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Upcycling waste apples into platform chemicals: pilot-scale production of lactic and succinic acids

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Abstract

The conversion of renewable biological resources into value-added products is essential for supporting the transition to a sustainable bioeconomy. This study explores the use of waste apples—a byproduct generated in large quantities along the fresh apple supply chain—as feedstock for the production of two industrially relevant platform chemicals: lactic acid (LA)

and succinic acid (SA). A screening identified *Heyndrickxia coagulans* A203 and *Actinobacillus succinogenes* DSM 22257 as the most suitable strains for LA and SA production from apple biomass. A simplified procedure, omitting costly centrifugation and sterilization steps between enzymatic liquefaction and fermentation, was successfully scaled up, resulting in 73.8 g·L⁻¹ LA with a yield of 0.91 g·g⁻¹ and 36.8 g·L⁻¹ SA with a yield of 0.69 g·g⁻¹. The results demonstrate the potential of waste apples as a low-cost, effective feedstock for the biotechnological production of high-value platform chemicals, contributing to the valorization of agri-food waste within a sustainable and circular bioeconomy.

1. Background

The transition toward a circular bioeconomy necessitates the development of innovative strategies aimed at minimizing waste and enabling the conversion of residual biomass into value-added products [1]. In response to this global challenge, numerous initiatives promoting circularity and sustainability have been implemented globally. Among these, the Bioeconomy Strategy of the European Union (EU) is notable for supporting the use of biowaste—including food and agricultural residues—as feedstock for the production of biobased products such as platform chemicals, biomaterials, and bioenergy [2]. Within this context, the valorization of agri-food waste into high-value biobased compounds represents a viable approach to waste reduction and sustainable resource management [3, 4].

Among the various types of agri-food waste, surplus and discarded apples—a byproduct generated throughout the fresh apple supply chain—represent promising feedstock due to their availability and biochemical composition [5]. The EU, one of the world's leading apple producers, harvested 11.5 million tons in 2023 [6], a substantial quantity that also generates significant waste. In fact, estimates indicate losses of 20.3% in primary production (pre- and postharvest), 1.6% in processing and manufacturing, 9.6% in distribution, and

17.7% in consumption [7]. Once these apples are no longer suitable for food or feed use due to safety, quality, or technical constraints and in line with the cascade use principle outlined in the EU Waste Framework Directive [8], they can be redirected to the production of platform chemicals, presenting a valuable opportunity for resource recovery within the framework of biorefineries [3, 9].

Platform chemicals are significant because of their versatility as intermediates that can be transformed into valuable compounds across multiple industries [10]. In particular, lactic acid (LA) and succinic acid (SA) have diverse applications in the food and beverage, pharmaceutical, and cosmetic sectors. Moreover, their use as monomers in the production of polylactic acid (PLA) and polybutylene succinate (PBS) has attracted increasing interest, as these bioplastics offer environmentally friendly alternatives to fossil-based materials [11, 12].

Owing to its content of fermentable sugars and nutrients [5], apple biomass represents a compelling substrate for fermentation processes aimed at the production of LA and SA. LA production has focused primarily on the use of apple pomace at the laboratory scale under batch and repeated-batch operation modes [13, 14], whereas SA production has focused mainly on process modeling and technoeconomic analyses related to the utilization of apple pomace [15, 16]. These studies provide an initial assessment of the potential of apple biomass as a substrate for LA and SA production; however, research on pilot-scale processes involving waste apples is currently nonexistent. Although pilot-scale studies have been conducted with other types of food waste [17-20], no such studies have been carried out on waste apples, highlighting a critical gap in the development of strategies for repurposing this residual biomass.

The efficiency of fermentation processes depends not only on the substrate but also the selection of appropriate microbial strains. *Heyndrickxia coagulans* (formerly *Bacillus coagulans*) is commonly used for LA production due to its high yields and productivities.

This microorganism has been evaluated on a variety of substrates and has demonstrated the ability to ferment both C5 and C6 sugars and to tolerate a broad range of inhibitory compounds that are commonly present in biomass-derived media [14, 21, 22]. Additionally, its ability to operate at elevated temperatures reduces the risk of contamination, further enhancing its suitability for industrial applications [20]. With respect to SA production, compared with other strains, such as *Basfia succiniproducens*, *Actinobacillus succinogenes* has emerged as a promising candidate due to its ability to convert both pentoses and hexoses into SA and achieve higher yields [23, 24].

Based on the above background, this study investigates the use of waste apples as feedstock for the pilot-scale production of LA and SA. A screening was performed using LA- and SA-producing bacteria to identify the most efficient strain for converting apple biomass. A simplified and less energy-intensive process employing the selected strains (*H. coagulans* A203 and *A. succinogenes* DSM 22257) was effectively scaled up, representing an innovative approach to exploring and establishing the pilot-scale production of LA and SA from waste apples while providing a comprehensive account of the entire process.

2. Methods

2.1. Raw material and preparation

Jonagold apples (*Malus domestica* ‘Jonagold’), purchased from Werder Frucht GmbH (Groß Kreutz, Germany), were provided by the Department of System Process Engineering at the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB, Potsdam, Germany) after they reached conditions unsuitable for consumption. A portion of the apples had already undergone natural fermentation, and others showed visible mechanical damage. In addition, signs of insect infestation were detected, further compromising their suitability for direct

consumption. The apples were pressed using a screw press (Type Cv, VETTER Maschinenfabrik GmbH & Co. KG, Kassel, Germany), with a sieve pore size of 0.8 mm and a gap width of 10 mm. The resulting apple mash (AM) contained $71.6 \pm 1.5 \text{ g}\cdot\text{L}^{-1}$ fructose, $21.5 \pm 0.7 \text{ g}\cdot\text{L}^{-1}$ glucose, and $7.5 \pm 0.3 \text{ g}\cdot\text{L}^{-1}$ sucrose and was frozen at $-20 \text{ }^{\circ}\text{C}$ until further use. The solid fraction, composed mainly of peel and seeds, was not evaluated in this study.

