ADVANCES IN AGRICULTURAL AND FOOD RESEARCH JOURNAL

Original Research Article

Techno-Economic Analysis of Nata de Coco as a Supporting Medium for Immobilizing Pectinase in Guava Juice Clarification

Nurul Azira Zahari[1], Mohd Noriznan Mokhtar[1,2]*

[1]Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

[2]Laboratory of Processing and Product Development, Institute of Plantation Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author: Mohd Noriznan Mokhtar, Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia; noriznan@upm.edu.my

Abstract: Enzymatic clarification using pectinase is a promising method to reduce cloudiness and viscosity in fruit juices. However, pectinase faces challenges like poor stability, limited reusability, and potential juice contamination. While immobilization could address these issues, its feasibility for industrial-scale guava juice clarification remains understudied. This study aims to explore the techno-economic aspects of using immobilized pectinase derived from nata de coco (NDC) for guava juice clarification. Initially, the focus lies on pectinase immobilization, morphology analysis, reusability, and reaction kinetics. Subsequently, an economic evaluation of the integrated guava juice process design with immobilized pectinase was conducted. After immobilization, results demonstrated a notable immobilized yield of 55.32% protein with 62.78% pectinase activity. Immobilized pectinase achieved a 61% reduction in turbidity. After the 6th cycle, the immobilized pectinase exhibited an impressive above 60.0% residual activity, indicating its potential for repeated use in the guava juice clarification process, enhancing its commercial viability. Furthermore, the study revealed a return on investment (ROI) of 20.19% and a payback period of 4.95 years, suggesting that the integration of immobilized pectinase could be financially beneficial for guava juice production. The sensitivity analysis highlighted that fluctuations in guava juice pricing significantly affect both ROI and net present value (NPV), emphasising the importance of market conditions and pricing strategies in financial decision-making. Therefore, these insights offer valuable guidance for optimising process design and enhancing project profitability in guava juice manufacturing.

Keywords: Guava juice; nata de coco; immobilized pectinase; enzymatic clarification; techno-economic analysis

Received: 22nd July 2024

Accepted: 16th December 2024

Available Online: 20th January 2025

Published: 24th June 2025

Citation: Zahari, N. A. & Mokhtar, M. N. Techno-economic analysis of nata de coco as a supporting medium for immobilizing pectinase in guava juice clarification. *Adv Agri Food Res J* 2025; 6(1): a0000556. https://doi.org/10.36877/aafrj.a0000556.

1. Introduction

Guava (*Psidium guajava L.*) is gaining recognition as a "superfood" in the agro-food industry due to its appealing characteristics, including health-promoting bioactive components and functional elements (Verma *et al*., 2013). Renowned for its nutritional richness, guava contains high levels of ascorbic acid (50–300 mg/100 g fresh weight) and a variety of carotenoids such as phytofluene, β-carotene, and lycopene (Mercadante *et al*., 1999). A previous study also investigated the guava extract content, revealing great levels of total phenolic (31–115 mg garlic acid equivalent/100 g fresh weight), total flavonoid (36–318 mg quercetin equivalent/100 fresh weight), and antioxidant activity; DPPH (4–14 μmol TE/g fresh weight), ABTS (2.5–12 μmol TE/g fresh weight) and FRAP (11.6–41 μmol FeSO4/g fresh weight) (Suwanwong & Boonpangrak, 2021), which can vary across different guava cultivars.

The extracted fruit juice often faces unwanted turbidity due to the suspension of polysaccharide particulates, primarily pectin, originating from the primary and inner cell walls (Deng *et al*., 2019). Pectin, categorised as a hydrocolloid, poses a challenge to the quality of guava juice when stored for prolonged periods, potentially affecting its commercial viability (Bhattacharjee *et al*., 2017). Elevated turbidity and viscosity can diminish consumer acceptance, particularly in specific markets, whether as pure guava juice or in blends with other juices (Ninga *et al*., 2018). Pectin's association with plant polymers and cell debris, characterised by a fibre-like molecular structure, complicates juice clarification, often leading to fouling issues during membrane filtration processes (Ninga *et al*., 2018).

