mixture), temperature (27 and 45°C), and reaction time (72, 120, 168 hours). The process involved oligomerization and ring-opening polymerization (ROP) to obtain PLA. The highest amount of LA was 2618.81 mg/L when working with a mixture of cassava peel and stone during 120 hours of fermentation at 45°C. During polymerization, 70.05% of the obtained material corresponded to PLA, and through thermal analysis, were determined: Glass transition temperature ($T_g = 91.81^\circ\text{C}$), melting temperature ($T_m = 178.77^\circ\text{C}$), and degradation temperature ($T_d = 286.09^\circ\text{C}$). Although the characteristics of the obtained material still differ from those reported for commercial PLA, the study revealed that the selected waste is a potential resource for producing a biopolymer.

Graphical abstract



Keywords

Bioplastic
Polylactic acid
Waste recovery

Article history

Received 08 Jan 2026
Revised 01 Mar 2026
Accepted 04 Mar 2026
Available online 27 Jun 2026

Handling Editor: Luiz C. Silva Filho

1. Introduction

Agricultural and agro-industrial waste are organic materials generated during the harvest and industrial transformation of the arable crop, respectively [1]. Sometimes,

these wastes are burned accidentally or as a cultivation practice by the farmers [2]. These wastes have great potential for transformation into high-value-added products, such as

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biofuels, bioactive compounds, and biopolymers [3].

Beekeeping is defined as the study of the honey bee to obtain economic benefits through the different products obtained from its breeding, which generates an organic by-product of the production of beeswax called pit. Like other agricultural waste or by-products from agri-food industries, the pit is used as organic fertilizer [4] without ruling out other applications.

Agricultural waste is lignocellulosic biomass, a sustainable resource for use as raw material in the development of a variety of bioproducts. Currently are recent developments in biorefineries that use these biomasses to produce biofuels, platform molecules, bioplastics, and a varied group of biobased materials. These types of developments fit into the concepts of circular economy and bioeconomy, which are presented as an alternative to the traditional production model to provide sustainable development possibilities that are ecologically friendly to the environment [5].

Among the byproducts of agricultural waste used as a substrate for manufacturing, there are corn, wheat, soybeans, potatoes, and cassava, among others, which tend to be a viable option in biopolymer extraction processes because they are ecological and because of their easy degradation in the environment [6]. Bioplastics obtained from cassava residue are an alternative to reduce the use of fossil resources in the production of petroplastics and propose a sustainable value chain for cassava [7].

As an alternative to traditional plastics, interest in bioplastics is constantly growing. The bioplastics are a family of materials with different properties and applications. These can be biobased, biodegradable, or meet both characteristics [8]. The global production of bioplastics in 2024 was around 2.47 Mt (56.3% biobased biodegradable and 43.7% biobased non-biodegradable), and it is expected that by 2029, bioplastic production will reach 5.73 Mt (66% biobased biodegradable and 34% biobased non-biodegradable). Of the total biodegradable biobased plastic production in 2024, 37.1% corresponded to polylactic acid (PLA), estimating 42.3% in 2029 [9].

PLA is a biodegradable, compostable, and non-toxic polymer with interesting properties for the industry. It is emerging as a promising raw material for the production of products under the concept of green plastic. It is a polymer with great application potential in various areas, including chemistry, medicine, pharmaceuticals, biotechnology, and others [10].

The precursor of polylactic acid is lactic acid (LA), 2-hydroxypropionic acid, which exists in two stereoisomers: the right-handed D-(+)-lactic acid or d-lactic acid ((R)-lactic acid) and the left-handed L-(-)-lactic acid or l-lactic acid ((S)-lactic acid). They are produced by microbial fermentation or chemical synthesis. In chemical synthesis, both isomers are produced in equal quantities, while in the fermentation process, any lactic acid type is produced depending on the microorganism used. The LA obtained is purified with one of the following techniques: nanofiltration, electrodialysis, and reactive distillation. An oligo (lactic acid) is synthesized by polycondensation to give rise to lactide (l-lactide, d-lactide, and meso-lactide). The polymerization of PLA begins with these monomers by ring opening or polycondensation in the presence of metal and organic catalysts. The resulting stereo forms of PLA (poly(l-lactide), poly(d-lactide), and poly(dl-lactide)), as well as their properties, depending on the stereoisomeric form of the lactide and the catalyst used for its

synthesis [11].

