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Polyhydroxyalkanoates production from food waste using *Lactobacillus casei*

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Abstract

Background: Food waste significantly impacts global economic and environmental systems and poses health risks. Addressing both the accumulation of waste and the demand for eco-friendly products, this study investigated the production of polyhydroxyalkanoates (PHAs) from food waste, integrating sustainable waste management with biopolymer manufacturing.

Methods: *Lactobacillus casei* was cultured in MRS broth supplemented with seven different carbon-rich food wastes, including whey, grape peel, and orange rind. The cultures were incubated at 37°C, with growth and biomass monitored over 48 hours. PHAs were extracted using sodium hypochlorite-chloroform dispersion and chloroform methods, followed by quantitative analysis and Fourier Transform Infrared Spectroscopy (FTIR).

Results: The highest PHA yield was observed from grape peel, generating 1.21 and 0.98 mg/L with sodium hypochlorite-chloroform and chloroform methods, respectively. Orange rind also produced significant yields. FTIR analysis confirmed the presence of essential functional groups, verifying the molecular integrity of the PHAs and their suitability for bioplastic applications.

Conclusion: The study demonstrates the efficacy of using *L. casei* to convert food waste into PHAs, varying by substrate. This approach not only provides a method to reduce food waste but also produces biodegradable plastics, contributing to a circular economy. Further optimization of substrate conditions could enhance PHA yields, increasing the economic and environmental benefits of this technology.

Keywords: *Lactobacillus casei*, Food loss and waste, Fourier transform infrared, Polyhydroxyalkanoates, Circular economy

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Introduction

The global food waste crisis presents a formidable challenge, leading to an estimated annual loss of $940 billion and contributing to 10% of global greenhouse gas emissions (1). Predominantly originating from households, food services, and retail sectors, approximately 931 million tonnes of waste are generated annually (2). Fruits and vegetables contribute significantly to this waste due to their high spoilage rates (3). The improper management of such waste not only results in methane emissions that exacerbate climate change but also leads to nutrient runoff, causing severe water pollution and soil degradation (4,5).

Various organic compounds found in food waste, such as whey, grape peel and pulp, orange peel, carrot pomace, beetroot pulp, and boiled rice water, pose substantial environmental challenges (6). Their disposal can lead to methane emissions, soil contamination, and further water pollution, intensifying the food waste crisis (7,8). Traditional waste management methods—anaerobic digestion, composting, landfilling, and incineration—are often inadequate. Anaerobic digestion, while mitigating waste volume, remains costly and contributes to methane emissions (9). Composting, though beneficial, is time-consuming, resource-intensive, and not suitable for all waste types (10). Landfilling and incineration release toxic gases and pollutants, posing sustainability and health challenges (11).

The principles of the waste hierarchy and circular economy offer more sustainable waste management pathways. Strategies focusing on prevention, reuse, recycling, recovery, and disposal aim to maximize resource utilization and minimize waste (12,13). Furthermore, the successful application of these principles in Sweden illustrates their potential in reducing greenhouse gas emissions and enhancing resource recovery (14). In this context, polyhydroxyalkanoates (PHA) represent a viable solution, turning food waste into biodegradable plastics. Biodegradable plastics are polymers that can be broken down by microorganisms

into natural by-products, such as water, carbon dioxide, and biomass within a defined time frame under specific environmental conditions, reducing their environmental persistence compared to traditional plastics (15). Food waste serves as a feedstock for PHA production, with efficiency determined by its composition, concentration, purity, and the microbial biocatalysts used. During the anaerobic degradation of food waste, volatile fatty acids (VFA) are produced as key intermediates, which are then utilized by microorganisms for efficient PHA synthesis. Direct use or pretreatment may be required to optimize microbial conversion (6). Bacteria from various classes, such as Actinobacteria, Bacteroidetes, Cyanobacteria, Deinococcus-Thermus, Firmicutes, and Proteobacteria, are known to produce different types of PHA using diverse carbon sources (16). The study by Choi et al (17) elucidated the biochemical pathways involved in PHA synthesis, highlighting the two-step process of hydroxyacyl-CoA generation and its subsequent polymerization into PHA, along with the various microbial enzymes involved in this metabolism. *Haloferax mediterranei*, a wild-type strain, has been evaluated for PHA production using whey hydrolysate in a life cycle analysis (18). While they achieved a PHA content of 50% (w/w) per cell dry weight, limitations included extended culture times (>100 h) and a low conversion efficiency of 0.8% at pilot scale. However, halophilic microbes offer advantages such as high salt tolerance, enabling semi-sterile bioprocessing (19).

