SCIENTIA
AGRICULTURAE
BOHEMICA

Article

High-Efficiency Bioethanol Production from Maradol Papaya Waste: Batch Fermentation and Fractional Distillation

Eduardo, Duque-Dussán[1, 2]*; Paula A., Figueroa-Varela[3]; Sergio, Muñoz-Salazar[4]

[1] Department of Sustainable Technologies, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 00 Prague, Czechia. Duque_Dussan@ftz.czu.cz

[2] Discipline of Postharvest, National Coffee Research Center of Colombia – Cenicafé, km 4 vía Antigua Chinchiná – Manizales, Manizales, Caldas, Colombia. Eduardo.Duque@cafedecolombia.com

[3] Biological Sciences Department, School of Applied Sciences and Engineering, EAFIT University, Medellín, Colombia. pfiguer2@eafit.edu.co

[4] Instituto Colombiano de Normas Técnicas – ICONTEC, Bogotá, Colombia. smunozs@icontec.net

* Correspondence: Duque_Dussan@ftz.czu.cz; Eduardo.Duque@cafedecolombia.com (Colombia)

Abstract: Postharvest losses of tropical fruits represent an underexplored feedstock for renewable fuels. This study evaluates the feasibility of converting discarded *Carica papaya* L. var. Maradol pulp into fuel-grade ethanol via batch fermentation and fractional distillation. Peeled fruit (4.0 kg) was pasteurized, adjusted to 22 °Brix with brown sugar, and inoculated at 1×10^6 cells mL^{-1} with *Saccharomyces cerevisiae* Ethanol Red™. Triplicate fermentations were performed at 30 ± 0.2 °C for 192 h, and kinetic parameters were determined by HPLC, GC-FID, and a modified Gompertz model. Soluble sugars declined from 205 to 17 g L^{-1} within 96 h, yielding 92.3 ± 1.8 g L^{-1} ethanol. Volumetric productivity and ethanol yield reached 1.04 ± 0.05 g L^{-1} h^{-1} and 0.46 ± 0.01 g g^{-1}, respectively—90 % of the theoretical maximum. Distillation with a 64–78 °C heads cut produced a 95.4 ± 0.6 % v v^{-1} ethanol distillate, with methanol, water, Sulphur, and acidity within ASTM D4806 limits. The process delivered 68.4 ± 1.3 g ethanol kg^{-1} fresh fruit (≈ 1.45 MJ kg^{-1}) and vinasse with a C:N ratio of 8.1, suitable for fertigation or anaerobic digestion. *Kluyveromyces marxianus* showed thermotolerance and moderate yields, while *Pichia kudriavzevii* achieved higher concentrations. Native isolates like *Lactobacillus plantarum*, *Acetobacter tropicalis*, and wild *Saccharomyces* spp. support co-culture strategies. These results position Maradol papaya waste as a viable, low-cost substrate for decentralized bioethanol production and circular bioeconomy systems.

Keywords: circular bioeconomy; ethanol yield; fuel quality; process kinetics; tropical fruit residues

Received for publication on May 22, 2025

Accepted for publication on June 23, 2025

1. Introduction

Tropical and subtropical zones dominate global fruit production because their relatively stable warm climates allow year-round cultivation of a broad spectrum of horticultural crops (Ahmad and Chwee, 2008; Kader and Yahia, 2011; Mukhametzyanov et al., 2023). Recent statistical reports attribute more than half of the world's fresh-fruit output to Latin America, Asia, and sub-Saharan Africa, regions that collectively sustain local food security while supplying high-value export markets (Ahmad and Chwee, 2008; Galán Saúco et al., 2014; Mohamed et al., 2011; Wu et al., 2024). Beyond nutrition, fruit agriculture reinforces rural economies by generating employment and providing renewable feedstocks for agro-industrial valorisation. Climate variability and price volatility further emphasize the importance of diversifying fruit-derived value chains to safeguard farmer livelihoods (Mancero-Castillo et al., 2024; Parajuli et al., 2019). As sustainability imperatives intensify, these production hubs face the dual challenge of expanding output and mitigating the environmental burdens associated with post-harvest losses and biomass disposal (Kader and Yahia, 2011; Mitra, 2018; Roussos, 2024).

Among commercially important tropical fruits, papaya (*Carica papaya* L.) is particularly significant (Burns et al., 2023; Koul et al., 2022). This fast-growing crop offers high yields, short maturation cycle: from flowering to harvest in approximately 5 to 6 months (Misnan et al., 2024; Queiroz et al., 2024; Zhou et al., 2021), and an exceptional nutritional profile rich in fermentable sugars, principally glucose, fructose, and sucrose, alongside valuable enzymes such as papain (Singh and Sudhakar Rao, 2011; Zhou et al., 2021). Global papaya production surpassed 14 million tonnes in 2023, with more than 95 % originating from tropical countries (FAOSTAT, 2023; Katyal et al., 2024; Mendoza-Grimón et al., 2024). The variety "Maradol", is widely cultivated across the tropics because of its elevated °Brix values, sweet flavour, and robustness under smallholder conditions (Vij and Prashar, 2015; Zhou et al., 2021). Continuous flowering and fruiting ensure a steady supply of raw material, enabling flexible integration into biotechnological processes whenever market surpluses or quality-rejection events arise (Amran et al., 2021; Katyal et al., 2024; Koul et al., 2022; Vij & Prashar, 2015). Additionally, the biochemical composition of papaya pulp, rich in fermentable sugars such as glucose, fructose, and sucrose, along with trace minerals and amino acids (Zhou et al., 2021), has been shown to support rapid growth of ethanologenic yeasts, reducing both fermentation time and exogenous nutrient requirements (I Nengah Muliarta et al., 2023; Kantiyok et al., 2021).

Despite these advantages, papaya suffers from extreme perishability; post-harvest losses frequently range between 20 % and 40 %, depending on infrastructure, ambient temperature, and handling practices (Karoney et al., 2024; Paull et al., 1997; Sivakumar and and Wall, 2013). Quality degradation during storage, transport, or retail leads to routine discarding of physical imperfect or over-ripe fruit, creating sizeable streams of organic waste (Karoney et al., 2024; Sivakumar and and Wall, 2013). In Colombia alone, seasonal gluts can generate more than 60000 tons of discarded papaya each year, representing an unused biochemical resource (Paternina et al., 2022). If unmanaged, this biomass decomposes anaerobically, releasing methane and nutrient-rich leachates that threaten local environmental quality (Vinod et al., 2023). Nevertheless, the very attributes that accelerate spoilage, high moisture content and readily fermentable sugars, render papaya residues excellent substrates for microbial ethanol production, aligning waste valorisation with circular-bioeconomy principles (Bhatt et al., 2025; Campos et al., 2020).