Polylactic acid is available on the market and is a safe material when decomposed, producing H₂O, CO₂, and humus without damaging the environment [12]. Its mechanical and physical properties, biocompatibility, and processability make it comparable to synthetic plastics such as polypropylene and polyethylene terephthalate, which are obtained conventionally [13]. Some studies on the production of bioplastics report cassava use in obtaining a material with optimal characteristics as an alternative to polystyrene [6]. On the other hand, cassava starch and dried fruit peel waste were used to prepare a biodegradable plastic film with good characteristics of flexibility and transparency [14].

The beeswax residue was used in previous research as a coating on biodegradable starch/PLA trays to improve their mechanical and water vapor barrier properties [15], together with kaolin and glycerol in thermoplastic starch, to improve the physical and structural properties of the material [16], being a promising material in improving the barrier properties of biodegradable polymers [17]. The use of biodegradable composite materials made with cassava starch and beeswax mixtures reinforced with fibrous agricultural waste is also recorded [18].

Based on this background, cassava, and beeswax waste were used to obtain PLA and make use of the waste generated in agricultural activity while generating a product of added value within the framework of the bioeconomy and the circular economy.

2. Material and Methods

2.1 Pre-treatment Materials

In this research, cassava peel waste, beeswax, and pit waste were used. The samples were supplied by a restaurant and an Agroindustrial Ecological Farm located in Manabí, province of Ecuador. The starch extracted from the cassava residue, the cassava residue flour, the pit, and the beeswax were hydrolyzed in acid (H₂SO₄) and alkaline (NaOH) medium in different concentrations (Table 1). It evaluated the total reducing sugars (TRS) production in the indicated biomasses in a reaction time of 60 minutes to select of the highest yield for the fermentation stage. In this experimentation phase, the procedure used was described in previous research [19].

Table 1. Experimental design of hydrolysis.

Residue	Hydrolysis	Concentration (% w/v)
Cassava starch		
Cassava flour	Acid	3, 4, 5
Pit	Alkaline	
Beeswax		

2.2 Lactic fermentation

Before lactic fermentation, the microorganism was activated using lyophilized culture TCC-20, CHR Hansen consisting of *Lactobacillus helveticus* and *Streptococcus thermophilus*. 0.5 g of culture was activated with 50 mL of MRS broth and incubated at 37 °C for 24 h. 1 mL was taken to do 10 serial dilutions in triplicate, from 1x10⁻¹ to 1x10⁻¹⁰, respecting a final volume of 1 mL. 10 Petri dishes were labeled (one for each dilution in triplicate) using a clean and previously sterilized pipette 0.1 mL of each dilution was seeded in a Petri dish with nutrient agar. With the help of a *Digralsky* loop, it was moved across the plate previously sterilized in alcohol,

flamed, and cooled, and it was incubated for 48 h at 37 °C [20]. After the incubation period, a count was performed using the standard method for examining dairy products, thereby determining the number of colony-forming units (CFU) [21].

For the residue with the highest TRS content, 10 mL of sample was taken (individually and collectively) and placed in sterilized flasks at 15 psi and 121 °C for 15 minutes. The

sample was allowed to rest in a laminar flow chamber and 10 mL of the *Lactobacillus* culture with the highest concentration, maintaining a pH between 5.5 and 6.5 (Fig. 1). The samples were taken to the incubator under the established working conditions (Table 2), where minimum fermentation times of 3 days and up to 7 days are reported, where the optimization of fermentation conditions was evaluated [22, 23].