Enzymatic clarification involves the breakdown of pectin molecules into smaller oligalacturonans by pectinase, leading to the flocculation of pectin-protein complexes and resulting in significantly reduced pectin and viscosity levels in the juice. The enzymatically treated juice can then be easily clarified through centrifugation or filtration (Ninga *et al*., 2021). This process enhances key attributes of the juice such as clarity, aroma, and flavour (Harsh *et al*., 2014). Despite the high catalytic efficiency of pectinase, free enzymes pose challenges such as poor stability under operational conditions, limited reusability in industrial processes, and the potential presence of enzyme preparation compounds in the final food product (Garcia-Galan *et al*., 2011).

In situations where reaction conditions dictate enzyme activity, immobilization emerges as a viable strategy to tailor biocatalysts for specific applications (Sulaiman *et al*., 2015a). Recent advancements in enzyme immobilization have enabled the development of biocatalysts suitable for industrial use, enhancing catalytic properties even under challenging

conditions, facilitating enzyme recovery from reaction mediums, and promoting their reuse, thereby enhancing economic viability (Magro *et al*., 2019). The choice of support material and immobilization protocols play a critical role in preparing enzyme biocatalysts with high activity and operational stability (dos Santos *et al*., 2015). Nata de coco (NDC), a bacterial cellulose derived from coconut water through *Acetobacter xylinum* fermentation, exhibits significant potential as a support for pectinase immobilization. Its similarity to pure cellulose in terms of functional groups (-OH) allows for covalent interactions, making it suitable for various coupling reactions for spacer arm and ligand preparation (Cieh *et al*., 2017; Sulaiman *et al*., 2015b). Furthermore, NDC possesses unique properties such as high purity, crystallinity, and mechanical strength (Halib *et al*., 2012), further enhancing its suitability for enzyme immobilization. While NDC has been utilised in the immobilization of enzymes such as glucoamylase (Wu & Lia, 2008), laccase (Frazão *et al*., 2014), lysozyme (Bayazidi *et al*., 2018), and lipase (Dikshit & Kim, 2020), its potential in pectinase immobilization remains largely unexplored.

This immobilization technique could significantly improve the guava juice industry by enhancing enzyme stability, reducing processing costs, and increasing production efficiency. It would be particularly beneficial in regions with large-scale guava production, such as Southeast Asia and Latin America, where high juice clarity and extended enzyme reuse are critical for competitive market positioning. While several academic studies have explored the use of immobilized enzymes for fruit juice clarification, the absence of industrial processes employing immobilized enzymes presents a compelling challenge in developing stable and effective biocatalysts for this purpose (Magro *et al*., 2019). Moreover, the integration of simulation techniques for process design, coupled with techno-economic analysis, plays a crucial role in assessing the viability of proposed projects on an industrial scale (Do *et al*., 2014; Sikder *et al*., 2012). To date, no techno-economic study has been conducted on the implementation of such a promising system for guava juice processing, particularly utilising an immobilized pectinase reactor.

The present study intends to fill these gaps by focusing on two aspects: technical factors concerning the immobilized pectinase on NDC for guava juice clarification and the economic evaluation of integrated guava juice process design with the immobilized pectinase. For the first aspect, the study includes pectinase immobilization, morphology analysis, reusability, and reaction kinetics. The second aspect focuses on process design, internal rate of return (IRR), net present value (NPV), and sensitivity analysis for different

process aspects, including the number of batches per year, plant capacity, and price of main raw material and product.

2. Materials and Methods

2.1. Materials

NDC cube was obtained from Nandong Food Industry Sdn. Bhd., Sungai Besar, Selangor. The commercial enzyme used in this study is pectinase from *Aspergillus aculeatus*, Pectinex Ultra SPL (Novozymes, Denmark). The commercial enzyme is a blend of pectinases (main), hemicellulases, and beta-glucanases. Tropical white guava fruits were obtained from the local market. The fruits were ground and pressed using a filter cloth. Glutaraldehyde (GA) 25% was bought from Ajax Finechem (Australia) while 1,12-dodecanediame and pectin from citrus peel were bought from Sigma-Aldrich (Malaysia). Other chemicals used in this research were reagent grade.