2.2. Enzymatic treatment of apple mash

2.2.1. Screening of enzymes

To increase the fermentable sugar content in the AM and reduce its viscosity, the enzymes Pektinase L40 (ASA Spezialenzyme GmbH, Wolfenbüttel, Germany), Cellic CTec3 HS, Dextrozyme GA and Viskoferm (Novozymes A/S, Bagsvaerd, Denmark) were separately evaluated. The experiments were carried out in duplicate in shaking flasks containing 50 g of AM and incubated at the original pH (4.5), $50 \text{ }^{\circ}\text{C}$ and 150 rpm for 24 h. The enzyme dosage was $1.0 \text{ mL}_e\cdot\text{kg}_{\text{bm}}^{-1}$. The combination of Pektinase L40 and Cellic CTec3 was also evaluated under the same conditions at two levels: high loading ($1.0 \text{ mL}_e\cdot\text{kg}_{\text{bm}}^{-1}$ each) and low loading ($0.5 \text{ mL}_e\cdot\text{kg}_{\text{bm}}^{-1}$ each). A control experiment without the addition of enzymes was included for comparison.

2.2.2. Enzymatic liquefaction of apple mash for microorganism screening

After the most effective enzymes and their optimal concentrations were selected, the AM was enzymatically liquefied for subsequent use as a medium for microorganism screening. AM (1.0 or 2.0 kg) was transferred to a sterilized bioreactor (Biostat Bplus, Sartorius AG, Göttingen, Germany), where the enzymes Cellic CTec3 and Pektinase L40 were added at a concentration of $0.5 \text{ mL}_e\cdot\text{kg}_{\text{bm}}^{-1}$ each. The reaction was carried out at the original pH (4.5), 50

°C and 100 rpm for 24 h, after which the resulting medium was centrifuged at 5,000 rpm for 15 min to remove solid particles.

This process was repeated using 5.6 kg of AM to generate sufficient medium for inoculum cultivation in the prepilot validation tests and pilot-scale fermentations.

2.3. Screening of microorganisms

To evaluate whether AM can support microbial growth and to identify the most adaptable LA- and SA-producing strains, screenings were conducted.

For LA production, *Heyndrickxia coagulans* strains A35, A107, A138, A203, and A559, which were all isolated at ATB and preserved in its internal collection, were selected from 700 strains based on their ability to consume sucrose and their performance in previous studies [20, 21]. The strains were reactivated from cryostock in 5 mL of MRS broth (Merck, Darmstadt, Germany) at 50 °C for 16–18 h.

For SA production, all available SA-producing strains, *Actinobacillus succinogenes* DSM 22257 and *Basfia succiniproducens* DSM 22022, CCUG 57762, CCUG 57763, CCUG 57764, CCUG 57765, and CCUG 57766, obtained from the Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany) and the Culture Collection University of Gothenburg (Gothenburg, Sweden), were also tested. Reactivation from the cryostock was performed in 5 mL of tryptic soy broth (TSB) (Merck, Darmstadt, Germany) at 37 °C for 16–18 h.

Microorganism screening was conducted in duplicate in a Bioscreen C (Norden Logic Oy, Helsinki, Finland) optical density (OD) meter using AM supernatant (*Section 2.2.2*), both with and without supplementation of 5 g·L⁻¹ yeast extract (Ohly GmbH, Deutsche Hefewerke GmbH & Co. OHG, Hamburg, Germany). MRS broth served as the control for LA producers, whereas nutrient broth (Merck, Darmstadt, Germany) was used as the control for SA

producers. AM-based medium was adjusted to a pH of 6.5–7.0 with 20% v/v NaOH and subsequently sterile-filtered using a 0.2 µm filter, while the control medium was autoclaved following the manufacturer’s instructions. Measurements were taken automatically every 5 min for 24 h in 250 µL of medium with 20 µL of inoculum. The incubation temperature was set to 50 °C for LA bacteria and 37 °C for SA bacteria.

The best-performing strains were selected for screening in bioreactors.

2.4. Screening of microorganisms in bioreactors

2.4.1. Inoculum preparation

H. coagulans strains A35, A138, and A203 were used in LA fermentations. Inocula were cultivated in 60 mL of MRS broth with 0.67 g of Everzit Dol dolomite (Evers, Hopsten, Germany) as a buffer at 40 °C and 100 rpm for 15 h.

For SA fermentations, *A. succinogenes* DSM 22257 was initially grown in 25 mL of TSB at 37 °C and 150 rpm for 20 h.

2.4.2. Fermentation

Fermentations were performed in duplicate in 0.25 L working-volume bioreactors (EloFerm Duet, EloSystems GbR, Berlin, Germany).

LA fermentations were carried out in 0.25 L of nonsterile AM supernatant (Section 2.2.2) supplemented with 10 g·L⁻¹ powdered yeast extract and inoculated at 2% (v/v). The yeast extract concentration was increased relative to the OD-based microorganism screening (Section 2.3) to ensure adequate nutrient availability for the strain during fermentation, compensating for the high sugar content in the AM. The temperature was maintained at 50 °C, and the pH was controlled at 6.0 using 20% v/v NaOH with a stirring speed of 200 rpm.

The most efficient strain was further evaluated under identical conditions in the absence of yeast extract supplementation.