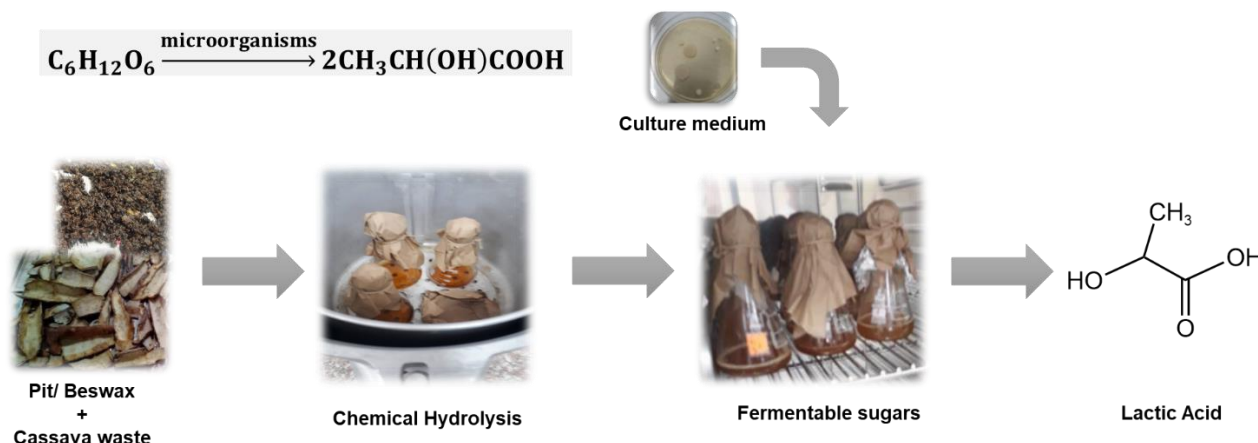


Fig. 1. Lactic fermentation process.

Table 2. Experimental design of the fermentation.

Residue	Temperature (°C)	Reaction time (h)
Cassava flour		72
Pit	27	120
Pit + Cassava flour	45	168

A mass balance was performed on the lactic fermentation process as a tool for validating analytical data and estimating variables for which direct analytical methods are not available [24]. The system was modeled as an isothermal, constant-volume batch reactor with no inflow or outflow during fermentation (Eq. 1), where C_i is the concentration of component i (g/L), r_i is the volumetric rate of formation or consumption (g/L.h), and dt is the time differential [25].

$$\frac{dC_i}{dt} = r_i \quad (1)$$

Cell growth is described by the specific growth rate μ (Eq. 2), where X is the biomass concentration (g/L), and μ is the specific growth rate (h^{-1}). Lactic acid production is described with the Luedeking–Piret model (Eq. 2), where P is the product concentration (g/L), α is the production coefficient associated with growth, and β is the production coefficient not associated with growth [26]. Although this equation formally defines the product generation term in the dynamic balance, α and β were not estimated in this work due to the lack of complete kinetic profiles.

$$\frac{dX}{dt} = \mu X \quad (2)$$

$$\frac{dP}{dt} = \alpha \frac{dS}{dt} + \beta X \quad (3)$$

Substrate consumption is related to biomass (Eq. 4) and product (Eq. 5) formation through observed yield coefficients [25], where $Y_{X/S}$ is the biomass/substrate yield (g/g), and $Y_{P/S}$ is the product/substrate yield (g/g). From these definitions, the differential substrate balance is expressed according to Eq. (6), where S is the substrate concentration (g/L).

$$Y_{X/S} = \frac{\Delta X}{\Delta S} \quad (4)$$

$$Y_{P/S} = \frac{\Delta P}{\Delta S} \quad (5)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \frac{dX}{dt} - \frac{1}{Y_{P/S}} \frac{dP}{dt} \quad (6)$$

The initial parameters for biomass (X), product formation (P), and substrate consumption (S) correspond to the initial conditions of the fermentation process (Table 3). For the overall cumulative balance, the yield coefficients reported in the literature were used (Table 4). μ_{max} , K_s , and K_P were not used, as these parameters correspond to dynamic kinetic models (Monod or product inhibition) and are not necessary for the overall cumulative balance.

Table 3. Initial fermentation conditions.

Variable	Value (g/L)
Initial biomass (X_0)	0.02
Initial substrate concentration (S_0)	180.00
Initial product concentration (P_0)	0.00

Table 4. Yield coefficients used.

