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Advancements in PLA-Based Bioplastics: Enhancing Mechanical Properties with Protein Additive for a Sustainable Circular Economy

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ABSTRACT

Conventional plastics present significant environmental challenges due to their non-biodegradable nature. This study evaluated polylactic acid (PLA) as a sustainable alternative, focusing on lactic acid synthesis from orange peel powder (OPP) and its blending with wheat gluten (WG) for bioplastic production. Orange peels were sun-dried, hydrolyzed and characterized by FTIR, confirming cellulose and protein content suitable for fermentation. A 10% hydrolyzed OPP concentration produced the highest lactic acid yield, which was extracted and purified. PLA synthesized via direct poly condensation showed thermal stability above 250 °C (DSC). Mechanical testing revealed that a 60:40 PLA/WG blend offered superior tensile strength and flexibility. SEM confirmed uniform polymer distribution, while solubilization and migration tests indicated the effects of solvents and temperature on film stability. These findings highlight the potential of PLA/WG composites as eco-friendly packaging materials, providing a scalable alternative to petroleum-based plastics and contributing to sustainable waste management.

Key words: PLA, wheat gluten, lactic acid, bioplastic, biodegradation

INTRODUCTION

Petrochemical-derived plastics are widely used in food packaging due to their production efficiency and favourable mechanical properties such as malleability and ductility (Yuan *et al*., 2022). However, their non-biodegradable nature poses serious environmental challenges to rivers, soils and seas (Stubbins *et al*., 2021). Plastic waste also threatens ecosystems and human health through non-biocompatible components, highlighting the urgent need for alternatives, as recycling alone has not curbed plastic accumulation (Teixeira *et al*., 2021). Biodegradable plastics from natural sources offer a viable solution (Bouftou *et al*., 2024). Polysaccharide-based biopolymers are especially promising for food packaging due to their abundance, biodegradability and favourable chemical and mechanical properties (Acharjee *et al*., 2023; Chauhan *et al*., 2024).

Poly lactic acid (PLA), synthesized via lactic acid (LA) polymerization, is a leading biodegradable polymer due to its renewability, degradability and food-grade classification (Abdullah *et al*., 2019). Beyond packaging, PLA is widely applied in biomedical fields such as surgical sutures, tissue engineering and drug delivery (Ebrahimi and Ramezani, 2022). LA can be produced through fermentation of renewable substrates such as starchy residues, lignocellulose and food waste (Huang *et al*., 2023). Citrus waste, particularly orange peel, represents an abundant yet underutilized substrate, rich in pectin and cellulose and suitable for fermentation (Bustamante *et al*., 2019; Ricci *et al*., 2019). Global citrus processing generates ~31.2 million tonnes of by-products annually, often discarded despite their potential for high-value applications (Sambudi *et al*., 2022; Suri *et al*., 2022). Around 90% of LA is produced through microbial fermentation using diverse strains including lactic acid bacteria, Bacillus, *E. coli* and fungi (Abedi and Hashemi, 2020; Huang *et al*., 2023). Although PLA is bio absorbable and compostable, modifications are needed to tailor its performance for food applications. Wheat gluten, a low-cost by product of the food industry, enhances PLA due to its availability, viscoelasticity and high degradation rates (Carvajal-Piñero *et al*., 2019). Plasticizers

improve film properties, with glycerol being more effective than water due to its stability and compatibility (Alonso-González *et al*., 2021). This study explored PLA–wheat gluten blends with glycerol to improve film characteristics, promoting sustainable use of agricultural by products and aligning with SDG 12 on responsible consumption and production.