Likewise, recombinant *Escherichia coli* was found as the most efficient bacterium for PHA production from whey, requiring no hydrolysis due to endogenous enzymes like β-galactosidase (20). Using *E. coli* engineered with *Alcaligenes latus* PHA production genes, Ahn et al (21) achieved high PHB production rates (2.6–4.6 g/h) from concentrated whey, with cell densities reaching 200 g/L in fed-batch systems and final PHA content up to 87% (w/w), significantly simplifying downstream processing. *Lactobacillus casei*, a bacterium prevalent in food waste, shows potential in the PHA production from complex substrates, presenting opportunities for both sustainable waste management and bioplastic production (22-24). This research aimed to harness the untapped potential of food waste—particularly from milk, fruits, and vegetables—for producing PHA, thereby addressing both waste reduction and the creation of sustainable, eco-friendly plastics (25). By integrating the principles of the circular economy, our research seeks to reduce the environmental impact of food waste through innovative repurposing strategies, enhance the sustainability of plastic production by developing biodegradable alternatives, optimize the process of PHA production from various food waste substrates to maximize yield and efficiency, and contribute to global sustainability efforts by transforming food waste into valuable biopolymers.

Materials and Methods

Preparation of Inoculum

The probiotic strain *Lactobacillus casei* ATCC 393TM was sourced from the American Type Culture Collection (ATCC, Virginia, U.S.). An inoculum concentration of 1% (v/v) was prepared in a culture tube using de Man, Rogosa and Sharpe (MRS) broth. The culture was incubated at 37°C for 24 hours, during which the pH was adjusted to 6.5 ± 0.2 to optimize growth conditions (26). This prepared inoculum was subsequently used to ferment the food wastes in the following steps.

Preparation of Fermentation Media

Food wastes were processed to prepare various fermentation media. Milk from a local farm was allowed to curdle; after curdling, it was sterilized by autoclaving at 121°C and 15 psi for 15 minutes. The whey was separated using a muslin cloth or a 20 µm sieve and then clarified by centrifugation at 1500 rpm for 15 minutes (27). Plant materials, including grape peel, grape pulp after juicing, beetroot pulp, carrot pulp, and orange rind, were collected and blended into a coarse paste to facilitate carbohydrate extraction. These were then mixed with water in a 1:4 ratio and gently heated to 50°C to extract soluble carbohydrates without boiling to prevent degradation. Rice water and potato extract were prepared using the same ratio, but were boiled at 100°C for 30 minutes to facilitate the breakdown of starch into simpler sugars.

All prepared extracts underwent filtration through muslin cloth, followed by autoclaving at 121°C and 15 psi for 15 minutes and clarification by centrifugation at 6000 rpm for 3 minutes (26). The processed extracts were cooled and stored at 4-5°C until used in fermentation experiments.

To standardize experimental conditions, eight different media formulations were prepared using MRS broth as the control medium (Table 1). Each medium was created by dissolving MRS broth powder in 30 ml of the prepared food waste extracts, ensuring consistency across all samples. The pH of each medium was adjusted to 6.5 ± 0.2, and subsequently sterilized by autoclaving at 121°C and at 15 psi pressure for 15 minutes. The carbohydrate content in the food waste and media was determined using the Anthrone method (28) before the fermentation process. Similarly, the protein content was estimated using the Lowry method (29), which enables the quantification of proteins before fermentation, providing insights into organic compounds in food waste and media.

Table 1. Media formulations used in the study (M_2 – Whey, M_3 – Grape peel, M_4 – Grape pulp, M_5 – Beetroot pulp, M_6 – Carrot pulp, M_7 – Orange rind, M_8 – Rice water)

Components	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8
MRS broth (in g)	1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66
Food waste (ml)		30	30	30	30	30	30	30
Water (ml)	30	-	-	-	-	-	-	-

Fermentation Process

The fermentation process was initiated by inoculating each of the prepared media with approximately 1% (v/v) of the *Lactobacillus casei* ATCC 393TM inoculum. The fermentation was conducted in an incubator set at 37°C for 48 hours. To monitor microbial growth, samples were collected at 0, 24, and 48 hours. Turbidity measurements at 600 nm were taken at these intervals to facilitate the construction of growth curves. The pH levels were regularly checked and maintained at 6.5 ± 0.2 throughout the fermentation to ensure optimal growth conditions, following the protocols outlined by Kaavessina et al (26). Upon the completion of fermentation, the media were cooled to 4°C for preservation. All fermentation processes were performed in duplicate to ensure reproducibility of the results.