SA fermentations were conducted using 0.20 L of sterilized (121 °C for 15 min) AM supernatant (*Section 2.2.2*) supplemented with 10 g·L⁻¹ of yeast extract and 1 g·L⁻¹ of NaHCO₃ (VWR Chemicals, Darmstadt, Germany), added as a 25 mL sterile solution. The inoculum was added at 10% v/v, and fermentations occurred at 37 °C, with the pH maintained at 6.7 using 20% w/v Na₂CO₃ at a stirring speed of 200 rpm. A different setup for SA fermentation was required to meet the metabolic needs of the microorganism, which included a carbon source to support carbon fixation and sterile conditions due to its lower optimal growth temperature. Additionally, preliminary tests (unpublished data) indicated a limitation in cell performance at higher sugar concentrations, which further justified the use of smaller quantities of AM and its subsequent dilution. SA fermentations without yeast extract supplementation were also conducted under the same conditions detailed previously.

2.5. Prepilot-scale validation test

Following strain selection, a simplified procedure was tested in duplicate in 1.0 L working-volume bioreactors (Biostat Bplus, Sartorius AG, Goettingen, Germany) prior to implementation at the pilot scale. Unlike the approaches described in *Sections 2.2.2* and *2.4.2*, enzymatic liquefaction and fermentation were sequentially performed in the same bioreactor, without centrifugation or sterilization (for SA) of the medium prior to fermentation. Inoculum and fermentation procedures were conducted as follows.

2.5.1. Inoculum preparation

To serve as the preculture for LA fermentations, *H. coagulans* A203 was cultivated under sterile conditions for 15 h in a 0.2 L working-volume bioreactor (EloFerm Duet, EloSystems

GbR, Berlin, Germany) using 160 mL AM supernatant (*Section 2.2.2*) and 10 g·L⁻¹ yeast extract supplied as a 40 mL solution under the same conditions detailed in *Section 2.4.2*.

A. succinogenes DSM 22257 was grown for 16 h in 0.25 L working-volume bioreactors, as outlined in *Section 2.4.2*.

2.5.2. Enzymatic liquefaction and fermentation

For LA fermentations, 1.0 kg of AM underwent enzymatic liquefaction under the conditions described in *Section 2.2.2*. After 24 h, the medium was directly supplemented with 10 g·L⁻¹ powdered yeast extract, and fermentation was initiated at the temperature, pH, stirring speed, and inoculum concentration conditions specified in *Section 2.4.2*.

SA fermentations were conducted with 0.8 kg of AM. Following enzymatic liquefaction, the medium was heated to 85 °C for 15 min to inhibit the growth of competing microorganisms at a mild fermentation temperature (37 °C). The medium was then supplemented with 10 g·L⁻¹ yeast extract and 1 g·L⁻¹ NaHCO₃, added as a 0.1 L sterile solution. The fermentation parameters were consistent with those detailed in *Section 2.4.2*.

2.6. Process scale-up

The scaling-up of the process was carried out in a Biostat UD bioreactor (B. Braun Biotech International, Germany) for both LA and SA fermentations, each performed in duplicate. Compared with the lab-scale protocol (*Section 2.5*), the pilot-scale procedure required modifications to handle the increased working volume. The overall strategy remained consistent with the lab-scale protocol presented in *Section 2.5* and is summarized below. However, the inocula were prepared in larger bioreactors to ensure sufficient cell density, and medium handling required careful monitoring to maintain homogeneity and prevent contamination.

226

227 2.6.1. *Inoculum preparation*

228 For LA pilot-scale fermentations, *H. coagulans* A203 was cultivated for 15 h in a 0.5 L
229 working-volume bioreactor (EloFerm Duet, EloSystems GbR, Berlin, Germany), as described
230 in *Section 2.5.1*.

231 Inocula for SA fermentations were also prepared following the procedure in *Section*
232 *2.5.1*, with the distinction that cultivation was carried out in a 2.1 L working-volume
233 bioreactor (Biostat Bplus, Sartorius AG, Goettingen, Germany).

234

235 2.6.2. *Enzymatic liquefaction and fermentation*

236 During the pilot-scale experiments, a total of 30.0 kg of AM was used for 30.0 L of LA
237 fermentation, and 20.0 kg of AM was used for 25.0 L of SA fermentation. The procedure
238 outlined in *Section 2.5.2*, comprising enzymatic liquefaction, inactivation (for SA), and
239 fermentation, was strictly followed, along with the corresponding fermentation parameters.

240

241 2.7. *Sampling and termination of fermentations*

242 For each fermentation described in *Sections 2.4.2, 2.5.2, and 2.6.2*, one sample was collected
243 at 2-h intervals, inactivated at 95 °C for 20 min, and stored at –20 °C for subsequent
244 quantification of sugars and organic acids. Fermentations were terminated when base
245 consumption ceased.

246

247 2.8. *Analytical methods*

248 Sugars and organic acid concentrations were determined by high-performance liquid
249 chromatography (HPLC) using an Azura system (Knauer, Berlin, Germany) equipped with a
250 Eurokat H column (300 mm × 8 mm, Knauer, Germany) with 0.01 N H₂SO₄ as the mobile

phase at a flow rate of $0.8 \text{ mL} \cdot \text{min}^{-1}$. For comparisons between two group means, a t test was performed. For comparisons among three or more group means, one-way ANOVA followed by Tukey's test was used to identify significant differences between groups. Yield was calculated as grams of product formed per gram of sugar consumed, and productivity was calculated based on the end of base consumption.