MATERIALS AND METHODS

The AR-grade chemicals used in this study ethanol (>99%, CAS No.: 64-17-5), methanol (>99% CAS No.: 67-56-1), sulfuric acid (CAS No.: 7664-93-9), glycerol (>99% CAS No.: 56-81-5), yeast extract (CAS No.: 8013-01-2), peptone (CAS No,: 100209-45-8), disodium hydrogen phosphate (98% CAS No.: 10028-24-7), ammonium chloride (>99.5% CAS No.: 12125-02-9), sodium chloride (>99% CAS No.: 7647-14-5), sodium hydroxide (CAS No.: 1310-73-2), magnesium sulphate (>99% CAS No.: 10034-99-8), calcium chloride (>97% CAS No.: 10043-52-4), potassium hydroxide (>85% CAS No.: 1310-58-3), calcium carbonate (>99% CAS No.: 471-34-1), xylene (>98% CAS No.: 1330-20-7), glucose (CAS No.: 50-99-7) and stannous chloride (98% CAS No.: 7772-99-8) were purchased from Himedia Labs Pvt. Ltd. Mumbai, India and Sigma-Aldrich Chemicals Pvt. Ltd. Banglore, India for the experimental work. Glycerol was used as fillers and plasticizers in the synthesis of bioplastic film. Double distilled water was employed to conduct the experiments.

Wheat grains were soaked in water and vinegar (10 ml/kg) with sugar (28.5 g/kg) and yeast (57 g/kg) at 35-40°C for two hours, rinsed three times and sun-dried. The dried grains were tempered to 16% moisture, ground into flour and stored in airtight containers. Dough prepared with 2% sodium chloride solution was immersed in water for 40 min and washed under running water to remove most starch.

Curd was collected from the local market of Hisar, Haryana, India, in sterilized flasks and serially diluted (10{ ² to 10{ v) under aseptic conditions. Using the spread plate technique on de Man, Rogosa, and Sharpe (MRS) medium, plates were incubated at 37 °C for 42 h. After growth, isolates were sub-cultured by streaking until pure colonies were obtained for biochemical identification.

Orange peel powder (OPP) was used as a substrate for lactic acid production. Orange peels collected from juice stalls in Hisar, Haryana, were sun-dried (5 days), powdered and hydrolyzed with 1 ml of 20% H, SO„ per solution, then heated in a boiling water bath for 20 min. The hydrolyzed powder was dried and used at concentrations of 5, 10 and 15%. Each was mixed with 50 ml mineral salt medium (M9), supplemented with 2% yeast extract and 1% peptone, adjusted to pH 6.0 with 4.0 M KOH and made up to 1000 ml. Culture flasks were inoculated with 2 ml bacterial consortium and incubated anaerobically at 37°C in triplicates. Samples were taken at 24-120 h. After centrifugation (4000 rpm, 10 min), supernatants were treated with CaCO*f* to form calcium lactate, which was then acidified with 1 M H, SO„ to release lactic acid. The filtrate was decolorized using activated charcoal and centrifuged (6000 rpm, 10 min) to remove impurities.

DNSA and colorimetric methods were used to determine total reducible sugars and lactic acid concentration, respectively. For sugars, absorbance was measured at 540 nm using a double-beam UV-Visible spectrophotometer. Lactic acid estimation involved placing 0.5 ml aliquots in screw-capped tubes, boiling for 10 min, and adding 3 ml concentrated H_2SO_4. After cooling, 100 µl para-phenyl phenol and 50 µl $CuSO_4$ reagent were added. Following dissolution and 30 min incubation at 30°C, absorbance was recorded at 570 nm.

Poly lactic acid was synthesized by direct poly condensation. Lactic acid (200 ml) was mixed with xylene (200 ml) and 0.01% stannous chloride catalyst. Under nitrogen, the reaction was run at 120°C for 3 h, and then at 130°C for 8-24 h until the volume was halved. Excess xylene was removed at 140°C, and the product was washed with methanol and dried to yield a white powder.

The chemical composition and functional groups of LA and PLA were analyzed using FTIR (Perkin-Elmer, Waltham, MA) with KBr pellets in the range of 400–4000cm. Morphology was examined by SEM (HITACHI S3400N, Japan), where samples were mounted on gold-coated stubs and imaged at 10 kV with 100× magnification.