Following the fermentation period, cells from all the media were harvested through centrifugation at 4000 rpm for 20 minutes, effectively separating the cell biomass from the liquid medium. The collected cell pellets were then dried at 60°C until they reached a constant weight, ensuring the complete removal of moisture. This procedure was critical for the accurate determination of dry biomass weight, which was calculated and expressed in grams per litre (g/L). This quantitative analysis of biomass production across the different media was vital for evaluating the efficacy of each substrate in supporting microbial growth and activity (23).

PHA Recovery and Purification

To effectively recover PHA from the cultured biomass, two distinct extraction methods were employed: The sodium hypochlorite-chloroform method and the chloroform method. These approaches were utilized to evaluate the comparative efficiency of each method in maximizing the yield and purity of the extracted PHA.

In the sodium hypochlorite-chloroform method, dried biomass was first ground into a fine powder using a mortar and pestle. One gram of this powdered biomass was then treated with a mixture consisting of 4 ml of sodium hypochlorite and 4 ml of chloroform. The mixture was stirred at 150 rpm at 20°C for 2 hours to ensure thorough integration. After this, 8 ml of water was added, and the mixture was centrifuged at 4000 rpm at 4°C for 3 minutes. This centrifugation formed three distinct layers: An aqueous top layer, a middle layer containing cell debris, and a dense organic bottom layer where the PHA was dissolved in chloroform. The organic layer was carefully extracted and transferred to a Petri dish, where the chloroform was allowed to evaporate, leaving behind the purified PHA. This method is noted for its effectiveness in isolating PHA from complex biomass matrices, providing a relatively pure product suitable for further analysis.

The second method, the chloroform method, simplified the extraction process by reducing the number of chemicals used. Here, the dried biomass was again reduced to a fine powder. About 10 ml of chloroform was added to 1 gram of this powder. The mixture was then placed in a shaker incubator set at 70°C and 150 rpm for 24 hours to ensure complete mixing and reaction. Following the incubation period, the mixture was stirred at 6500 rpm at 4°C for 15 minutes to facilitate phase separation. The organic phase containing the PHA was then carefully isolated and poured into a Petri dish for drying. This method emphasizes simplicity and efficiency, offering an alternative approach that still effectively extracts PHA from processed biomass.

Quantification and Characterization of PHA

The yield of PHA from different media was quantified using a spectrophotometric method, where 5 ml of sulfuric acid was added to 1 g of the extracted PHA. This mixture was heated at 100°C in a hot air oven for one hour to convert the PHA into crotonic acid. Crotonic acid working standards were prepared using sulfuric acid as a blank to calibrate the equipment. The absorbance of these samples and the standards was measured at 235 nm using a UV spectrophotometer, providing a quantitative measure of the PHA concentration (30). A one-way analysis of variance (ANOVA) was performed to evaluate the significance of differences in biomass production and PHA yield among the media. P-values < 1 were considered significant.

Following quantification, the chemical structure of the isolated polyhydroxyalkanoates was characterized using Fourier Transform Infrared Spectroscopy (FTIR). Samples were embedded in potassium bromide (KBr) discs, and their infrared spectra were captured with a Perkin-Elmer Fourier transform infrared spectrophotometer. The spectra were recorded across a wavenumber range from 400 to 4000 cm^{-1}, enabling the identification of characteristic functional groups. This analysis confirmed the polymer's composition, providing insights into its molecular structure and potential applications (23).

Results

Carbohydrates and Protein Estimation of Food Wastes and Media

Carbohydrate concentrations varied significantly among different food wastes. Grape peel exhibited the highest concentration at 10.23 g/L, while rice water contained the lowest one at 5.17 g/L, highlighting the substantial variability in fermentable sugar content (Figure 1a). In the fermentation media, the one containing grape peel extract (medium M3) showed the highest carbohydrate concentration at 13.85 g/L, compared to the medium containing only MRS broth (Medium M1), which had the lowest carbohydrate concentration at 3.62 g/L (Figure 1b). These findings highlight the impact of carbohydrate variability on the composition and functionality of

fermentation media, potentially affecting the growth kinetics and metabolic activities of *Lactobacillus casei* ATCC 393TM.

Similarly, protein concentrations in the selected food wastes also showed notable variation. Grape peel had the highest protein concentration at 5.31×10^{-3} g/L, whereas carrot exhibited the lowest at 2.00×10^{-3} g/L (Figure 2a). Among the fermentation media, the medium enriched with grape peel extract (medium M3) exhibited the highest protein concentration at 9.15×10^{-3} g/L. In contrast, the medium with only MRS broth (medium M1) had the lowest protein level at 4.30×10^{-3} g/L (Figure 2b). These variations highlight the significance of protein content in both food waste and fermentation media, as it has a substantial impact on microbial growth and metabolic activity. These factors are crucial for effective fermentation and subsequent PHA production, suggesting that optimal nutrient availability is key to enhancing biotechnological processes (31).