Variable	Value	Reference
$Y_{X/S}$, g/g	0.07 – 0.19	[27]
$Y_{P/S}$, g/g	0.74 – 0.89	

2.3 Separation, purification and quantification of lactic acid

For separation of the lactic acid produced, the solution obtained in the fermentation was filtered and centrifuged at 2500 rpm for 5 min in Falcon tubes. Once the centrifugation was completed, the supernatant was filtered twice: First using Whatman N° 42 filter paper and the next with microfiltration in a 1.2 μm pore membrane, both under vacuum [28].

The purification of lactic acid was done with simple distillation and a roto evaporator, reducing the operation time to obtain a purer final product. The procedure consisted of vacuum filtering the lactic acid and separating it at 100 °C [29]. After removing the water, the amount obtained was determined by the difference in volume.

The quantification of lactic acid was carried out using a modification of the method proposed by Zamanova *et al.* [30], using a ThermoFisher Scientific ACCELA liquid chromatograph equipped with a C18 reversed-phase column (5 μm ; 4.6 x 100 mm) and a UV-VIS diode array photodetector (PDA). The binary mobile phase used consisted of acetonitrile (A) and a 3×10^{-2} M solution of H_3PO_4 (B) at a ratio of 12:88 (v/v) with a constant flow of 0.7 mL/min. Detection was done at a wavelength of 210 nm with an injection volume of 20 μL and a controlled temperature of 40°C in the column and sample compartment. In the lactic acid determination, 88% (v/v) standard lactic acid and its respective calibration curve were used.

2.4 Sequential synthesis of lactide and polylactic acid from lactic acid

Lactic acid was extracted using a separatory funnel, with 100 mL of sample and 100 mL of solvent (petroleum ether). The esterification process was carried out with heating and stirring, for which 100 mL of the lactic acid and 5 mL of H_2SO_4 at 60% (v/v) were heated to 80°C for 35 min, then the temperature was increased to 120°C for 2 hours, and 5 mL of H_2SO_4 was added. After 7 hours of heating, 5 mL of H_2SO_4 is added again. Finally, after 9 hours of operation, the lactic acid diester called lactide was obtained [28].

The lactide obtained was polymerized through the ring opening (ROP) method. The lactide was reacted with 50 mL of methanol and 3 g of tin II chloride as a catalyst. This reaction occurs after 24 hours at 60°C [31]. The polymer was dried by evaporating the methanol at 70°C, adding acetone, and then vacuum filtering using a Watman No. 42 filter paper (Fig. 2).

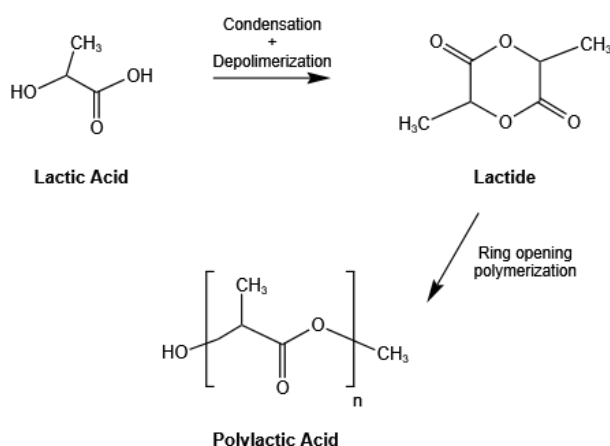


Fig. 2. Lactic fermentation process.

2.5 Determination and characterization of PLA

The amount of PLA obtained in the polymerization was known by solubilizing 0.5 g of the material in 50 mL of chloroform at room temperature for 2 hours. It was then titrated with 0.04 M ethanolic KOH solution, using phenolphthalein as an indicator. An excess of approximately 0.200 mL was added [32]. Back titration was carried out with 0.04 M HCl (isopropyl alcohol) solution. The percentage of polylactic acid was calculated using Eq. (7).

$$\% \text{ PLA} = \left(\frac{V_e \times C_{\text{KOH}} \times M_{\text{PLA}}}{m} \right) \times 100\% \quad (7)$$

Where V_e is the equivalent volume of added KOH ($V_{\text{KOH}} + V_{\text{excess}} - V_{\text{HCl}}$), C_{KOH} is the molar concentration of KOH, M_{PLA} is the molar mass of the PLA acid group, and m is the mass of the sample.