2.2. NDC Surface Activation and Pectinase Immobilization

The NDC cube was cut into 2–5 mm small cubes and dried in the oven at 120^{o}C overnight. About 2 g of dried NDC was submerged in 25 mL methanol solution containing 5 g sodium methoxide and 1 g of 1,12-dodecanediame in a round bottom flask. This coupling reaction step for the spacer arm on the support surface was followed according to the previous study (Sulaiman *et al.*, 2015b). The wet support (NDC—1,12-dodecanediame) was then stirred in 25 mL of 0.1 M potassium phosphate buffer (pH 8) containing 2.5% (v/v) of GA for 2 h at 25^{o}C. The precipitate was collected and washed with phosphate buffer. The wet activated support (NDC—1,12-dodecanediame—GA) was shaken with 30 mL of phosphate buffer solution (pH 5), then 0.2 mL of the pectinase solution was added and the whole mixture was stirred at 4^{o}C for 12 h. Any unbound pectinase was removed by washing with phosphate buffer until enzyme activity and protein content could not be detected in the filtrates.

2.3. Protein Content, Enzyme Activity, and Turbidity

The protein estimation was done according to the Bradford method proposed by previous work (Sulaiman *et al*., 2015b). Pectinase activity determination was done according to the titration assay method which also followed the previous study (Jiang *et al*., 2013). For the cloudiness or turbidity test, the guava juice sample was diluted with a 2-dilution factor and tested using a UV spectrophotometer (Ultrospec 3100 Pro, Amersham,

UK) based on the method used by previous study (Mousa, 2020). All analyses were conducted in replications.

2.4. Morphology

The surface morphologies of NDC, NDC—1,12-dodecanediame, NDC—1,12-dodecanediame—GA, and NDC—1,12-dodecanediame—GA—Pectinase were analysed and compared using Scanning Electron Microscopy (SEM S-3400N, Hitachi, Japan). The acceleration was set up in a range of 5 – 20 kV, and samples were sputter-coated with gold to avoid charging effects during SEM observations.

2.5. Reaction Kinetics

Immobilized enzyme (1 g) was added to 100 mL guava juice and incubated for 10, 20, 30, 40, 50, and 60 min (50°C). Immediately, each sample (top part) was pipetted (without immobilized enzyme particle) and was centrifuged at 10000×g for 5 min, and the supernatant was taken for turbidity analysis. The experiment was repeated by using free pectinase (0.05 mL), but its reaction was stopped by heating at 95°C for 5 min. Similarly, each sample was centrifuged, and the supernatant was analysed for turbidity, C_T (mg/mL). For the reaction kinetics study, a non-elementary kinetics model was used in which n-reaction order and reaction rate constant (k) were estimated by using graphical differentiation as Equation (1) below:

$$\ln\left(-\frac{dC_T}{dt}\right) = \ln k + n\ln C_T \quad (1)$$

2.6. Reusability of Immobilized Pectinase

About 1 g of immobilized pectinase was added to 50 mL of 1% pectin which was dissolved in 0.15 M NaCl (pH 7) and incubated at 50^{o}C for 1 h. Then the solution was titrated for enzyme activity analysis (Part 2.3). After that, the immobilized pectinase was collected and mixed with a newly prepared pectin solution to start a new cycle. The reactions were repeated until the 6th cycle and the activity of pectinase was measured.

2.7. Process Design Description

Process design, cost estimation, and economic evaluation were developed using the SuperPro Designer® (SPD) v12 package (Intelligent Inc., New Jersey, USA). Guava juice process simulation was developed and analysed for mass balance, capital cost estimation,

operating cost estimation, and economic evaluation. The process consists of fruit washing, grinding-pressing, clarification, centrifugation, blending, pasteurisation, and bottling. The base capacity of this study is 5000 kg/batch of guava fruit, and the annual operating time is 4350 hr (equivalent to 543 batches per year, generated by SPD). The guava juice processing was generally followed according to García (2018) with some modifications (e.g., reactor with immobilized pectinase and reaction kinetics).