Growth Curve of L. casei in Different Media

The growth dynamics of *L. casei* were monitored over 48 hours in eight different fermentation media. Interestingly, medium M7, which contains orange rind extract, demonstrated the highest growth rate despite having lower protein and carbohydrate concentrations compared to medium M3, which is supplemented with grape peel extract. This unexpected result suggests that components other than traditional macronutrients in the orange rind may create a more favourable environment for the growth of *L. casei*.

In contrast, medium M1, which consists solely of MRS broth, showed the least microbial growth. This highlights the substantial role that additional nutrients and bioactive compounds in food waste extracts play in enhancing microbial proliferation (Figure 3). These observations strongly indicate that food waste substrates significantly influence the growth patterns of *L. casei*, reinforcing the idea that integrating these substrates into fermentation media not only helps waste utilization but also promotes enhanced microbial fermentation processes. Such enhancements are crucial for the effective synthesis of bioproducts, suggesting a pivotal role for food waste in optimizing biotechnological applications (32).

Biomass Concentration Obtained from Different Media

The concentration of biomass in various fermentation media was assessed to evaluate the impact of substrate composition on cellular growth and the accumulation of organic compounds. Remarkably, medium M2, which contains whey, exhibited the highest biomass concentration at 43 g/L (Figure 4). This suggests that whey

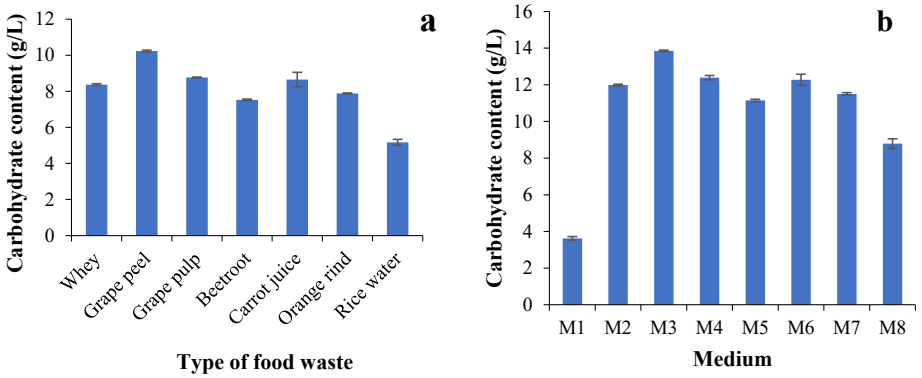

Figure 1. Diagram of carbohydrate content of (a) food wastes, (b) media (M1 – MRS broth, M2 – Whey, M3 – Grape Peel, M4 – Grape Pulp, M5 – Beetroot Pulp, M6 – Carrot Pulp, M7 – Orange Rind, M8 – Rice Water). Standard deviation is indicated for each bar.

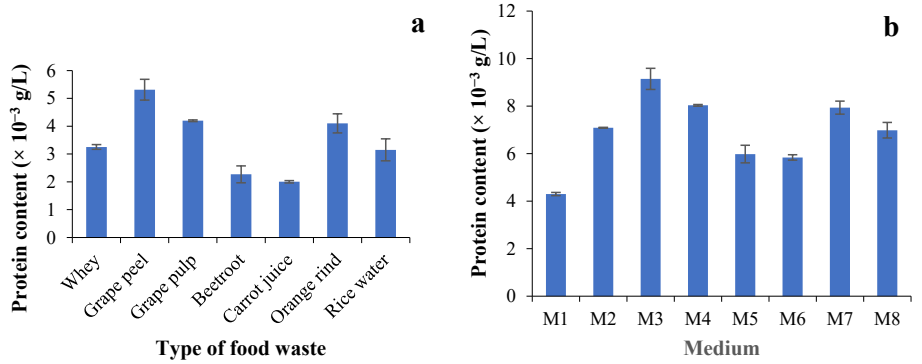

Figure 2. Diagram of protein content of (a) food wastes, (b) media (M1 – MRS broth, M2 – Whey, M3 – Grape Peel, M4 – Grape Pulp, M5 – Beetroot Pulp, M6 – Carrot Pulp, M7 – Orange Rind, M8 – Rice Water). Standard deviation is indicated for each bar.