DSC analysis was performed with a Q200 (TA Instruments, USA); ~6 mg samples were sealed in aluminum pans, hydrated overnight, and heated from 30-220 °C at 20 °C/min under nitrogen flow (25 mL/min).

TGA/DTG analyses were carried out on a TGA Q500 (TA Instruments, USA) using ~10 mg samples in alumina pans, heated to 800°C at 5°C/min with nitrogen balance/sample flow of 40/60 mL/min. All measurements were conducted in duplicate to ensure reproducibility.

Bioplastic films were prepared via solvent casting using PLA and wheat gluten (WG). PLA and WG were dissolved separately at 1% w/w in water glycerol solutions with gentle stirring to ensure uniformity. Blends of varying ratios (PLA/WG: 100/0, 80/20, 60/40, 50/50, 40/60, 20/80, and 0/100) were poured into silicon dishes and dried for 48 h initially at room temperature, followed by oven drying. The resulting films were then analyzed for mechanical and thermal properties.

Tensile strength of PLA/WG films was measured using a Universal Testing Machine (UTM, H10KS; Tinius Olsen, UK) following ASTM standards. Samples (150 mm, gauge length 25 mm) were tested at a crosshead speed of 10 mm/min, with results expressed in N/15 mm.

Morphology was examined by SEM (HITACHI S3400N, Japan), where samples were mounted on gold coated stubs and imaged at 10 kV with 100× magnification.

Overall migration was evaluated gravimetrically in food stimulants (distilled water, 3% acetic acid and 10% ethanol). Rectangular films (10 cm^2) were exposed to 10 ml of stimulant at 4, 20, and 40°C for three days. After evaporation, migration (OM, mg/dm^2) was calculated as

$$OM = (M1 - M2) / S$$

Where, M1 is the mass of residue, M2 the stimulant mass and S the film surface area. All tests were performed in triplicate.

Soil burial degradation test was conducted over 30 days. PLA/WG membranes measuring 4 x 4 cm were accurately weighed and then placed in a pot containing a mixture of vermicompost and soil in 20:80 ratios. The pots were then incubated for 30 days at room temperature. The modifications in the samples were captured using smart phone after they were removed, cleansed and desiccated after 30 days.

RESULTS AND DISCUSSION

Bacterial isolates obtained after repeated striking (10^{-3} and 10^{-6} dilutions) from curd were identified as *Lactobacillus* spp. based on colony morphology and biochemical tests (Fig. 1). Identification involved gram staining, catalase and oxidase reactions, motility, NaCl tolerance, milk coagulation, phenol resistance and endospore formation. The isolates were gram-positive, catalase-negative, non-motile rods without endospores. They coagulated milk, tolerated 0.4% phenol and showed growth in MRS broth containing 1-9% NaCl (Table 1).

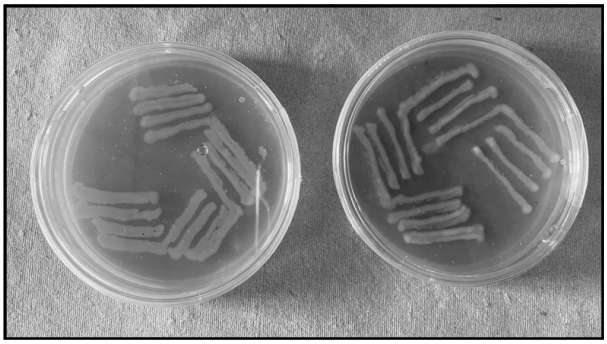

Fig. 1. Isolated bacteria from curd sample.