The present study characterizes the production of fuel-grade ethanol from discarded *C. papaya* var. Maradol pulp (Figure 1), through a controlled fermentation–distillation sequence conducted at laboratory scale. Critical process parameters, including yeast strain selection, thermal profile, and control treatments, were defined in accordance with industrial-biotechnology best practices, ensuring methodological transparency and reproducibility. The experimental process quantified ethanol yield, sugar-consumption kinetics, pH evolution, and fermentation efficiency, providing a coherent data set for scientific analysis. By demonstrating the potential of papaya waste as a low-cost renewable feedstock for decentralised biofuel production, this work contributes to the development of integrated biorefinery strategies aimed at reducing post-harvest losses, lowering greenhouse-gas emissions, and advancing sustainable energy solutions in tropical agro-ecosystems (Bhatt et al., 2025; Koul et al., 2022; Paternina et al., 2022; Sivakumar and and Wall, 2013).

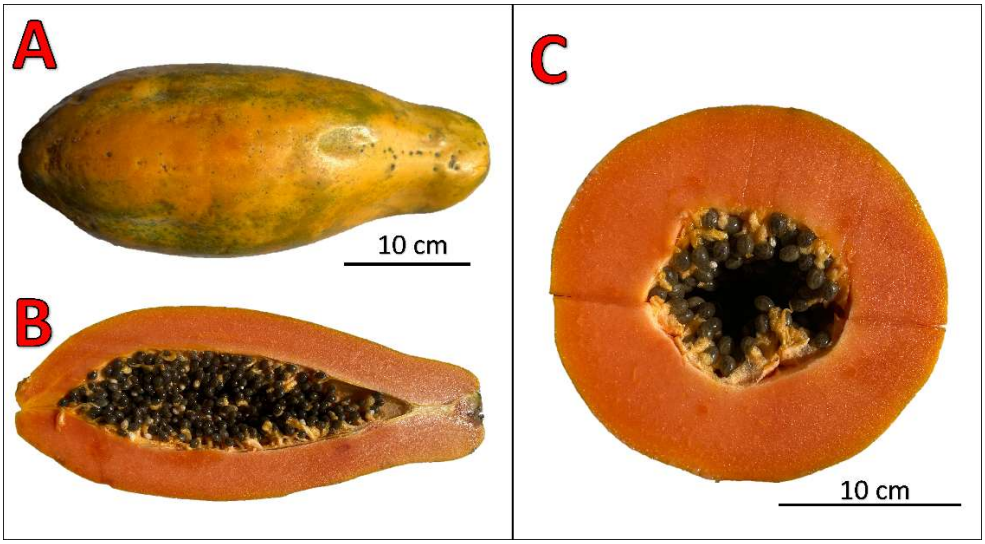

Figure 1. Maradol papaya used as fermentation feedstock. **A.** Whole fruit at maturity stage 6 (Paull and Duarte, 2011) showing characteristic yellow-orange peel with residual green mottling. **B.** Longitudinal cross-section exposing the orange, high-°Brix pulp and central seed cavity. **C.** Transverse cross-section highlighting pulp thickness (≈ 80 % w/w) relative to thin exocarp. Scale bars = 10 cm in panels A=B and C.

2. Materials and Methods

All experimental work was carried out between January and April 2025 in the Mechanical Engineering laboratories of the Universidad Autónoma de Manizales (Manizales, Colombia (5.06750 °N, 75.51000 °W)). Unless otherwise stated, reagents were analytical grade and deionised water (18 MΩ cm) was used throughout.

2.1. Raw material and pre-treatment

A batch of 4.0 kg of fully ripe *Carica papaya* L. var. Maradol was acquired from the local farmers' market in Manizales, Colombia within 24 h post-harvest. After washing, the fruit was peeled and deseeded, yielding 3.20 ± 0.05 kg of edible pulp, corresponding to ≈ 80 % pulp recovery on a fresh-weight basis. The pulp (16.8 ± 0.4 °Brix) was homogenised in a sterilised blender, diluted with

0.8 L of distilled water, pasteurised at 80 °C for 5 min, and cooled to 30 °C. Brown sugar (150 g kg^{-1} pulp) was then incorporated to raise the soluble-solids content to ≈ 22 °Brix, a level reported to sustain high ethanologenic activity (Sanchez and Cardona, 2008). Only the pulp was used as fermentation substrate. The peel and seeds were excluded to avoid the introduction of inhibitory compounds and ensure ethanol distillate quality. Their potential effects are discussed in Section 3.3.

2.2. Yeast strain and inoculum preparation

Fermentations employed industrial distiller's yeast *Saccharomyces cerevisiae* Ethanol Red® (Lesaffre, France). Dried yeast (10 g L^{-1}) was rehydrated for 15 min at 35 °C in sterile 0.5 % glucose solution, reaching 1.2×10^{8} cells mL^{-1}. The papaya must, was inoculated at 1×10^{6} cells mL^{-1}. Diammonium phosphate (0.4 g L^{-1}) (Sigma-Aldrich, Germany) supplied assimilable nitrogen (Sanchez and Cardona, 2008).

Cell density was determined using a Neubauer improved hemocytometer (Marienfeld Superior, Germany) under light microscopy (Leica DM500, Germany), without viability staining. Viability staining was not performed, as the yeast was used fresh from a commercial source and rehydrated under controlled conditions, ensuring high viability typical of industrial strains.

2.3. Fermentation set-up and operating condition

Fermentations were carried out in 4 L borosilicate bioreactors (Duran ®, Germany) fitted with a three-piece laboratory fermentation lock (2 mL sterile 20 % glycerol; cracking pressure < 1 kPa). Three inoculated batches (biological triplicates) and one uninoculated control were incubated at 30 ± 0.2 °C and 100 rpm for 192 h as reported in similar fruit fermentation systems (Sánchez and Cardona, 2008). Ten-millilitre samples were withdrawn every 24 h to quantify sugars, ethanol, pH, and biomass. Dissolved oxygen remained below 0.5 mg L^{-1} throughout the process, as determined using a portable DO meter (Hanna Instruments HI9147, Romania). This condition was maintained by sealing the bioreactors with fermentation locks and operating without active aeration; gentle mixing at 100 rpm minimized stratification without introducing oxygen.