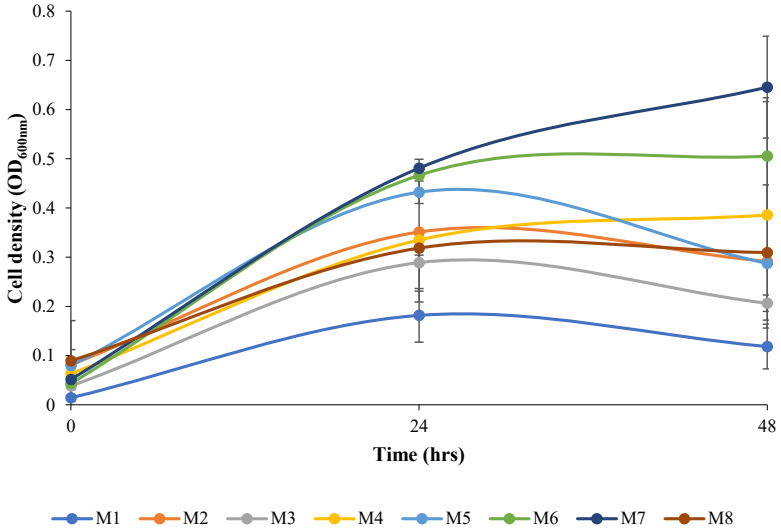

Figure 3. Diagram of growth curve of *L. casei* in different media (M_1 – MRS broth, M_2 – Whey, M_3 – Grape peel, M_4 – Grape pulp, M_5 – Beetroot pulp, M_6 – Carrot pulp, M_7 – Orange rind, M_8 – Rice water)

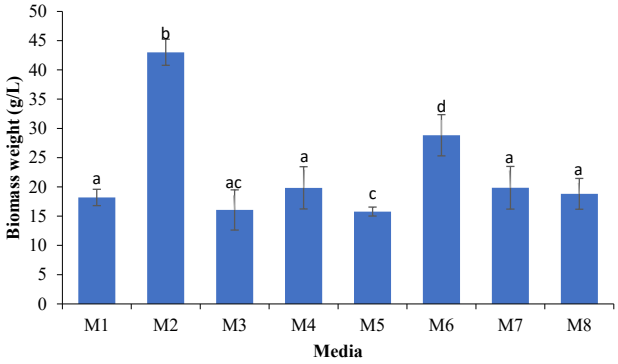

Figure 4. Diagram of biomass obtained from different media (M1: MRS broth, M2: Whey, M3: Grape peel, M4: Grape pulp, M5: Beetroot pulp, M6: Carrot pulp, M7: Orange rind, M8: Rice water). Standard deviation is indicated for each bar. Bars labeled with the same letters are not statistically different ($P > 0.05$).

provides not only essential nutrients but also favourable conditions for prolific microbial growth. In stark contrast, medium M1, which consists solely of MRS broth, yielded the lowest biomass concentration at 18.2 g/L.

Interestingly, while media M3 (grape peel) and M7 (orange rind) did not produce the highest biomass levels, they demonstrated moderate to high growth rates. This observation implies that factors beyond the initial nutrient concentration—potentially including the presence of bioactive compounds or unique micronutrients in food waste—may significantly influence biomass accumulation (33).

Effect of Different Food Wastes on PHAs Production

The production of PHAs was significantly influenced by the type of food waste substrate used in the fermentation media, as analyzed using two different extraction methods: sodium hypochlorite-chloroform and chloroform alone. Quantitative results, which are visually represented in Figure 5, demonstrate the variable efficacy of these methods. Medium M3, which includes grape peel extract, exhibited the highest PHA production, yielding 1.21 mg/L with the sodium hypochlorite-chloroform method and 0.98 mg/L with the chloroform method. These high yields underscore the grape peel's substantial impact on PHA production, likely due to its high carbohydrate and protein content, which enhances microbial activity and biopolymer synthesis. Similarly, medium M7, containing orange rind extract, also demonstrated significant PHA production, yielding about 1.14 mg/L and 0.88 mg/L with the two methods, respectively.

Conversely, medium M1, which contains only MRS broth and lacks supplementary food waste nutrients, showed the least PHA production. This stark contrast highlights the crucial role of additional nutrients from food waste in supporting the metabolic processes necessary for PHA synthesis. These findings emphasize the need to optimize food waste composition to enhance PHA yield effectively. Notably, the sodium hypochlorite-chloroform method generally resulted in higher PHA purity and yield than the chloroform method alone, indicating its superior effectiveness in extracting PHAs from complex biomass matrices. This comparative analysis not only sheds light on the potential of utilizing food waste substrates for sustainable bioproduct synthesis but also suggests that selecting and optimizing extraction methods are critical for maximizing PHA production efficiencies (34).