The characterization of PLA obtained was done by a simultaneous thermal analyzer: thermogravimetric analysis/differential scanning calorimetry (TGA/DSC). An empty capsule and a capsule with the sample to be studied were placed in the SDT Q200, EM 004 thermal analyzer. The samples were heated from the initial temperature to the final temperature at a speed of 10 °C/min, according to what is stated in the ISO 11357-3:2018 standard [33]. The changes in temperature and heat flow were measured and recorded to calculate the specific heat. In the analysis, air was the purge gas, the flow was 50 mL/min of N_2 , and the sample weight was approximately 9.2 g.

The process yield was determined by the ratio of the mass of the final product to the initial raw material [25], as calculated using equation (8). Where Y_{GLOBAL} is the process yield, m_{DB} is the mass of dry biomass (cassava residue + bone), and m_{PLA} is the mass of polylactic acid.

$$Y_{\text{GLOBAL}} = \frac{m_{\text{PLA}}}{m_{\text{DB}}} \quad (10)$$

3. Results and Discussion

3.1 Lactic fermentation

In the microbiological count, values of 16×10^8 CFU/g were found, comparable with related research, where it is considered that values between 10^7 and 10^8 correspond to fermented dairy products [34], guaranteeing adequate growth of the culture during the fermentation stage [35]. We worked with a concentration of 1×10^{-1} because it was the best concentration obtained among those evaluated.

Fermentation was carried out for the residue with the highest TRS content, this being the pit with 5% alkaline hydrolysis, followed by the cassava residue flour with 4% alkaline hydrolysis, with a concentration of 4.60 g/L and 3.09 g/L respectively [19]. The management of dairy culture with the studied waste allowed the production of LA under different operating conditions (Fig. 3). However, the best results were obtained when working at 45 °C at a lower temperature, this being a variable that influences the speed of the reaction. Although the microorganism used grew with the cassava and stone waste when using them as substrate individually, the highest concentration of LA (26188.1 mg/L) was obtained when combining them.

The performance in LA production and its final concentration depends on several factors such as microorganisms, genus, and species, fermentation conditions such as temperature, reaction time, pH, inoculum size, substrate concentration, and nutrients, in addition to the relationship between said factors [36-38]. In this sense, the combination of waste became an appropriate substrate for the biosynthesis of lactic acid and, together with the concentration of the inoculum and temperature, among others, stimulated the production of LA.

The time in which the highest production of lactic acid occurred in the fermentation process was 120 hours. This result is comparable with the production of lactic acid from sugar cane, where a higher yield was obtained with the same microorganism used in this research [39]. The fermentation time is an interesting variable in selecting the best lactic acid bacteria starter for the culture [40]. Higher inoculum concentrations require shorter fermentation times to reduce sugar levels than lower concentrations and vice versa, possibly due to inhibition and reduced efficiency of the fermentation process [38].

The highest production of LA was obtained at 45°C, which

reported the best results in previous research for the production of lactic acid [41]. Temperature is an aspect that influences cell growth and the concentration of lactic acid since its increase favors the hydrolysis of the substrate. Although lactic fermentation can be carried out at ambient conditions (15-27 °C), mesophilic (25-40 °C), thermophilic (40-65 °C), extreme thermophilic (65-80 °C) or hyper-thermophilic (> 80 °C), the optimal temperature will depend on the substrate, the inoculum, being for pure cultures between 30 and 45 °C [42].