2.4. Distillation and ethanol recovery

Fermented broths were vacuum-filtered through 0.5 mm stainless-steel mesh. Distillation was conducted in an 8 L stainless-steel pot still with a 300 mm Liebig condenser (coolant 16 ± 1 °C). Vapour temperature was monitored by a type-K thermocouple.

- Heads cut (64 – 78 °C): discarded to eliminate methanol.
- Hearts (78 – 92 °C): collected for analysis; methanol < 100 mg L^{-1} (GC-FID).
- Tails (> 92 °C): discarded.

2.5. Analytical methods

Soluble sugars (glucose, fructose, sucrose) were quantified by HPLC (Agilent 1260 Infinity, USA, Rezex RPM Monosaccharide, 80 °C, 0.6 mL min^{-1} H_2O). Ethanol was measured by GC-FID (DB-Wax column, injector 250 °C, detector 240 °C) using an Agilent 7890A (USA) gas chromatograph equipped with a flame ionization detector (FID) and controlled by Agilent ChemStation software. Volatile compound identification was performed by comparing retention times with those of analytical

standards. The NIST 98 and Wiley 275 spectral libraries were used for compound identification where applicable, and only compounds with a match similarity >90% were considered reliably identified. pH was monitored with a glass electrode (Mettler Toledo SevenCompact, Switzerland).

2.6. Kinetic calculations

Volumetric ethanol productivity (Q_{EtOH}), specific yield ($Y_{P/S}$), and sugar-conversion efficiency were calculated from concentration–time data (Fagundes et al., 2024), where C_{EtOH} is ethanol concentration and C_S is total soluble sugar concentration (Equations 1 and 2).

$$Q_{EtOH} = \frac{\Delta C_{EtOH}}{\Delta t} \ [\mathrm{g\ L^{-1}\ h^{-1}}] \tag{1}$$

$$Y_{P/S} = \frac{C_{EtOH,final} - C_{EtOH,0}}{C_{S,0} - C_{S,final}} \ [\mathrm{g\ g^{-1}}] \tag{2}$$

Fermentation curves were fitted to the modified Gompertz model to estimate maximum specific growth rate (μ_{max}), and lag phase (λ) as seen in Equation 3:

$$C_{EtOH}(t) = C_{max}\, exp\left\{- exp\left[\frac{\mu_{max}\, e}{C_{max}}(\lambda - t) + 1\right]\right\} \tag{3}$$

with C_{max} being the asymptotic ethanol concentration.

2.7. Statistical analysis

All assays were performed in triplicate. Results are reported as mean ± standard deviation. Statistical significance was evaluated by one-way ANOVA followed by Tukey's post hoc test at $\alpha = 0.05$ (R 4.3.0). ANOVA was applied only to final values of ethanol concentration at 192 h, volumetric productivity (Q_{EtOH}), and ethanol yield on substrate ($Y_{P/S}$), comparing biologically independent treatments (e.g., inoculated vs. uninoculated). Time-course data were excluded from ANOVA to respect the assumption of independence and were instead modeled using the modified Gompertz equation. Assumptions of normality and homoscedasticity were verified using Shapiro–Wilk and Levene's tests, respectively.

2.8. Alternative microorganisms and culture potential

While *S. cerevisiae* Ethanol Red™ was the primary strain used in fermentation due to its high ethanol tolerance and GRAS designation, exploratory tests were conducted to assess the growth and ethanol productivity of alternative ethanologenic and acid-tolerant microorganisms. Pure cultures of *Kluyveromyces marxianus* ATCC 12424 and *Pichia kudriavzevii* CBS 573 were obtained from the

Colombian Microbial Culture Collection (CCCM) and grown in Yeast Extract Peptone Dextrose (YPD) broth (Merck, Germany) supplemented with 10% (v v^{-1}) papaya extract. Incubations were performed at 30 °C and 42 °C to evaluate thermotolerance and substrate assimilation. Growth was monitored via OD600 over 96 h, and ethanol content was measured by GC-FID at 48 h and 96 h.

Spontaneous fermentation trials using uninoculated papaya must were also performed to isolate native microbiota. Samples were plated de Man, Rogosa and Sharpe (MRS) agar (Oxoid, UK) and Glucose Yeast Extract Calcium Carbonate (GYC) agar (Himedia, India) under aerobic and anaerobic conditions at 30 °C. Isolates were identified using 16S rRNA and ITS sequencing.

3. Results and Discussion

3.1. Fermentation kinetics and model parameters

Sugar consumption and ethanol formation followed the expected sigmoidal pattern (Figure 2), 92 % of the initial soluble sugars were metabolised within the first 96 h, after which the curve levelled off, indicating substrate exhaustion. The modified Gompertz model reproduced the experimental data with an average R^2 of 0.994, confirming that *S. cerevisiae* exhibited typical batch-growth behaviour on papaya pulp.

HPLC analysis revealed that glucose, fructose, and sucrose were initially present at 78 ± 3 g L^{-1}, 66 ± 2 g L^{-1}, and 61 ± 2 g L^{-1}, respectively. Glucose and fructose were consumed rapidly and fell below detection limits by 96 h. Sucrose hydrolyzed progressively and was nearly depleted by 120 h. The overall trend reflects efficient substrate utilization by *S. cerevisiae*, consistent with the observed ethanol accumulation.

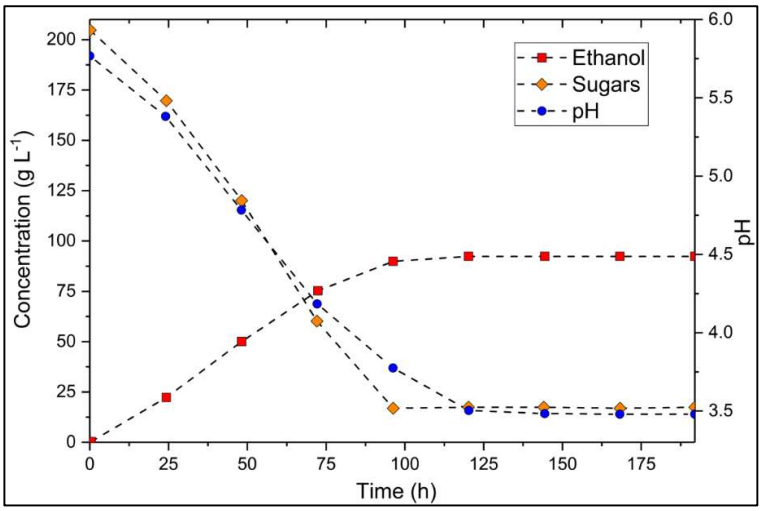

Figure 2. Fermentation kinetics at 30 °C. Residual sugars decline sigmoidally while ethanol rises to 92 g L^{-1}; pH (right axis) drops from 5.8 to 3.5. Curves are triplicate means ± SD (n = 3).