Characterization of PHAs by FTIR

The FTIR spectra of PHAs produced by *L. casei* across various media (M1 – MRS broth, M2 – Whey, M3 – Grape peel, M4 – Grape pulp, M5 – Beetroot pulp, M6 – Carrot pulp, M7 – Orange rind, M8 – Rice water) demonstrated the presence of characteristic functional groups (Figure 6; Table 2). The spectra consistently exhibited an absorption

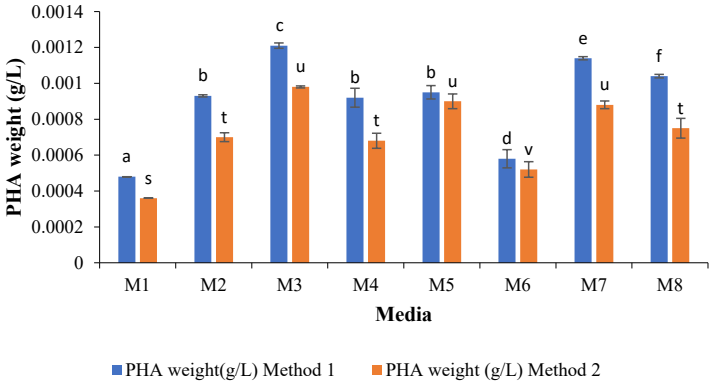

Figure 5. Diagram of PHA yield obtained using the Sodium Hypochlorite-Chloroform Grape peel, M4: Grape pulp, method, and the Chloroform method across different media (M1: MRS broth, M2: Whey, M3: M5: Beetroot pulp, M6: Carrot pulp, M7: Orange rind, M8: Rice water). Standard deviation is indicated for each bar. Bars labelled with the same letters within individual methods are not significantly different from each other ($P > 0.05$).

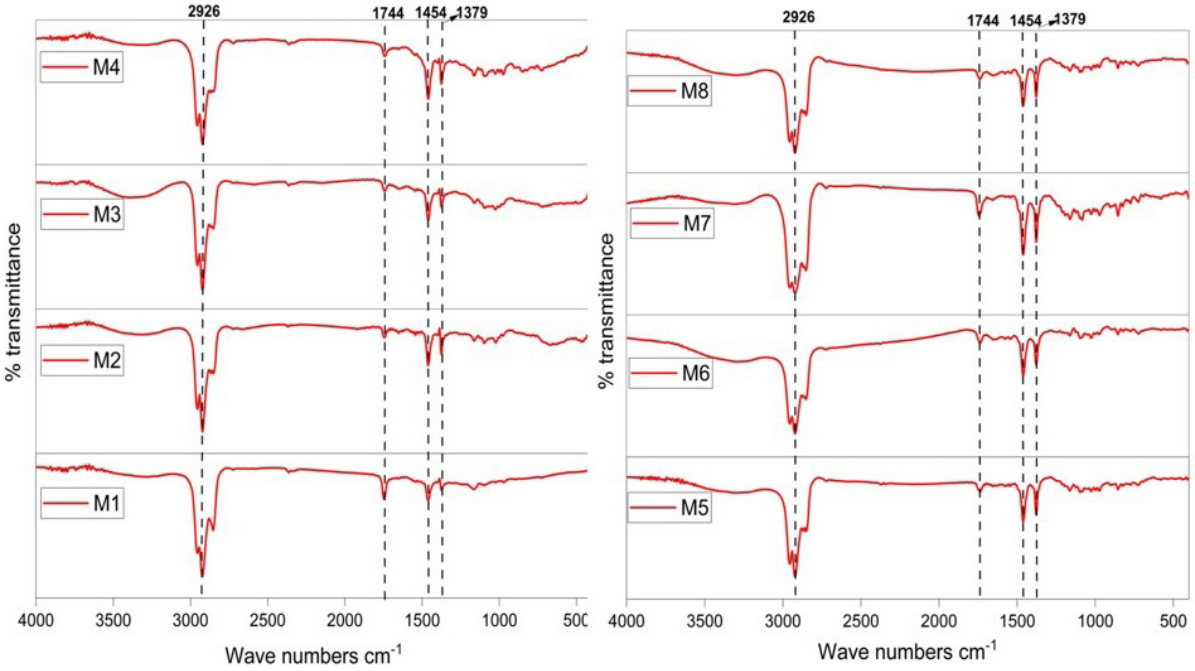

Figure 6. FTIR spectrum of PHA produced by *L. casei* ATCC 393TM utilizing different media (M1: MRS broth, M2: Whey, M3: Grape peel, M4: Grape pulp, M5: Beetroot pulp, M6: Carrot pulp, M7: Orange rind, M8: Rice water). M0 is a blank spectrum.