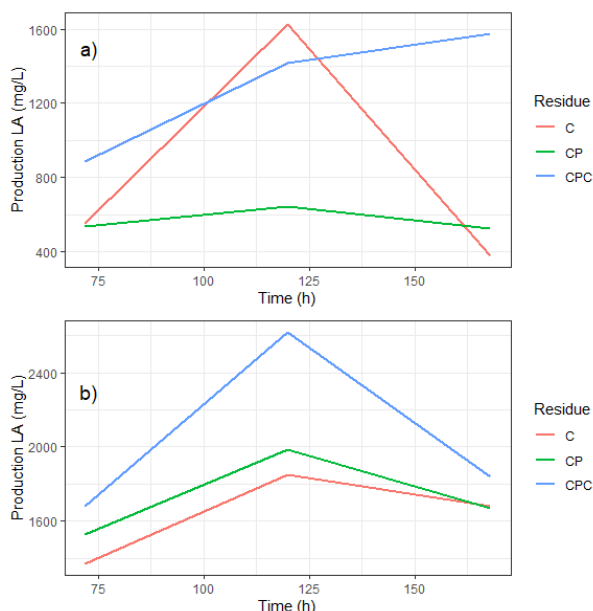


Fig. 3. Lactic acid production (C: pit, CP: cassava flour, CPC: cassava flour +pit): a) 27°C, b) 45°C.

The amount of lactic acid obtained was 159 mL, and after purification, a volume of 69 mL of LA was recorded. The same amount was obtained with both separation techniques, and the separation time was shorter when using the roto evaporator. The purification of LA is necessary before polymerization since the fermentation broth contains a large amount of water, and the biomass has to be separated from the solid particles to obtain high-purity PLA [43, 44].

3.2 Mass balance and process yield

The mass balance of the fermentation process was performed using the initial conditions described for biomass, substrate, and product, with average yield values as reported in the literature ($Y_{P/S} = 0.82$ g/g; $Y_{X/S} = 0.13$ g/g) [27, 45, 46]. The estimated substrate consumption required to produce 26.19 g/L of lactic acid was 31.94 g/L, leading to a final substrate concentration of 148.06 g/L from an initial concentration of 180 g/L. Under these conditions, the calculated biomass formation was 4.15 g/L, reaching a final concentration of 4.17 g/L from an initial value of 0.02 g/L. The substrate consumed indicates that approximately 82% of the carbon was used for lactic acid formation and 13% for cell growth. The remaining fraction is associated with maintenance requirements and formation of secondary metabolites, which is consistent with the stoichiometric balances described for lactic fermentation processes [25]. Although the death phase of the bacteria and the total consumption of the substrate were not observed, related research reports the production of lactic acid, even when the

substrate is not completely consumed [47].

The process yield calculated for LA production from the cassava residue-stone mixture was 0.50 g PLA/g dry biomass. This value indicates that 50% of the initial dry biomass was converted into the final polymer product, integrating the efficiencies of pretreatment, hydrolysis, lactic fermentation, and polymerization. The definition of overall yield applied in lignocellulosic biorefinery processes is consistent with the technological schemes described for the conversion of the biomass into lactic acid and its subsequent transformation into PLA, allowing for comparison of the overall process efficiency independently of the intermediate partial yields [12, 45].

3.3 Obtaining polylactic acid and its characterization

Most APL production processes use the most efficient conversion of lactide (the cyclic dimer of lactic acid) through ring-opening polymerization, which in turn allows obtaining a high molecular weight polymer [48, 49]. With the ROP method, through the controlled polymerization of lactic acid, 10 grams of polylactic acid were obtained. The amount of PLA present was quantified using the titratable acidity method, and a percentage of polylactic acidity of 70.05% + 0.51 was determined, lower than that recorded in another research [32].

A further increase in the percentage of acidity found in PLA indicates that it has been effectively injected into the lactic acid chain [32]. It allows for providing a production alternative from the use of this plant biomass, developing innovative formulations that satisfy human needs and are friendly to the environment, responding to the problems presented by the volumes of this waste, and achieving a productive model that does not generate detriment to ecosystems.

Fig. 4 shows the thermogram of the material. A peak begins at T1, corresponding to an exothermic process where heat is released into the system. In T2, heat is recovered. There is a crystallization temperature since the polymeric crystals melt, and it is necessary to absorb heat from the environment. Finally, an exothermic process is observed with the release of heat that may be associated with the degradation of the polylactic acid or the material.