Key kinetic descriptors are summarised in Table 1. The maximum specific growth rate, μ_{max} = 0.28±0.02 h^{-1}, is slightly higher than values reported for mango peel hydrolysate (0.24 h^{-1}) (Ampah et al., 2022; Awodi et al., 2022) and jackfruit waste (0.21 h^{-1}) fermented under comparable conditions

(Suo et al., 2024). The short lag phase (λ=6.3±0.4h) and high volumetric productivity (Q_{EtOH} =1.04±0.05g $L^{-1}h^{-1}$) suggest that the Maradol matrix imposes neither nutrient limitations nor inhibitory stresses on the yeast (Bai et al., 2008).

Table 1. Kinetic performance of *S. cerevisiae* on Maradol papaya must (mean ± SD, n=3)

Parameter	Symbol	Value	Unit
Maximum ethanol concentration	C_{max}	92.3 ± 1.8	g L^{-1}
Volumetric productivity	Q_{EtOH}	1.04 ± 0.05	g L^{-1} h^{-1}
Ethanol yield on substrate	$Y_{P/S}$	0.46 ± 0.01	g g^{-1}
Maximum specific growth rate	μ_{max}	0.28 ± 0.02	h^{-1}
Lag phase	λ	6.3 ± 0.4	h
Residual sugars (120 h)	–	17 ± 2	g L^{-1}

The ethanol yield on substrate ($Y_{P/S}$=0.46±0.01 g g^{-1}) reached 90 % of the theoretical maximum, outperforming banana, pineapple and mixed-citrus waste fermentations reported in the literature (0.38–0.42 g g^{-1}) that used similar inoculation and nutrient regimes (Casabar et al., 2019; Guerrero et al., 2018; Patsalou et al., 2019). The residual sugar of 17 ± 2 g L^{-1} at 120 h is largely attributable to non-fermentable oligosaccharides, corroborating HPLC profiles obtained for tropical-fruit substrates rich in pectic side chains (Freitas et al., 2021; Van Buggenhout et al., 2009; Voragen et al., 2009).

3.2. Ethanol recovery and distillate quality

The stabilised biomass profile (Figure 3) confirmed fermentation completion prior to distillation; the hearts fraction represented 90 ± 3 % of the condensate mass with an average ethanol strength of 95.4 ± 0.6 % v v^{-1} (Table 2). Methanol was held below 85 mg L^{-1}, two orders of magnitude under the ASTM D4806 limit (5000 mg L^{-1}), validating both the peel-removal strategy and the 64 – 78 °C heads cut.

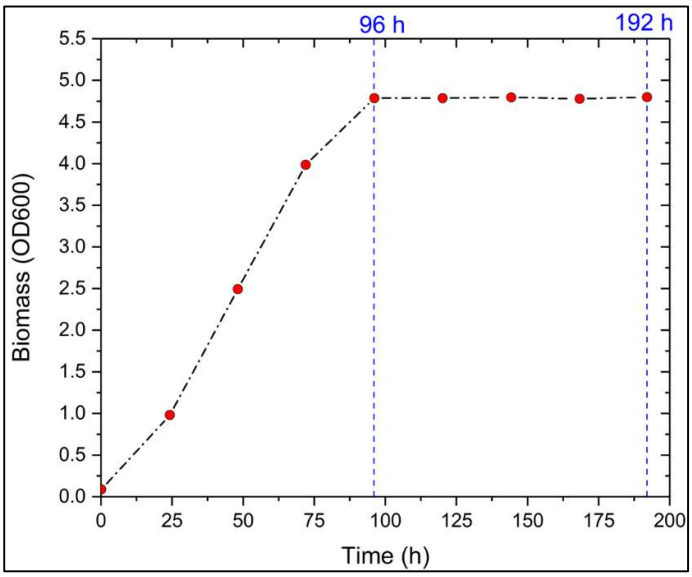

Figure 3. Yeast biomass growth during fermentation. Optical density (OD_{600}) increases exponentially to 4.8 ± 0.2 by 96 h and then plateaus, mirroring sugar depletion; means ± SD, n = 3.

Higher alcohols remained under 0.15 % v v^{-1}, mirroring values seen in cane-molasses distillates and indicating that protein-derived fusel-oil formation was minimal (Ingledew, 2015; Mousdale, 2018). Such low congener levels are advantageous for downstream dehydration, as they reduce the load on molecular-sieve beds or azeotropic extractants (Din et al., 2021).

Table 2. Comprehensive chemical composition of the ethanol hearts fraction obtained from Maradol papaya fermentation, compared with ASTM D4806 fuel-ethanol limits.

Parameter / Compound	Unit	This study (mean ± SD, $n = 3$)	ASTM D4806 limit*	Compliance
Ethanol	% v v^{-1}	95.4 ± 0.6	≥ 92.1	✓
Water (Karl-Fischer)	% v v^{-1}	4.2 ± 0.3	≤ 5.0*	✓
Methanol	mg L^{-1}	85 ± 4	≤ 5000	✓
Higher alcohols (propanol + iso-butanol + iso-amyl)	% v v^{-1}	0.15 ± 0.02	–**	✓
Sulphur	mg kg^{-1}	8 ± 1	≤ 30	✓
Acidity (as acetic acid)	mg KOH g^{-1}	0.0018 ± 0.0002	≤ 0.007	✓

* ASTM D4806 expresses dryness through ethanol purity and a visual "water and sediment" test; a laboratory threshold of ≤ 5 % v v^{-1} water is commonly applied prior to final molecular-sieve dehydration.

** ASTM D4806 sets no explicit numerical limit for fusel (higher) alcohols; the low values measured here are typical of fruit-derived distillates and do not hinder downstream dehydration.