Table 2. Major peaks identified in the FTIR spectra of PHAs

Major Peaks (cm^{-1})	Groups	Attributes	References
2926, 2983	$-CH_3$ and $-CH_2$	Asymmetric and symmetric stretching of hydrocarbon chains	(36)
1379	$-CH_3$	Methyl group vibration, part of the polymer's structure	(36)
1454	-CH2	Methylene group vibration, part of the polymer's structure	(36)
922	Alkyl stretch	Characteristics of the alkyl groups in PHAs	(37)
1228 - 1279	C-O-C	Carbon-oxygen-carbon stretching of esters, part of the polymer	(38,39)
1720 - 1744	Ester C=O	Ester carbonyl group, indicative of the PHA backbone	(40,41)
1600	Carboxylic stretch vibration	Indicative of carboxylic groups in the polymer	(42,43)

peak corresponding to ester carbonyl (C=O) groups around 1744 cm^{-1}, indicative of the ester linkages within the PHA polymer backbone (35). Additionally, the spectra showed peaks at 2926 cm^{-1} and 2963 cm^{-1} attributed to the asymmetric and symmetric stretching vibrations of CH_2 and CH_3 groups, respectively. These aliphatic stretches are characteristic of the hydrocarbon chains in PHAs, as noted in previous studies (36).

Absorption bands were also observed at 1379 cm^{-1} and 1454 cm^{-1}, corresponding to the aliphatic $-CH_3$ and

-CH_2 groups, respectively (36). These bands indicate the polymer's methyl and methylene vibrations, further corroborating the presence of PHA. The spectra also revealed peaks between 1228 $cm^{-1}$and 1279 cm^{-1}, characteristic of the carbon-oxygen-carbon (C-O-C) stretching of esters (37). This is consistent with the ester linkages in the PHA polymer. The absorption band at 922 cm^{-1} was predicted to be due to the alkyl stretch, aligning with previous research findings (38).

These common peaks across all media indicate that *L. casei* can produce PHA with consistent chemical structures, regardless of the medium used. The characteristic functional groups identified by FTIR analysis—such as ester C=O, CH_3, CH_2, and hydroxyl (-OH) groups—confirm the successful synthesis of PHAs (39). The FTIR analysis of PHAs produced by *L. casei* ATCC 393™ in different media revealed the presence of consistent functional groups, including ester carbonyl (C=O), aliphatic CH_2, CH_3, and hydroxyl (-OH) groups. These findings confirm the successful synthesis of PHA across all tested media, demonstrating the robustness of *L. casei* in producing PHAs with a stable chemical structure.

Discussion

The significant variability in carbohydrate and protein concentrations across different food wastes highlights the potential of these substrates to influence fermentation processes (44). The findings of this study align with previous research, which suggests that nutrient-rich substrates can enhance microbial metabolic activities. The higher carbohydrate and protein contents in grape peel compared to other wastes like rice water and carrot indicate that substrates with rich nutrient profiles can significantly boost the activity of microorganisms such as *Lactobacillus casei* ATCC 393TM (45). Similar results were observed in other studies, where nutrient-rich food wastes, when fermented, were found to boost microbial bioproduct yield (46). This finding reinforces the importance of substrate selection in bioprocess optimization.

The growth dynamics of *L. casei* in various media reveal the crucial role of substrate composition. Interestingly, the high growth rate in the orange rind extract medium (M7), despite its lower carbohydrate and protein concentrations compared to the grape peel extract medium (M3), suggests that factors beyond macronutrient content, such as bioactive compounds or micronutrients, play a significant role in promoting microbial growth. These components may include vitamins, antioxidants, and other phytochemicals known to enhance microbial health and metabolic activity. The presence of such compounds suggests that the composition of food waste substrates might include key factors that can optimize microbial growth, even in media with relatively lower carbohydrate and protein content (47).

The evaluation of biomass concentration reinforced the importance of substrate composition (48). Medium M2 (whey) yielded the highest biomass concentration, indicating its superior nutrient profile for supporting microbial growth (49). The moderate biomass levels in media M3 and M7 further suggest that factors beyond simple nutrient content, such as specific bioactive compounds, significantly influence biomass accumulation. These results highlight that, while macronutrient content is essential, the presence of certain bioactive compounds plays a significant role in biomass production, suggesting that food waste substrates containing these compounds could provide enhanced microbial growth conditions.