Table 1. Biochemical and physiological characteristics of isolated bacteria

Bio-chemical and physiological characteristics	Isolates
Gram stain	+
Motility test	Non-motile
Catalase test	-
Endospore test	-
0.4% phenol test	+
Milk coagulation	+
NaCl tolerance	+

Dough prepared from pretreated wheat grains was soaked in water for 40 min and washed under tap water until starch was completely removed. Salt addition improved gluten binding, reduced lipid binding and removed salt-soluble proteins. The resulting viscoelastic material was identified as wheat gluten (WG) using FTIR (Fig. 2). Spectral analysis showed a moderate band at 3417/cm (O–H stretching of amino acids), a peak at 2928/cm (–CH_2– stretching) and the amide-I region at 1638-1620/cm (C=O stretching of peptide bonds) (Dhaka and Khatkar, 2016). Additional bands were observed at 1546/cm (amide-II, N–H) and 1449/cm (C–H bending). Absence of starch-specific bands confirmed complete starch removal. The FTIR spectrum of WG closely resembled that of commercially available wheat gluten.

Orange peel contains insoluble polysaccharides (cellulose, hemicellulose) and soluble sugars

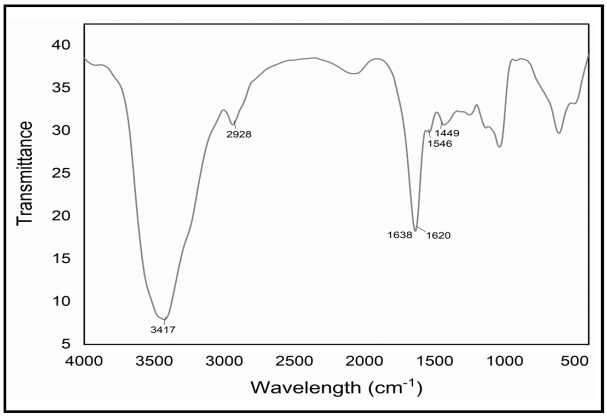

Fig. 2. FTIR spectrum of wheat gluten.

such as arabinose, fructose, galactose, galacturonic acid and glucose. FTIR analysis of dried orange peel (Puccini *et al*., 2016) revealed peaks typical of lignocellulosic materials (Fig. 3). The band at 3240/cm corresponded to O–H stretching, while 2928/cm related to symmetric/asymmetric C–H vibrations. Non-ionic –COOH groups appeared at 1738/cm and the C=O stretch at 1618/cm. A peak at 1247/cm corresponded to COO{ and aliphatic stretching (Rathinavel and Saravanakumar, 2021). $–CH_2–$ and $–CH_3–$ vibrations caused bending at 1423/ cm, with additional bands at 2862/cm (C–H) and 1053/cm (C–O–H/C–O–R), characteristic of cellulose and hemicellulose (Malook, 2019).

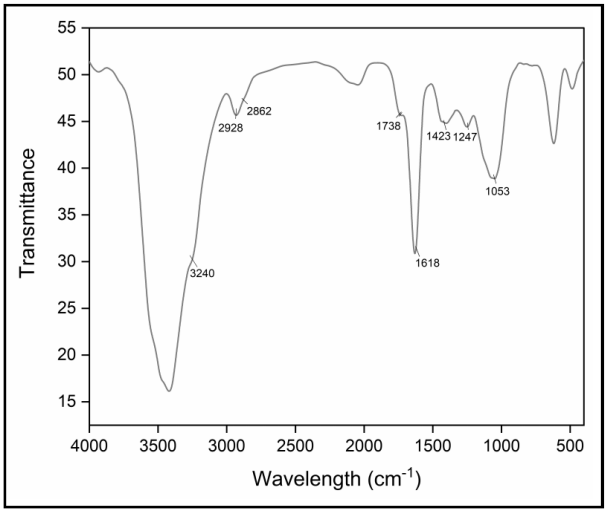

Fig. 3. FTIR spectrum of orange peel showing the functional groups.