3. Results and discussion

3.1. Enzymatic treatment of apple mash

Although enzymes are widely used in bioconversion processes to enhance overall performance, for instance, by increasing the availability of fermentable sugars [20, 21] or degrading inhibitory compounds [25], in the case of AM, the application of various enzymes did not result in a significant increase in total reducing sugars (TRS) compared with the control ($p > 0.05$), as shown in Table 1. This outcome is likely attributed to the absence of starch, which is typically converted into glucose, fructose and sucrose during apple ripening [26], as well as the minimal levels of cellulose and hemicellulose in the dry matter, which was also relatively low ($11.1 \pm 0.0\%$). Consequently, the amylase-based preparation Dextrozyme GA, along with cellulase- and hemicellulase-based enzyme cocktails Cellic CTec3 and Viskoferm, had limited substrate available for hydrolysis.

Table 1. Total reducing sugars (TRS) after the enzymatic treatment of the apple mash.

Enzyme	TRS ($\text{g} \cdot \text{L}^{-1}$)
No enzyme (control)	95.7 ± 0.3^a
Pektinase L40	95.7 ± 1.1^a
Dextrozyme GA	97.4 ± 2.3^a
Cellic CTec3	95.6 ± 1.9^a
Viskoferm	94.1 ± 1.6^a
Cellic CTec3 + Pektinase L40 (high loading)	95.5 ± 0.7^a
Cellic CTec3 + Pektinase L40 (low loading)	96.1 ± 1.4^a

The values represent the mean $\pm$ standard deviation. Different letters indicate statistically significant differences between means according to Tukey's test ($p < 0.05$).

Whereas the TRS remained unchanged (Table 1), a considerable reduction in viscosity was observed when Cellic CTec3 was evaluated individually. High viscosity represents a disadvantage in fermentation medium, as it can impair mass transfer, leading to gradients and unequal cell exposure to optimal conditions, thereby reducing process efficiency [20]. Moreover, elevated viscosity can hinder downstream processing, particularly separation operations such as filtration, resulting in lower yields [27]. Although Pektinase L40 reduced viscosity to a lesser extent, its inclusion was still considered relevant, as pectinases are commonly used in fruit mash processing to improve yields and reduce viscosity [28]. Because no difference in medium viscosity was observed between the higher and lower enzyme concentrations, the combination of Pektinase L40 and Cellic CTec3 at a low loading ($0.5 \text{ mL}_e \cdot \text{kg}_{\text{bm}}^{-1}$) was selected for the enzymatic liquefaction of the AM.

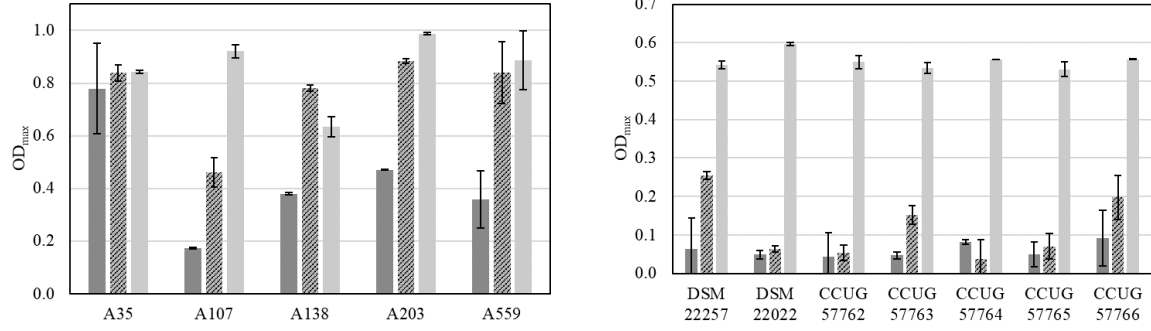
3.2. Screening of microorganisms

The screening of microorganisms based on OD measurements provides a preliminary assessment of the ability of a substrate to support microbial growth, as well as the adaptability of different strains to it, as reflected in biomass production [23]. The maximum OD values ranged from 0.173–0.988 for LA-producing strains and from 0.037 to 0.603 for SA-producing strains (Figure 1). Based on the maximum OD relative to that of the control medium (Figure 1) and the performance reported in previous studies [21], *H. coagulans* strains A35, A138 and A203 were selected for screening in bioreactors, and *A. succinogenes* DSM 22257 was chosen for subsequent SA fermentations. Notably, the superiority of *A. succinogenes* over *B. succiniproducens* in terms of SA production has already been reported [23, 24].

Figure 1. Growth of lactic acid- and succinic acid-producing strains in apple mash media.