3.3. Yield on raw fruit and comparative assessment

Normalised to fresh-fruit mass, the process delivered 68.4 ± 1.3 g ethanol per kg of papaya (energy yield ≈ 1.45 MJ kg^{-1}). This compares favourably with bioethanol yields in other studies (Kantiyok et al., 2021) and from mature plantain pulp (62 g kg^{-1}) and sweet-sorghum juice (65 g kg^{-1}) processed similarly (Rutto et al., 2013; Uchôa et al., 2021). The superior output is linked to papaya's high intrinsic °Brix and low lignocellulosic barrier, which facilitate direct fermentation without enzymatic saccharification.

The exclusion of peel and seeds from the fermentation substrate was guided by both compositional and functional considerations. Pilot trials using whole fruit (pulp + peel) resulted in significantly elevated methanol concentrations (>320 mg L^{-1}), even after applying 4 % heads cut. This effect is attributed to the high pectin content of papaya peel, whose methyl ester groups are hydrolyzed and converted to methanol during fermentation. Removing the peel reduced methanol levels by approximately 75 %, allowing a smaller heads discard (2 %) and improving ethanol recovery. Similarly, the seeds were excluded due to their content of phenolic compounds, fatty acids, and alkaloids such as benzyl isothiocyanate, which are known to exhibit antimicrobial activity or inhibit yeast metabolism (Amran et al., 2021; Malathi et al., 2024). Although papaya seeds may be valorised separately (e.g., for oil extraction), their presence was found to be incompatible with efficient ethanol production under the studied conditions (Das and Prasad, 2024; Parimi et al., 2024).

3.4. Process integration and valorisation pathways

With Q_{EtOH} exceeding 1 g L^{-1} h^{-1}, a 5-day batch cycle is feasible in resource-limited settings, aligning with weekly harvest intervals often observed among smallholders (Mungai et al., 2016). The residual vinasse, rich in nitrogen (5.2 ± 0.3 g L^{-1}) and with a C:N ratio of 8.1, can be valorised as a fertigation supplement or anaerobic-digestion feed. A block-flow mass-and-energy diagram (Figure 4) illustrates these loops, underscoring the compatibility of papaya bioethanol with circular-bioeconomy objectives and integrated waste-management schemes.

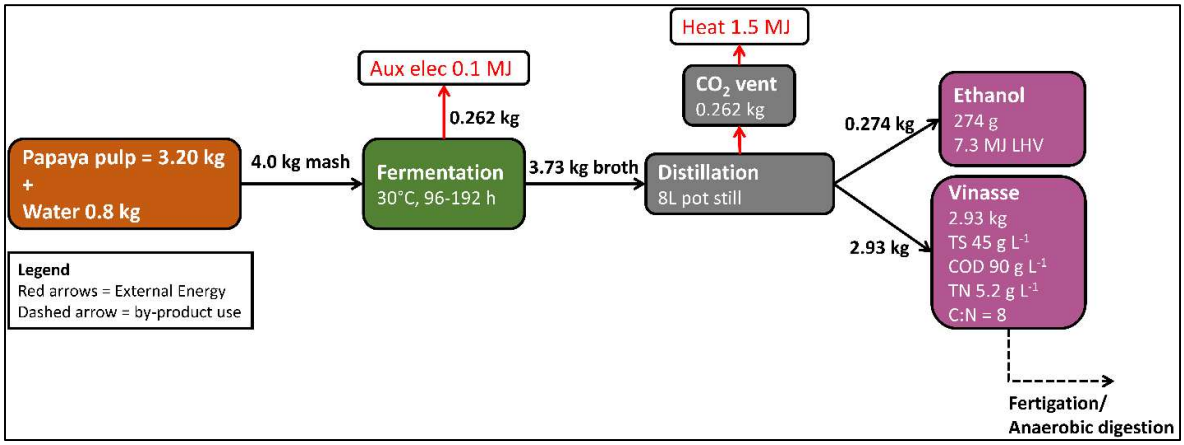

Figure 4. Mass- and energy-balance for a 4 kg papaya batch. Fermentation of 3.20 kg pulp + 0.80 kg water uses 0.10 MJ electricity and releases 0.262 kg CO_2; distillation adds 1.5 MJ heat and yields 0.274 kg ethanol (7.3 MJ LHV) and 2.93 kg vinasse (TS 45 g L^{-1}, COD 90 g L^{-1}, TN 5.2 g L^{-1}, C: N ≈ 8). Red arrows indicate energy inputs; dashed arrow shows vinasse valorisation.

The ethanol produced through this process can be used directly as a fuel blend component in rural or off-grid settings, supporting energy access in smallholder communities. Its compliance with ASTM D4806 standards enables integration into local biofuel markets, particularly where conventional feedstocks are limited or economically unviable. Moreover, in low-income regions, this bioethanol offers practical benefits as a clean-burning cooking fuel, reducing reliance on firewood or charcoal and helping to mitigate indoor air pollution and deforestation. Its application in small-scale cookstove systems aligns with circular bioeconomy goals by converting postharvest papaya waste into a locally produced, renewable energy source.

3.5. Alternative microorganisms and culture potential

K. marxianus demonstrated moderate ethanol production (46.2 ± 2.1g L^{-1}) and high thermotolerance, maintaining > 80% viability at 42 °C. *P. kudriavzevii* displayed slower growth kinetics but reached ethanol concentrations of 52.8 ± 1.9 g L^{-1} at 30 °C, suggesting potential for use in extended fermentation processes and could be advantageous in low-tech fermentation environments (de Moura Ferreira et al., 2022; Mo et al., 2019).

In addition, native isolates recovered from spontaneous fermentation included *Lactobacillus plantarum*, *Acetobacter tropicalis*, and wild *Saccharomyces* spp. *L. plantarum*, a facultative heterofermentative lactic acid bacterium, could help lower pH during early fermentation stages,

inhibiting contaminants and stabilizing microbial dynamics (Chen et al., 2017). *A. tropicalis*, although an obligate aerobe, is known to oxidize residual sugars and ethanol to acetic acid under aerobic conditions, potentially contributing to post-fermentation valorisation pathways or redox balance (Díaz-Muñoz and De Vuyst, 2022). Wild *Saccharomyces* strains may offer metabolic diversity or stress tolerance traits valuable for multi-strain fermentation (Ge et al., 2025). Although these microorganisms were not co-cultured in the present study, their roles suggest promising avenues for future research on metabolic synergy, pH modulation, and side-stream valorisation in mixed-culture bioethanol systems (Nady et al., 2025; Rappaport et al., 2024; Romanens et al., 2020).(Rappaport et al., 2024; Romanens et al., 2020)

4. Conclusions

The present study demonstrates that peeled *Carica papaya* L. var. Maradol pulp is a highly effective substrate for bioethanol production. Batch fermentation with *S. cerevisiae* Ethanol Red™ achieved a final ethanol titre of 92.3 ± 1.8 g L^{-1}, a volumetric productivity (Q_{EtOH}) of 1.04 ± 0.05 g L^{-1} h^{-1}, and an ethanol yield on substrate ($Y_{P/S}$) of 0.46 ± 0.01 g g^{-1} (≈ 90 % of theoretical). On a fresh fruit basis, the process yielded 68.4 ± 1.3 g ethanol kg^{-1} (≈ 1.45 MJ kg^{-1}), surpassing comparable tropical fruit residues. Notably, the workflow required neither enzymatic saccharification nor complex nutrients, and fermentation was completed in under five days at 30 °C.