Orange peel powder hydrolyzed with 20% H_2SO_4 was tested at 5, 10 and 15% concentrations, with pH maintained at 6 to support Lactobacillus growth (Kasirajan *et al*., 2019). At 5% substrate concentration, bacteria first utilized available glucose, with biomass increasing from 24 to 48 h and reaching 42.1 g/l, while reducing sugars decreased from 37 to 21.2 g/l and lactic acid peaked at 12.2 g/l. After 72 h, sugar depletion led to stationary growth, reducing sugars dropped to 14.5 g/l, and lactic acid stabilized near 16 g/l, with no further increase (Fig. 4 a). In contrast, 10% substrate concentration supported higher production, with biomass rising to 83.45 g/l and lactic acid reaching 35.87 g/l, while reducing sugars steadily decreased from 62.74 to 11.89 g/l. Maximum lactic acid production of 37.9 g/l was recorded at 96 h, after which both biomass and acid yield slightly declined (Fig. 4b). At 15% concentration, however, excessive substrate inhibited fermentation, limiting lactic acid output to only 8.27 g/l and biomass to 28.24 g/l (Fig. 4c). These findings suggest that substrate overload hampers bacterial activity, while moderate concentration enhances fermentation efficiency. Consistent with earlier studies (Chenebault *et al*., 2022), the most favourable condition was 10% substrate concentration with 96 h incubation, yielding the highest lactic acid output.

The experiment was conducted under optimized Lactobacillus sp. conditions and the 96 h culture was centrifuged at 4000 rpm for 10 min to remove cells. Excess $CaCO_3$ was added to the supernatant to neutralize the acid (pH 5-6), forming calcium lactate, followed by mineral acid precipitation and decolorization with activated carbon for 3-5 h (Krishnamurthy and Amritkumar, 2019). The purified solution was analyzed by FTIR (Fig. 5), confirming lactic acid functional groups. Peaks were observed at 1632/cm (C=O), 3345/cm (O–H), 2856/cm (C–H), and 1220/cm (C=O asymmetric with CH_3 vibrations) (Kumar *et al*., 2020; Vignesh Kumar *et al*., 2022). Additional peaks at 1730, 1124, 1043 and 823/cm matched those reported in lactic acid detection studies (Pãucean *et al*., 2017).

Purified lactic acid was successfully polymerized into PLA via poly condensation, lactide formation and polymerization. The reaction, carried out under nitrogen with xylene and catalyst, yielded a white powder that was confirmed as PLA by FTIR (Fig. 7a). Spectra exhibited characteristic ester peaks at 1724 and 1119/cm (–COO{ and –CO–) and $–CH_3$ stretching at 2977/cm, while the broad –OH