a

b

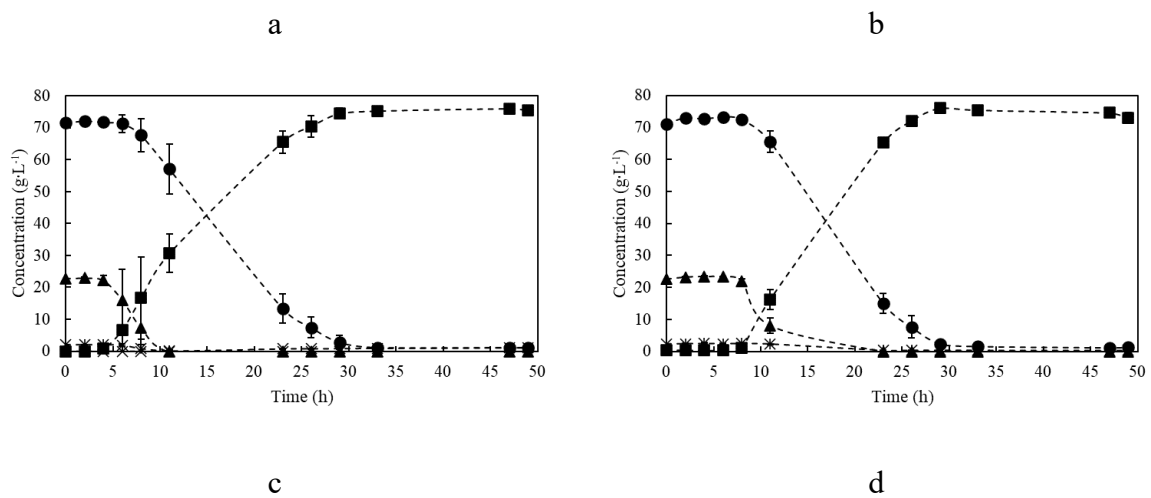


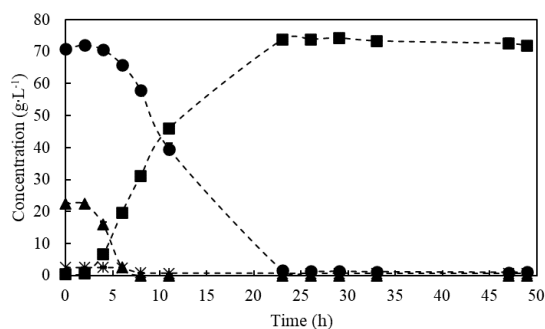
Lactic acid-producing strains (a) and succinic acid-producing strains (b). Apple mash (dark gray bar), apple mash supplemented with 5 g·L⁻¹ yeast extract (striped bar), and control medium (light gray bar). The error bars represent standard deviations.

3.3. Screening of microorganisms in bioreactors

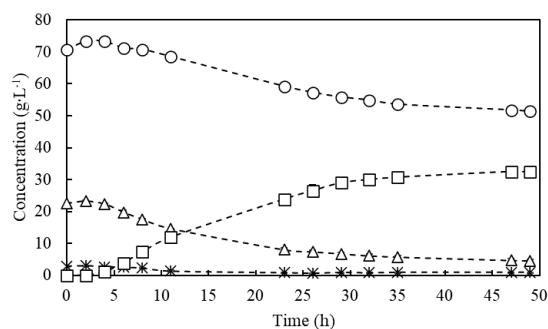
The screening of microorganisms in bioreactors enables the identification of the best-performing strain under actual fermentation conditions, ensuring high yields and productivities and, consequently, a more effective process [20, 21]. The fermentation performance of LA strains selected in the initial OD-based assessment is illustrated in Figure 2a-c, which show that LA production ceased between 20 and 30 h when nearly all the sugars had been consumed.

Figure 2. Sugar consumption and product formation in apple mash fermentations.

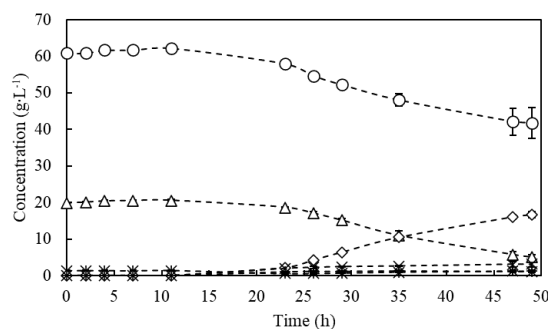
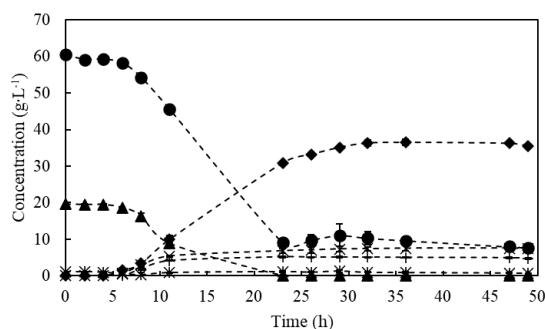




e



f



10 g·L⁻¹ yeast extract (solid symbols) and no yeast extract (open symbols). *H. coagulans* A35 (a), A138 (b), A203 (c, d), and *A. succinogenes* DSM 22257 (e, f). Fructose + malic acid (●), glucose (▲), sucrose (*), lactic acid (■), succinic acid (◆), acetic acid (x), and formic acid (+). The error bars represent standard deviations.

LA production ranged from 71.8–75.5 g·L⁻¹, with yields varying between 0.91 and 0.94 g·g⁻¹ (Table 2). No significant differences were observed in LA concentration or yield among the strains ($p > 0.05$). However, strain A203 exhibited higher productivity, reaching 3.5 g·L⁻¹·h⁻¹, compared with 2.5 g·L⁻¹·h⁻¹ for strains A35 and A138. Additionally, longer lag phases were observed for strains A35 and A138 (Figure 2a-c).

Table 2. Lactic and succinic acid production in apple mash fermentations by *H. coagulans* and *A. succinogenes* strains.

Strain	Product (g·L ⁻¹)	Yield (g·g ⁻¹)	Productivity (g·L ⁻¹ ·h ⁻¹)
<i>H. coagulans</i> A35	75.5 ± 0.2	0.94 ± 0.01	2.5 ± 0.1
<i>H. coagulans</i> A138	73.0 ± 1.5	0.92 ± 0.03	2.5 ± 0.1
<i>H. coagulans</i> A203	71.8 ± 0.2	0.91 ± 0.01	3.5 ± 0.0
<i>H. coagulans</i> A203 (no yeast extract)	32.3 ± 0.1	0.83 ± 0.06	0.7 ± 0.0

<i>A. succinogenes</i> DSM 22257	35.6 ± 0.3	0.69 ± 0.02	1.1 ± 0.1
<i>A. succinogenes</i> DSM 22257 (no yeast extract)	16.7 ± 0.8	0.70 ± 0.15	0.3 ± 0.0

The values represent the mean ± standard deviation.