PHA production varied considerably depending on the food waste substrate used. Medium M3 (grape peel extract) achieved the highest PHA yield, which can be attributed to its high carbohydrate and protein content that promotes microbial activity and biopolymer synthesis. The lower yields in medium M1 reaffirm the necessity of nutrient-rich substrates for effective PHA production. This confirms that food waste substrates with higher nutrient content lead to better biopolymer synthesis, making them more efficient for PHA production. Additionally, the comparative analysis of extraction methods demonstrated that higher PHA yields obtained using the sodium hypochlorite-chloroform method compared to the chloroform-only method can be attributed to the more effective breakdown of cellular material and release of PHAs by the former method (50). Sodium hypochlorite acts as a strong oxidizing agent, facilitating the disruption of cell walls and membranes, thereby improving PHA recovery. The superior performance of the sodium hypochlorite-chloroform extraction method suggests that further optimization and scaling of this method could improve the economic viability of PHA production (51). Exploring alternative, less hazardous oxidative agents or combining physical disruption techniques with chemical extraction could further enhance efficiency and sustainability.

FTIR analysis confirmed the consistent presence of characteristic PHA functional groups across all media. In line with previous studies, the main peak for standard PHA is typically observed between 1724–1744 cm^{-1}, corresponding to the ester carbonyl (C=O) group, a key feature of the PHA backbone (40,41). In our samples, a strong absorption peak was consistently detected at 1744 cm^{-1}, aligning with this standard range and confirming the presence of ester C=O bonds. Additionally, aliphatic CH_2 and CH_3 stretching vibrations were observed at 2926 and 2983 cm^{-1}, respectively, further validating the presence of hydrocarbon chains in the polymer structure. These findings are consistent with earlier reports of PHAs produced by different microbial strains and substrates under varying conditions (35,36,42,43). This analysis further validates the consistent quality of PHA produced

from food waste substrates, suggesting that microbial fermentation of food waste is a reliable method for synthesizing bioplastics.

Further research should investigate the potential of optimizing the extraction process to enhance yield and purity while minimizing the use of harsh chemicals. Exploring alternative, environmentally friendly oxidative agents or combining physical and chemical extraction techniques could offer a more sustainable approach. Additionally, a detailed analysis of the residual impurities in the PHA samples, particularly those indicated by the hydroxyl group absorption, could provide insights into refining the purification process. This approach would not only enhance the quality and applicability of PHAs but also contribute to the development of more sustainable and eco-friendly biopolymer production methods.

Conclusion

This study investigated the potential of various food wastes as substrates for PHAs production using *Lactobacillus casei*, investigating the impacts of food wastes on the microbial growth kinetics and PHA production. Significant variations in carbohydrate and protein concentrations were observed among different media, with grape peel (medium M_3) exhibiting the highest values, correlating with enhanced microbial growth and substrate utilization. Despite lower nutrient content compared to M_3, orange rind (medium M_7) led to the highest growth rate for *L. casei*, suggesting additional nutritional or bioactive components enhancing microbial growth. Whey-based media (M_2) yielded the highest biomass production, indicating its role in fostering microbial proliferation. PHA production peaked in media containing grape peel (medium M_3), emphasizing the importance of substrate selection for optimal production. FTIR spectroscopy confirmed the presence of typical PHA functional groups, validating their suitability for biodegradable material applications. This research advocates for utilizing food waste as sustainable PHA substrates, stressing the importance of substrate selection and fermentation optimization for maximizing yield and quality. Overall, it contributes to sustainable biotechnology and waste management practices, promoting the circular economy and reducing environmental impacts in line with global sustainability efforts.

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Authors' contributions

Conceptualization: Kaviya Amuthasekaran, Naseer Hussain.
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Formal analysis: Kaviya Amuthasekaran, Naseer Hussain.
Funding acquisition: Naseer Hussain.
Investigation: Kaviya Amuthasekaran, Naseer Hussain.
Methodology: Kaviya Amuthasekaran, Naseer Hussain.
Project administration: Naseer Hussain.
Resources: Naseer Hussain.
Software: Kaviya Amuthasekaran.
Supervision: Naseer Hussain.
Validation: Naseer Hussain.
Visualization: Kaviya Amuthasekaran.
Writing–original draft: Kaviya Amuthasekaran.
Writing–review & editing: Naseer Hussain.

Competing interests

There are no competing interests.

Ethical issues

None.

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