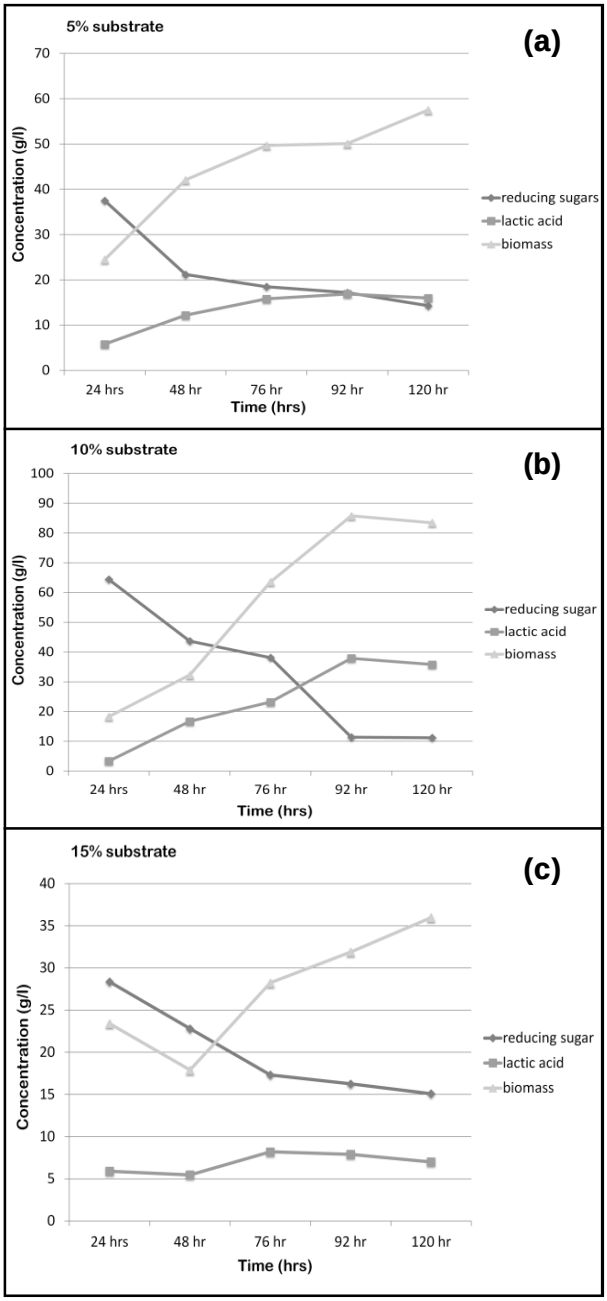

Fig. 4.(a). The effect of reducing sugar content on the formation of lactic acid and biomass at 5% of the substrate concentration, (b) the effect of reducing sugar content on the formation of lactic acid and biomass at 10% of the substrate concentration and (c) the effect of reducing sugar content on the formation of lactic acid and biomass at 15% of the substrate concentration.

peak of lactic acid (~3354/cm) nearly vanished, indicating successful polymerization. DSC analysis revealed thermal stability above 250°C, with a degradation peak

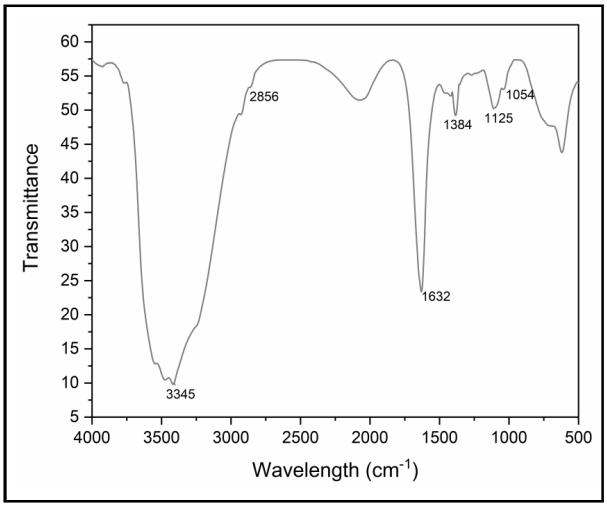

Fig. 5. FTIR graph of extracted lactic acid.

at 292.6°C and energy requirement of 390.1 J/g (Fig. 6). SEM images showed ductile fracture features with fibrils, layers and microvoids, confirming uniform granule dispersion (Fig. 7b). TGA demonstrated minimal mass loss (~1.4%) up to 200°C, major degradation (~97.5%) between 200-400°C and negligible residue beyond 400 °C, highlighting PLA's thermal resilience and biodegradability. These results collectively indicate that the synthesized PLA possesses well-defined chemical, thermal and morphological properties suitable for eco-friendly packaging applications, combining stability under moderate thermal conditions with efficient biodegradation, supporting its potential as a sustainable alternative to conventional plastics.

Blend films of varying compositions (i.e., weight ratios of 100/0, 80/20, 60/40, 50/50, 40/60, 20/80 and 0/100 between wheat gluten and PLA) were made by pouring a combination of solutions with a predetermined composition onto a silicon dish. Every cast film was allowed to dry for a total of 48 h first at room temperature and then in a hover. They were then examined to determine their mechanical and thermal characteristics.