H. coagulans A203 has been evaluated in previous studies. When fermenting defatted rice bran hydrolysate, strain A203 produced 71.2 g·L⁻¹ LA, with a yield of 0.88 g·g⁻¹ and a productivity of 2.97 g·L⁻¹·h⁻¹ [21]. It also demonstrated the ability to utilize pasta waste hydrolysate, producing approximately 44 g·L⁻¹ LA from 55 g·L⁻¹ sugars [20]. These findings highlight the efficiency of strain A203 in utilizing diverse substrates and further support *H. coagulans* as a promising candidate for the biotechnological production of LA. Given its high LA concentration, yield, productivity, and strong performance in previous studies, strain A203 was selected for pilot-scale LA production using AM.

Following strain selection, the need for nutrient supplementation was evaluated (Figure 2c and d). After 49 h of fermentation without the addition of yeast extract, only partial sugar consumption was observed, resulting in 32.3 g·L⁻¹ LA, and the productivity decreased to 0.7 g·L⁻¹·h⁻¹. The influence of yeast extract on SA fermentation was also assessed using *A. succinogenes* DSM 22257, as shown in Figure 2e and f. The absence of supplementation led to significant decreases in the SA concentration and productivity, from 35.6 g·L⁻¹ and 1.1 g·L⁻¹·h⁻¹ to 6.7 g·L⁻¹ and 0.3 g·L⁻¹·h⁻¹, respectively. Comparative values are presented in Table 2.

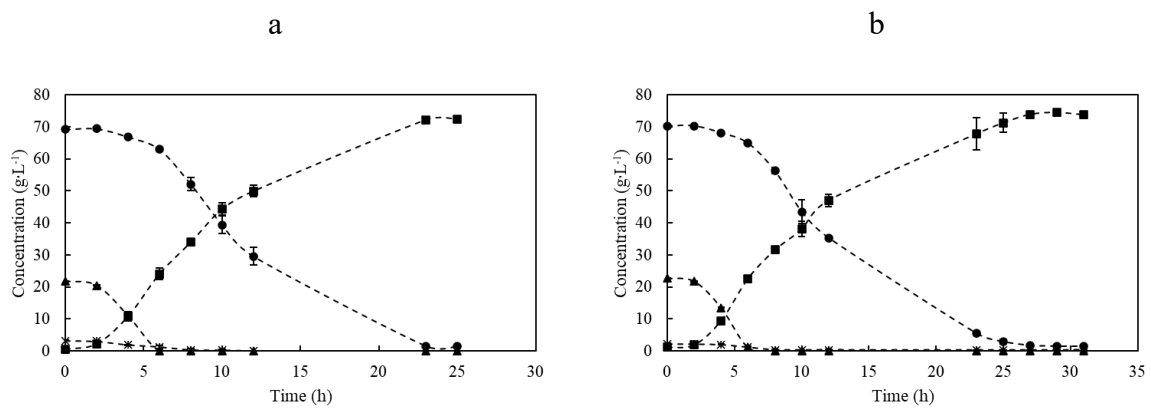
These results suggest that AM lacks sufficient nutrients to meet the demands of *H. coagulans* A203 and *A. succinogenes* DSM 22257 during fermentation, indicating the need for supplementation. Notably, higher ODs were observed in supplemented media in the preliminary strain screening (Figure 1). Because nutrient supply is essential for AM fermentation, cost-effective alternatives to yeast extract are promising for reducing overall production costs [11]. Alternative nitrogen sources, such as wine lees, urea, and ammonium chloride, have been evaluated in apple pomace fermentations for LA production. Moreover,

the use of agro-industrial byproducts, such as tomato pomace, has shown potential to enhance fermentation performance, surpassing that achieved with yeast extract [14].

3.4. Pilot-scale lactic acid fermentation

The implementation of a lab-scale validation step is important to ensure that process simplifications do not compromise process stability, increase contamination risk, or reduce product yield prior to progressing to pilot-scale application [29]. The progress of the fermentation conducted in the prepilot-scale validation trial, in which the AM, following viscosity reduction, was directly supplemented and inoculated, is shown in Figure 3a.

Figure 3. Lactic acid fermentation of apple mash by *H. coagulans* A203 at the laboratory and pilot scales.



Laboratory scale (a) and pilot scale (b). Fructose + malic acid (●), glucose (▲), sucrose (*), and lactic acid (■). The error bars represent standard deviations.

LA production reached $72.4 \text{ g}\cdot\text{L}^{-1}$, with a yield of $0.93 \text{ g}\cdot\text{g}^{-1}$ and a productivity of $3.4 \text{ g}\cdot\text{L}^{-1}\cdot\text{h}^{-1}$ (Table 3). No significant differences in LA concentration, yield, or productivity were observed compared with the fermentation conducted in the previous step (*Section 3.3*) ($p > 0.05$), indicating that the applied method maintained process performance. The elimination of the sterilization step was achievable because of the elevated temperatures tolerated by the thermophilic strain *H. coagulans* employed in this study, which reduced the risk of

contamination commonly associated with mesophilic microorganisms [11, 14]. Furthermore, although solid particles remaining after hydrolysis can negatively affect fermentation performance by causing inhibitory effects, mass transfer limitations, and operational issues in the bioreactor [30], the results indicate that centrifugation was not necessary in this process. Additional processing steps, such as centrifugation and sterilization between hydrolysis and fermentation, can significantly increase production costs and reduce the economic competitiveness of bioproducts compared with petrochemical alternatives, particularly at larger scales [20]; therefore, these results highlight the potential of the implemented procedure for efficient and cost-effective LA production.