Fractional distillation, including a 64–78 °C heads cut, produced a hearts fraction that met all ASTM D4806 requirements: ethanol 95.4 ± 0.6 % v/v, methanol 85 ± 4 mg L^{-1}, water 4.2 ± 0.3 % v/v, sulphur 8 ± 1 mg kg^{-1}, and acidity 0.0018 ± 0.0002 mg KOH g^{-1}. These results underscore the importance of peel removal for methanol control. The nitrogen-rich vinasse (C: N ≈ 8) offers additional valorisation potential as a fertigation medium or anaerobic digestion feed, making the process well suited to decentralised, smallholder contexts. Integrating solar-assisted heat supply or biogas-fired boilers, as illustrated in the process flow analysis, could further reduce the carbon footprint and advance circular bioeconomy objectives by diverting papaya post-harvest losses from uncontrolled decomposition to renewable fuel.

Future work should scale the system to pilot level, explore continuous or fed-batch modes to boost Q_{EtOH}, couple on-site membrane dehydration for anhydrous ethanol, and conduct life cycle and techno-economic assessments. Valorising co-products such as seed oil and peel pectin could enhance overall sustainability and profitability. Finally, culture-dependent screening of spontaneous fermentation batches revealed the presence of lactic acid bacteria (*Lactobacillus plantarum*) and acetic acid bacteria (*Acetobacter tropicalis*), both capable of modulating pH and metabolite profiles. While these organisms were controlled in the current study, future experiments could explore co-culture strategies or metabolic engineering to enhance carbon flux, reduce inhibitors, or valorise side streams.

Author Contributions: EDD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing - original draft, Writing, review and editing. PAFV: Data curation, Formal analysis, Investigation, Validation, Writing - original draft. SMS: Data curation, Formal analysis, Writing review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This project has received funding from the European Union's Horizon EUSPA-2022-SPACE research and innovation actions under grant agreement No101131859 (COMUNIDAD).

Acknowledgments: The authors gratefully acknowledge Dr. Héctor A. Tinoco N. (Department of Mechanical Engineering, Universidad Autónoma de Manizales) for generously providing laboratory access and valuable technical advice. We also thank the laboratory staff for their assistance with equipment maintenance and safety oversight.

Conflicts of Interest: The authors have no conflicts of interest to disclose.

Data availability statement: Data supporting the findings of this study are available on request from the corresponding author.

5. References

1. Ahmad I, Chwee CP (2008): An overview of the world production and marketing of tropical and subtropical fruits. Acta Horticulturae, 787, 47–58. https://doi.org/10.17660/ActaHortic.2008.787.3
2. Ampah J, Dzisi KA, Addo A, Bart-Plange A (2022): Drying kinetics and chemical properties of mango. International Journal of Food Science, 2022(1), 6243228. https://doi.org/10.1155/2022/6243228
3. Amran AH, Zaidi NS, Syafiuddin A, Zhan LZ, Bahrodin MB, Mehmood MA, Boopathy R (2021): Potential of Carica papaya seed-derived bio-coagulant to remove turbidity from polluted water assessed through experimental and modeling-based study. Applied Sciences, 11(12), 5715. https://doi.org/10.3390/app11125715
4. Awodi PS, Ogbonna JC, Nwagu TN (2022): Bioconversion of mango (Mangifera indica) seed kernel starch into bioethanol using various fermentation techniques. Heliyon, 8(6), e09707. https://doi.org/10.1016/j.heliyon.2022.e09707
5. Bai FW, Anderson WA, Moo-Young M (2008): Ethanol fermentation technologies from sugar and starch feedstocks. Biotechnology Advances, 26(1), 89–105. https://doi.org/10.1016/j.biotechadv.2007.09.002
6. Bhatt SC, Negi S, Chauhan M, Mishra S, Rizwanuddin S, Naik B, Gupta AK, Kumar V, Kumar V (2025): Exploring papaya byproducts: A step toward circular economy and sustainability. ACS Food Science & Technology, 5(1), 50–60. https://doi.org/10.1021/acsfoodscitech.4c00872
7. Burns P, Saengmanee P, Doung-Ngern U (2023): Papaya: The versatile tropical fruit. In Sarwar Khan M (ed.), Tropical Plant Species and Technological Interventions for Improvement. IntechOpen. https://doi.org/10.5772/intechopen.104624
8. Campos DA, Gómez-García R, Vilas-Boas AA, Madureira AR, Pintado MM (2020): Management of fruit industrial by-products—A case study on circular economy approach. Molecules, 25(2), 320. https://doi.org/10.3390/molecules25020320
9. Casabar JT, Unpaprom Y, Ramaraj R (2019): Fermentation of pineapple fruit peel wastes for bioethanol production. Biomass Conversion and Biorefinery, 9(4), 761–765. https://doi.org/10.1007/s13399-019-00436-y
10. Chen Y, Huang Y, Bai Y, Fu C, Zhou M, Gao B, Wang C, Li D, Hu Y, Xu N (2017): Mixed cultures of Saccharomyces cerevisiae and Lactobacillus plantarum in alcoholic fermentation affect physicochemical and sensory properties of citrus vinegar. LWT, 84, 753–763. https://doi.org/10.1016/j.lwt.2017.06.032
11. Das U, Prasad SS (2024): Papaya waste as a nutraceutical product. In Tomer V, Chhikara N, Kumar A, Panghal A (eds.), Nutraceuticals from Fruit and Vegetable Waste. Wiley, pp. 425–439. https://doi.org/10.1002/9781119803980.ch16