The PLA/WG 60/40 (Table 3) blend exhibited the highest tensile strength (11.63 N/15 mm) and elongation at break (71.67%), representing a 49.1% improvement over pure PLA. Tensile strength decreased slightly with higher WG content but remained above that of pure PLA or WG, as the PLA matrix reinforced the composite. Excessive WG (50:50) led to poorly bonded, densely packed regions, reducing

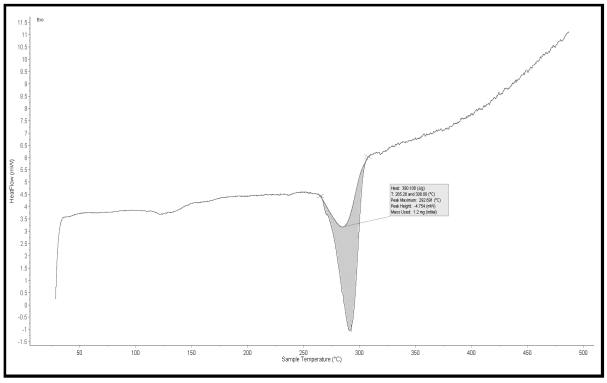

Fig. 6. DSC curve of produced PLA.

compatibility (Mendes *et al*., 2016). Both PLA and WG are biodegradable, and incorporating WG offers a cost-effective way to produce PLA-based bioplastics. Similar enhancements in mechanical strength were observed in PLA/chitosan blends at 60/40 ratios (Kasirajan *et al*., 2019).

Table 3. Mechanical characteristics of the membrane made of PLA/WG in different combinations

PLA/Wheat gluten	Tensile strength (N/15 mm)	Elongation (%)
100/0	7.80	160.74
80/20	8.24	140.00
60/40	11.63	71.67
50/50	9.96	85.58
40/60	7.40	221.36
20/80	6.22	252.23
0/100	4.09	280.89

PLA scanning microscopy (Fig. 8a) showed regular granule distribution, while PLA/WG films (Fig. 8b) displayed minor fractures and microvoids from solvent casting. The PLA matrix embedded in WG laminations appeared smoother due to better plasticizer incorporation. Void forms were obtained when hydrogen bonding in PLA chains weakened at gelatinization temperatures, allowing water infiltration into hydroxyl groups (Maulida *et al*., 2018). The surface morphology resembled that of other PLA-based bioplastics (Kasirajan *et al*., 2019).

The total migration of PLA/WG (60:40) into food stimulants distilled water, 10% ethanol and 3% acetic acid was evaluated at 4, 20 and 40°C (Table 4). According to Van't Hoff's rule, diffusion rates doubled with every 10°C increase, enhancing the interaction between the bioplastic and simulants (Sirait *et al*., 2019). At 4°C, no migration was observed, indicating good stability under refrigerated conditions. Slight migration occurred at 20°C for ethanol and acetic acid, suggesting moderate diffusion of components. At 40°C, significant migration was recorded for all

Table 4. Total migration from plasticized PLA/WG (60:40) films into food stimulants

Thermal treatment	Stimulant	Migration (mg/dm²)
4°C	Distilled water	Nd*
	Ethanol (10%)	Nd
	Acetic acid (3%)	Nd
20°C	Distilled water	Nd
	Ethanol (10%)	45.3
	Acetic acid (3%)	9.2
40°C	Distilled water	221.5
	Ethanol (10%)	563.2
	Acetic acid (3%)	297.6

*Nd: Not detected.