Table 3. Lactic acid production in apple mash fermentations by *H. coagulans* A203 at the laboratory and pilot scales.

Scale	Lactic acid (g·L ⁻¹)	Yield (g·g ⁻¹)	Productivity (g·L ⁻¹ ·h ⁻¹)
Laboratory scale	72.4 ± 0.0	0.93 ± 0.01	3.4 ± 0.3
Pilot scale	73.8 ± 0.1	0.91 ± 0.00	2.7 ± 0.1

The values represent the mean ± standard deviation.

Considering the outcomes of the process implemented at the laboratory scale, LA production from AM was scaled up. The analysis revealed that malic acid, which is naturally present in AM and coincident with the fructose peak in the chromatogram, was fully consumed by strain A203, whereas minor concentrations of fructose and sucrose remained in the fermentation broth. This led to the production of 73.8 g·L⁻¹ LA, a yield of 0.91 g·g⁻¹, and a productivity of 2.7 g·L⁻¹·h⁻¹. Although there were no significant differences in productivity between the laboratory and pilot scales, scaling up can lead to lower productivities due to differences in mixing efficiency and limitations in heat and mass transfer, which can result in gradients in pH, temperature, and nutrients, thus affecting microbial behavior [31].

LA production from apple biomass has focused primarily on the use of apple pomace at the laboratory scale. The fermentation of apple pomace hydrolysate by *H. coagulans* DSM 2314 resulted in 40.72 g·L⁻¹ LA, with a yield of 0.96 g·g⁻¹ and a productivity of 0.58 g·L⁻¹·h⁻¹

[14]. The superior outcomes obtained with AM (Table 3) are likely due to its higher sugar availability and lower content of inhibitory compounds, which are typically formed during lignocellulose pretreatment, promoting more efficient fermentation and increasing both the product concentration and the fermentation rate.

Although pilot-scale studies using apple residues are currently lacking, similar processes have been explored with other types of food waste. Fermentation of food waste composed of fruits, vegetables, carbohydrates, meat, and dairy products resulted in 68.5 g·L⁻¹ LA, with a yield of 0.38 g·g⁻¹ total solids and a productivity of 0.95 g·L⁻¹·h⁻¹ [17]. The utilization of pasta waste hydrolysate by *H. coagulans* A559 produced 47.67 g·L⁻¹ LA, achieving a yield of 0.81 g·g⁻¹. However, during upscaling, a 10-h increase in the lag phase was attributed to residual solid particles remaining after hydrolysis [20], an issue not encountered during the scale-up of AM fermentation. Therefore, the enhanced performance observed in this study demonstrates the potential of waste apples as a viable substrate for effective and sustainable LA production.

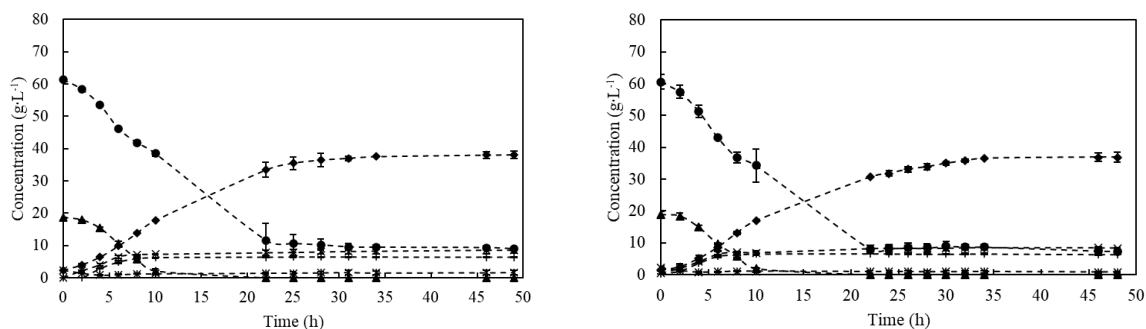
3.5. Pilot-scale succinic acid fermentation

Unlike *H. coagulans*, *A. succinogenes* grows optimally at 37–39 °C [12], a range that also favors the proliferation of competing microorganisms, which can negatively impact fermentation performance and outcomes [32]. In this study, microbial contamination was controlled by introducing a simplified thermal inactivation step (80 °C) following enzymatic liquefaction, and the performance during the subsequent fermentation at the laboratory scale is presented in Figure 4a.

Figure 4. Succinic acid fermentation of apple mash by *A. succinogenes* DSM 22257 at the laboratory and pilot scales.

a

b



Laboratory scale (a) and pilot scale (b). Fructose + malic acid (●), glucose (▲), sucrose (*), succinic acid (◆), acetic acid (x), and formic acid (+). The error bars represent standard deviations.

Compared with LA fermentation, SA fermentation began with a lower initial sugar concentration due to dilution from the addition of nutrient solution and inoculum. Consequently, the fermentation resulted in 38.1 g·L⁻¹ SA, with a yield of 0.69 g·g⁻¹ and a productivity of 1.1 g·L⁻¹·h⁻¹ (Table 4). Acetic and formic acids were also detected at final concentrations of 8.5 g·L⁻¹ and 6.3 g·L⁻¹, respectively. No significant differences in SA concentration, yield, or productivity were observed compared with fermentation conducted with centrifuged and autoclaved medium, demonstrating the efficacy of the implemented procedure, which included the inactivation step. This is because most contaminating microorganisms cannot survive prolonged exposure to 80 °C, as this temperature exceeds the thermal limits of their cellular structures and enzymes, leading to protein denaturation and membrane disruption [33]. In addition, inactivation at 80–85 °C is a common method to reduce the microbial load without the high energy demands and specialized equipment required for sterilization, which is typically conducted at 121 °C under pressure [20]. Moreover, the milder conditions contribute to the preservation of nutrient integrity [21]. The results also indicate that the exclusion of the centrifugation step did not affect fermentation outcomes.