12. de Moura Ferreira MA, da Silveira FA, da Silveira WB (2022): Ethanol stress responses in Kluyveromyces marxianus: Current knowledge and perspectives. Applied Microbiology and Biotechnology, 106(4), 1341–1353. https://doi.org/10.1007/s00253-022-11799-0
13. Díaz-Muñoz C, De Vuyst L (2022): Functional yeast starter cultures for cocoa fermentation. Journal of Applied Microbiology, 133(1), 39–66. https://doi.org/10.1111/jam.15312
14. Din NAS, Lim SJ, Maskat MY, Mutalib SA, Zaini NAM (2021): Lactic acid separation and recovery from fermentation broth by ion-exchange resin: A review. Bioresources and Bioprocessing, 8(1), 31. https://doi.org/10.1186/s40643-021-00384-4
15. Fagundes VD, Machado ÊL, Schneider RCS, Colla LM (2024): Life cycle assessment of bioethanol production from banana, potato and papaya waste. The International Journal of Life Cycle Assessment, 29(10), 1846–1862. https://doi.org/10.1007/s11367-024-02342-6
16. FAOSTAT (2023): Crops and livestock products. Food and Agriculture Organization of the United Nations, Rome. https://www.fao.org/faostat/en/#data/QCL
17. Freitas CMP, Coimbra JSR, Souza VGL, Sousa RCS (2021): Structure and applications of pectin in food, biomedical, and pharmaceutical industry: A review. Coatings, 11(8), 922. https://doi.org/10.3390/coatings11080922
18. Galán Saúco V, Herrero M, Hormaza JI (2014): Tropical and subtropical fruits. In Dixon GR, Aldous DE (eds.), Horticulture: Plants for People and Places, Vol. 1. Springer, pp. 123–157. https://doi.org/10.1007/978-94-017-8578-5_5
19. Ge Y, Wu Y, Aihaiti A, Wang L, Wang Y, Xing J, Zhu M, Hong J (2025): Metabolic pathways of yeast and acetic acid bacteria during fruit vinegar fermentation and their influence on flavor. Microorganisms, 13(3), 477. https://doi.org/10.3390/microorganisms13030477
20. Guerrero AB, Ballesteros I, Ballesteros M (2018): Potential of agricultural banana waste for bioethanol production. Fuel, 213, 176–185. https://doi.org/10.1016/j.fuel.2017.10.105
21. I Nengah Muliarta, Setianingsih NLPP, Sudiarta WS, Prawerti DAD, Somariana IK, Suwarmadi Putra IKS (2023): Predicting baker's yeast dosage and fermentation time for bioethanol production from papaya skin waste. Formosa Journal of Sustainable Research, 2(8), 1881–1898. https://doi.org/10.55927/fjsr.v2i8.4831
22. Ingledew WM (2015): Wallowing with the yeasts used to make alcohol. Journal of the American Society of Brewing Chemists, 73(3), 209–222. https://doi.org/10.1094/ASBCJ-2015-0614-01
23. Kader AA, Yahia EM (2011): Postharvest biology of tropical and subtropical fruits. In Yahia EM (ed.), Postharvest Biology and Technology of Tropical and Subtropical Fruits, pp. 79–111. Woodhead Publishing. https://doi.org/10.1533/9780857093622.79
24. Kantiyok BD, Mohammed SSD, Yabaya A (2021): Fruit wine produced from pawpaw (Carica papaya) and watermelon (Citrullus vulgaris) using Saccharomyces cerevisiae isolated from fermented foods. Science World Journal, 16(4). https://www.ajol.info/index.php/swj/article/view/222456
25. Karoney EM, Molelekoa T, Bill M, Siyoum N, Korsten L (2024): Global research network analysis of fresh produce postharvest technology. Postharvest Biology and Technology, 208, 112642. https://doi.org/10.1016/j.postharvbio.2023.112642
26. Katyal M, Singh R, Mahajan R, Sharma A, Gupta R, Aggarwal NK, Yadav A (2024): Valorization of papaya peel waste for production of nanocellulose by Novacetimonas hansenii BMK-3. Biotechnology and Applied Biochemistry, 72(4). https://doi.org/10.1002/bab.2706

27. Koul B, Pudhuvai B, Sharma C, Kumar A, Sharma V, Yadav D, Jin J-O (2022): Carica papaya L.: A tropical fruit with benefits beyond the tropics. Diversity, 14(8), 683. https://doi.org/10.3390/d14080683
28. Malathi VM, Abhimannue AP, Sharma K, Muricken DG, Tomy TL (2024): Papaya seeds: Nutraceutical potential and health-promoting activities. In Valorization of Fruit Seed Waste, pp. 285–313. Elsevier. https://doi.org/10.1016/B978-0-443-15535-2.00009-8
29. Mancero-Castillo D, Garcia Y, Aguirre-Munizaga M, Ponce de Leon D, Portalanza D, Avila-Santamaria J (2024): Dynamic perspectives into tropical fruit production: A review. Frontiers in Agronomy, 6, 1482893. https://doi.org/10.3389/fagro.2024.1482893
30. Mendoza-Grimón V, Amorós R, Fernández-Vera JR, Lopes da Veiga E, Palacios-Díaz MdP (2024): Utilising reclaimed water for papaya cultivation in Cape Verde: A case study. Agronomy, 14(11), 2726. https://doi.org/10.3390/agronomy14112726
31. Misnan NM, Afzan A, Omar MH, Low KH (2024): Flavonoid variability in Carica papaya var. Sekaki leaf maturation and its association with sexual differentiation. Natural Product Communications, 19(7), 1934578X241260175. https://doi.org/10.1177/1934578X241260175
32. Mitra SK (2018): Climate change impacts and mitigation strategies for tropical and subtropical fruits. Acta Horticulturae, 1216, 1–12. https://doi.org/10.17660/ActaHortic.2018.1216.1
33. Mo W, Wang M, Zhan R, Yu Y, He Y, Lu H (2019): Kluyveromyces marxianus developing ethanol tolerance through adaptive evolution. Biotechnology for Biofuels, 12(1), 63. https://doi.org/10.1186/s13068-019-1393-z
34. Mohamed Z, AbdLatif I, Mahir Abdullah A (2011): Economic importance of tropical and subtropical fruits. In Yahia EM (ed.), Postharvest Biology and Technology of Tropical and Subtropical Fruits, pp. 1–20. Woodhead Publishing. https://doi.org/10.1533/9780857093622.1
35. Mousdale D (2018): Biorefineries: Industrial perspectives and challenges. In Fermentation Microbiology and Biotechnology. CRC Press.
36. Mukhametzyanov RR, Ostapchuk TV, Dzhancharov TM, Ivantsova NN, Vasileva EN (2023): Changes in global production and trade of major tropical fruits. In Popkova EG et al. (eds.), Digital Agriculture for Food Security, pp. 147–153. Springer. https://doi.org/10.1007/978-3-031-27911-9_17
37. Mungai LM, Snapp S, Messina JP, Chikowo R, Smith A, Anders E, Richardson RB, Li G (2016): Smallholder farms and sustainable intensification potential. Frontiers in Plant Science, 7, 1720. https://doi.org/10.3389/fpls.2016.01720
38. Nady DS, Abdel-Halim S, Hegazy MEF, El-Desouky MA, Hanna DH (2025): Use of Carica papaya waste as bio-adsorbent for sewage wastewater treatment. Biomass Conversion and Biorefinery, 15(10), 15921–15938. https://doi.org/10.1007/s13399-024-06300-y
39. Parajuli R, Thoma G, Matlock MD (2019): Environmental sustainability of fruit and vegetable production supply chains. Science of the Total Environment, 650, 2863–2879. https://doi.org/10.1016/j.scitotenv.2018.10.019
40. Parimi MR, Gantala DD, Deepala NA, Bikkina SSY, Reddy JMK, Vangalapati M (2024): Optimizing bromelain extraction from green papaya peel waste. Environmental Science and Pollution Research. https://doi.org/10.1007/s11356-024-35435-8
41. Paternina GA, Luna FV, Bermudez AA (2022): Nutraceutical, thermophysical and textural characteristics of papaya and incidence for post-harvest management. Heliyon, 8(4), e09231. https://doi.org/10.1016/j.heliyon.2022.e09231