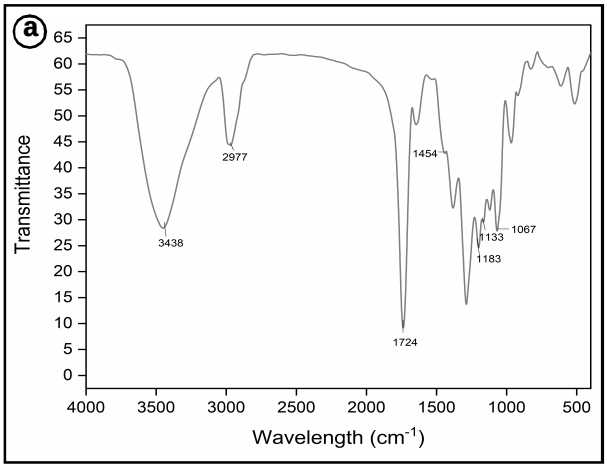

Fig. 7. (a) FTIR spectrum of the produced PLA by direct condensation polymerization process and (b) Scanning microscopic image of PLA at length scale: 1 µm.

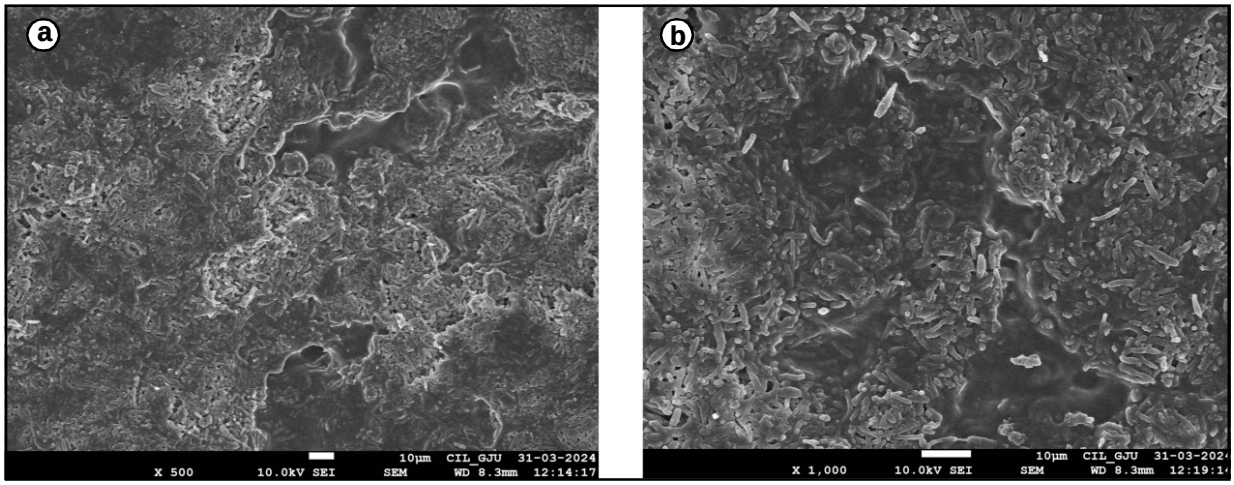

Fig. 8. Scanning microscopic images a) PLA/WG 60:40 at 500x and (b) PLA/WG 60:40 at 1000x

three simulants, attributed to the increased mobility of plasticizers within the PLA/WG matrix. These results indicated that higher temperatures accelerated diffusion and migration of additives, reflecting a relatively low affinity between glycerol and the bioplastic, and highlight the importance of temperature considerations in packaging applications.

CONCLUSION

Mixing PLA with WG as a bioplastic material for packaging is a viable option, as it is safe for humans and other living organisms, cost-effective and environmentally-friendly. The optimal composition for PLA/WG is 60/40 wt%, where the composite achieved its highest tensile strength. This study demonstrated that increasing the WG content enhanced the tensile strength of the composite up to a certain point, after which further WG loading reduced the material's ability to withstand impact. Based on these findings, this bioplastic was suitable for producing food containers and straws, although it should not be exposed to high temperatures and should be used in applications where its fragility and brittleness are not a concern.

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