Table 4. Succinic acid production in apple mash fermentations by *A. succinogenes* DSM 22257 at the laboratory and pilot scales.

Scale	Succinic acid (g·L ⁻¹)	Acetic acid (g·L ⁻¹)	Formic acid (g·L ⁻¹)	Yield (g·g ⁻¹)	Productivity (g·L ⁻¹ ·h ⁻¹)
Laboratory scale	38.1 ± 1.1	8.5 ± 1.0	6.3 ± 0.2	0.69 ± 0.01	1.1 ± 0.4
Pilot scale	36.8 ± 1.5	8.3 ± 0.3	6.4 ± 0.2	0.69 ± 0.02	1.0 ± 0.1

The values represent the mean ± standard deviation.

Considering these findings, SA production from apple pomace was scaled up. Sugar consumption and product formation followed the same trends observed at the laboratory scale (Figure 4b). Sugars were nearly fully consumed, resulting in 36.8 g·L⁻¹ SA with a yield of 0.69 g·g⁻¹ (Table 4), while the productivity reached 1.0 g·L⁻¹·h⁻¹. Acetic acid and formic acid were also produced, with final concentrations of 8.3 g·L⁻¹ and 6.4 g·L⁻¹, respectively. Additionally, approximately 5.0–6.0 g·L⁻¹ of malic acid was formed, and no fructose remained in the fermentation broth. No significant differences in LA, acetic acid, or formic acid concentrations, yield, or productivity were observed, indicating that the scale-up process had no negative impact on fermentation performance.

Studies on SA production from apple biomass have involved mainly theoretical investigations, relying on process modeling and technoeconomic analyses of apple pomace utilization by *A. succinogenes*. SA production has been assessed using life cycle assessment (LCA) to identify environmental impacts and process bottlenecks [15], and biogas coproduction has been proposed as a promising strategy [16]. Like LA, the pilot-scale production of SA by *A. succinogenes* was investigated using other food wastes. The utilization of oat pomace and acid whey resulted in 19.6 g·L⁻¹ SA, achieving a yield of 0.77 g·g⁻¹ and a productivity of 0.27 g·L⁻¹·h⁻¹ [18]. In turn, 38.99 g·L⁻¹ SA was produced from industrial candy waste, with a yield of 0.70 g·g⁻¹ and a productivity of 1.26 g·L⁻¹·h⁻¹ [19]. In comparison with these values, the results of the current study demonstrate competitive performance and highlight the potential of waste apples as a substrate for SA biosynthesis.

Overall, the findings provide valuable data for the scaling up and implementation of biotechnological strategies aimed at the valorization of agri-food waste within the framework of biorefineries. The growing field of intelligent biorefineries, which integrates artificial intelligence and machine learning for adaptive control, offers further optimization by continuously adjusting processes in response to feedstock variability. This enables more efficient resource management, improves process stability and yield, and reduces costs. Future trends include the development of genetically engineered microbial strains designed to increase fermentation rates and product diversity, as well as the development of sustainable systems that minimize waste and energy consumption. In addition to these upstream innovations, efficient downstream processing is essential for recovering and purifying bioproducts, ensuring their transformation into commercially viable products [34]. Together, these advancements highlight the potential of integrated biorefinery approaches that not only convert agri-food residues into high-value bioproducts but also contribute to a more circular and sustainable bioeconomy.

4. Conclusions

Pilot-scale production of LA and SA from apple biomass was successfully demonstrated. Preliminary screening identified *H. coagulans* A203 and *A. succinogenes* DSM 22257 as the most suitable candidates for LA and SA production, respectively, when apple mash was used as the substrate. An alternative fermentation procedure confirmed the feasibility of conducting the process without costly centrifugation and sterilization steps. Pilot-scale LA production resulted in 73.8 g·L⁻¹ LA, a yield of 0.91 g·g⁻¹ and a productivity of 2.7 g·L⁻¹·h⁻¹. For SA, 36.8 g·L⁻¹ was achieved, corresponding to a yield of 0.69 g·g⁻¹ and a productivity of 1.0 g·L⁻¹·h⁻¹. These results validate the potential of apple biomass as a feedstock for LA and SA production, covering microorganism selection, process development, and pilot-scale

fermentation. Overall, this study supports the integration of agri-food residues into biotechnological processes aimed at generating high-value products, contributing to the ongoing efforts to implement a circular and sustainable bioeconomy. Moving forward, continued advancements in microbial strain development and process optimization will be crucial for enhancing product yields and achieving scalable, cost-effective production, further promoting biorefinery approaches.

List of abbreviations

AM: apple mash

LA: lactic acid

mL_e·kg_{bm}⁻¹: milliliters of enzyme per kilogram of biomass

SA: succinic acid

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

L.P.R.C.P.: Writing – original draft, Writing – review and editing, Methodology, Validation, Formal analysis, Data curation, Visualization. **R.S.:** Writing – review and editing, Methodology, Validation, Investigation, Data curation. **A.O.W.:** Writing – review and editing, Conceptualization, Supervision. **B.S.:** Writing – review and editing, Supervision. **K.K.:** Writing – review and editing, Conceptualization, Supervision, Project administration, Funding acquisition.

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