42. Patsalou M, Samanides CG, Protopapa E, Stavrinou S, Vyrides I, Koutinas M (2019): Citrus peel waste biorefinery for ethanol and methane. Molecules, 24(13), 2451. https://doi.org/10.3390/molecules24132451
43. Paull RE, Duarte O (2011): Tropical Fruits. CABI.
44. Paull RE, Nishijima W, Reyes M, Cavaletto C (1997): Postharvest handling and losses during marketing of papaya. Postharvest Biology and Technology, 11(3), 165–179. https://doi.org/10.1016/S0925-5214(97)00028-8
45. Queiroz RF, Oster AH, De Oliveira Silva E, Aroucha EMM (2024): Physical–chemical parameters and maturation of Formosa papaya. Proceedings of the National Academy of Sciences, India Section B, 94(5), 949–958. https://doi.org/10.1007/s40011-024-01654-9
46. Rappaport HB, Senewiratne NPJ, Lucas SK, Wolfe BE, Oliverio AM (2024): Metabolic roles of acetic acid bacteria in sourdough microbiomes. mSystems, 9(10), e00537-24. https://doi.org/10.1128/msystems.00537-24
47. Romanens E, Pedan V, Meile L, Schwenninger SM (2020): Anti-fungal Lactobacillus–Saccharomyces co-cultures influence cocoa bean fermentation. PLOS ONE, 15(10), e0239365. https://doi.org/10.1371/journal.pone.0239365
48. Roussos PA (2024): Climate change challenges in temperate and sub-tropical fruit tree cultivation. Encyclopedia, 4(1). https://doi.org/10.3390/encyclopedia4010036
49. Rutto LK, Xu Y, Brandt M, Ren S, Kering MK (2013): Juice, ethanol and grain yield of sweet sorghum cultivars. Journal of Sustainable Bioenergy Systems, 3(2). https://doi.org/10.4236/jsbs.2013.32016
50. Sánchez ÓJ, Cardona CA (2008): Trends in biotechnological production of fuel ethanol from feedstocks. Bioresource Technology, 99(13), 5270–5295. https://doi.org/10.1016/j.biortech.2007.11.013
51. Singh SP, Sudhakar Rao DV (2011): Papaya. In Yahia EM (ed.), Postharvest Biology and Technology of Tropical and Subtropical Fruits, pp. 86–126e. Woodhead Publishing. https://doi.org/10.1533/9780857092618.86
52. Sivakumar D, Wall MM (2013): Papaya fruit quality management during the postharvest supply chain. Food Reviews International, 29(1), 24–48. https://doi.org/10.1080/87559129.2012.692138
53. Suo H, Xiao S, Wang B, Cai Y-X, Wang J-H (2024): Conversion of jackfruit flake into value-added products by fermentation. Food Chemistry: X, 21, 101164. https://doi.org/10.1016/j.fochx.2024.101164
54. Uchôa PZ, Porto RCT, Battisti R, Marangoni C, Sellin N, Souza O (2021): Ethanol from banana harvest biomass: Three-waste fermentation and scale-up assessment. Industrial Crops and Products, 174, 114170. https://doi.org/10.1016/j.indcrop.2021.114170
55. Van Buggenhout S, Sila DN, Duvetter T, Van Loey A, Hendrickx M (2009): Pectins in processed fruits and vegetables: Part III—texture engineering. Comprehensive Reviews in Food Science and Food Safety, 8(2), 105–117. https://doi.org/10.1111/j.1541-4337.2009.00072.x
56. Vij T, Prashar Y (2015): A review on medicinal properties of Carica papaya Linn. Asian Pacific Journal of Tropical Disease, 5(1), 1–6. https://doi.org/10.1016/S2222-1808(14)60617-4
57. Vinod BR, Asrey R, Sethi S, Prakash J, Meena NK, Menaka M, Mishra S, Shivaswamy G (2023): Advances in physical treatments for papaya postharvest quality. eFood, 4(2), e79. https://doi.org/10.1002/efd2.79
58. Voragen AGJ, Coenen G-J, Verhoef RP, Schols HA (2009): Pectin, a versatile plant polysaccharide. Structural Chemistry, 20(2), 263–275. https://doi.org/10.1007/s11224-009-9442-z

59. Wu L, Yan X, Huang J, Xu X, Zhang S, Cui S, Chen X, Zeng Z, Huang S, Zeng Y, Weng Y, Lu B, Su D, Yang W, Li Y, Ma Y (2024): Sustainable fruit production: Challenges and optimization strategies. Agricultural Systems, 221, 104132. https://doi.org/10.1016/j.agsy.2024.104132
60. Zhou Z, Ford R, Bar I, Kanchana-udomkan C (2021): Papaya flavour profiling. Genes, 12(9), 1416. https://doi.org/10.3390/genes12091416