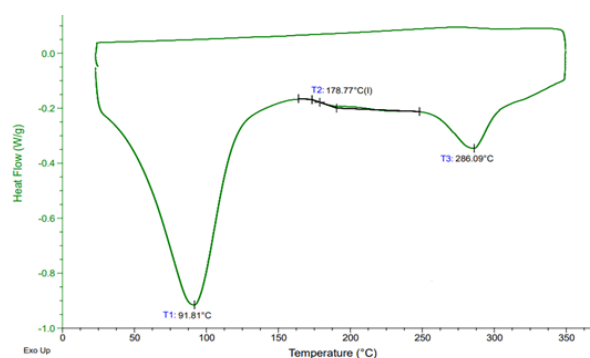


Fig. 4. Thermogram of the PLA obtained.

The thermal and calorimetric analysis allowed us to know the glass transition temperature ($T_g = 91.81$ °C), the melting/crystallization temperature ($T_m = 178.77$ °C), and the degradation temperature ($T_d = 286.09$ °C) of the material obtained. PLA is characterized by having a high melting temperature (160 °C), a glass transition temperature (60 °C), and an approximate degradation temperature of 320 °C [50].

In commercial samples of different types of PLA, glass

transition temperatures of 57.3 to 66.3 °C and fusion temperatures between 152.4 to 178.7 °C, the latter being a value close to that reported for the material obtained in this research. A lower glass transition temperature and a higher melting temperature are due to the spherical particle size of the polymer [51]. Commercial unmodified/graphene-modified PLA filaments recorded a T_g and T_m of 62/63 °C and 155/156 °C with an exothermic peak of 118/120 °C [52]. In a study where the behavior of commercial PLA used as raw material in the plastic industry was analyzed, a T_g and T_m of 61.9 °C and 153 °C were reported. When comparing these results with other investigations, it was evident that higher values were found for PLA with a higher molecular weight. A decrease in the T_g temperature is possibly due to the increase in the mobility of the polymer chains or chain breakage. An increase in T_g could occur due to greater packing in the amorphous zones in the annealed material, facilitating the formation of more crystalline zones [53].

The thermal properties of the material allow the establishment of the operating conditions of the transformation process to which this biopolymer will be subjected. When PLA has a crystallization point superior to 150 °C, it indicates that the molar mass is high and optimal for use in the production of thermoplastic filament, which can subsequently be used for three-dimensional printing with successful printing results [54].

The absence of spectroscopic (FTIR, NMR) and chromatographic (GPC/SEC) analyses represents a limitation of this work. Structural confirmation and molecular weight determination of PLA are inferred from process reproducibility and thermal characterization, rather than direct molecular analysis. Future studies may incorporate comprehensive structural and molecular characterization to strengthen material validation.

4. Conclusions

Poly(lactic acid) (PLA) is a biodegradable polymer that presents itself as an interesting alternative to synthetic polymers, especially when using waste raw materials within a circular economy and sustainability framework. In this research, the combination of cassava waste with the pit, under established fermentation conditions (45°C and approximately 124 h), achieved a yield of 26.19 g/L, the highest compared to the other conditions evaluated. The overall process yield, considering the fermentation stage and lactic acid recovery, was 50%, highlighting limitations in the system's efficiency, particularly in the lactic fermentation and purification stages. Although the yield was lower than that reported in similar studies, obtaining the monomer allowed for its subsequent polymerization using the ring-opening method, demonstrating the technical viability of the process. The synthesized PLA exhibited characteristics comparable to those reported in the literature, in terms of glass transition temperature and melting point, confirming the proper formation of the polymer. The results demonstrate that cassava waste constitutes a potential raw material for the production of lactic acid and its subsequent polymerization to PLA, making it necessary to optimize the fermentation and purification conditions to increase the yield and improve the competitiveness of the process on an industrial scale.

Author Contributions

All authors contributed to the study conception and

design. Material preparation, data collection and analysis were performed by Sofia Briones. The first draft of the manuscript was written by María Antonieta Riera and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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How to cite this article

Briones, S.; Riera, M. A. *Orbital: Electron. J. Chem.* **2026**, 18, 8. DOI: <http://dx.doi.org/10.17807/orbital.v18i1.24723>