It is assumed that the grinding phase breaks the guava cellular structure, exposing its constituents such as water, pomace, soluble solids, and suspended solids (mainly responsible for turbidity in juice), therefore it is depicted as a 'reaction' with the mass stoichiometry as the following Equation 2:

$$100\ \text{Guava} \rightarrow 12.9\ \text{Pomace} + 3.1\ \text{Suspended Solid} + 7.6\ \text{Soluble Solid} + 76.4\ \text{Water} \quad (2)$$

In the enzymatic clarification step, the reaction kinetics model parameters obtained from Part 2.5 were used in the SPD simulation. The stoichiometry equation used in this study is as Equation 3:

$$\text{Suspended Solid} \rightarrow \text{Clumped Solid} \quad (3)$$

2.8. Purchasing costs (PCs)

Equipment PCs were determined from various sources including Peters *et al*. (2003), local suppliers, the SPD database, and official traders' websites. Malaysia's import duty (6.1%), sale and service tax (SST) (10%) (ITA, 2024), and freight costs (10%) (Heinzle *et al*., 2006) were factored into the PC calculation. Additionally, unlisted equipment (e.g., pumps) can be estimated using a multiplier (e.g., 0.2 × PC) (Heinzle *et al*., 2006).

2.9. CAPEX and OPEX

Capital expenditure (CAPEX) estimation relied on the PC. Total plant cost (TPC), a part of direct fixed capital (DFC), comprises both total plant direct cost (TPDC) and total plant indirect cost (TPIC). TPDC, covering expenses such as equipment installation, process piping, instrumentation, electrical system, buildings, yard improvement, and auxiliary facility was determined using multipliers or fractions derived from solid-fluid processing plants (Peters *et al*., 2003). TPIC, including contractor fees contingency (CFC), working capital (WC), and start-up and validation costs (SC), were assessed using multiplier values from relevant literature (Heinzle *et al*., 2006; Peters *et al*., 2003) as detailed in Table 1.

Table 1. Key assumption used for the capital cost estimation (Peters *et al*., 2003; Heinzle *et al*., 2006)

Total Plant Direct Cost (TPDC)	
Installation	0.39 × PC
Piping	0.31 × PC
Instrumentation	0.26 × PC
Electrical Facilities	0.10 × PC
Buildings	0.29 × PC
Yard Improvement	0.12 × PC
Auxiliary Facilities	0.55 × PC
Total Plant Indirect Cost (TPIC)	
Engineering	0.25 × TPDC
Construction	0.35 × TPDC
Contractor's Fee and Contingency (CFC)	
Contractor's Fee	0.05 × TPC
Contingency	0.10 × TPC
Other Capital Costs	
Working Capital (WC)	30 days
Start-Up and Validation Cost (SC)	0.05 × DFC

Thus, the estimation of capital investment was derived from Equations 4-6 (Heinzle *et al*., 2006).

$$\text{Total Plant Cost (TPC)} = \text{TPDC} + \text{TPIC} \quad (4)$$

$$\text{Direct Fixed Capital (DFC)} = \text{TPC} + \text{CFC} \quad (5)$$

$$\text{Capital Investment} = \text{DFC} + \text{WC} + \text{SC} \quad (6)$$

Table 2. Cost of resources

Classification	Item	Cost/Price	Unit
Material	Guava fruit	500.00	US$/MT
	Additives (*e.g*., sugars and other ingredients)	0.50	US$/kg
	Carton box packaging	1.00	US$/kg
	Juice Packing Carton (1 L)	1.00	US$/kg
	Immobilized pectinase	60.00	US$/kg
Utilities (SPD database)	Clean water, 25°C	0.29	US$/MT
	Saturated steam, 152°C	12.00	US$/MT
	Chilled water, 5°C	0.40	US$/MT
	Electricity	0.10	US$/kWh
Laboratory & Quality Control		0.15 × Total Labour Cost	
Labour (Mokhtar, 2022)	Security Guard/General Staff (Certificate holder)	3.47	US$/h
	Clerk/Maintenance staff (Diploma holder)	4.07	US$/h
	Plant/Equipment operator (Diploma holder)	4.78	US$/h
	Executive officer/QC officer (Bachelor holder)	5.89	US$/h
	Plant manager (Bachelor holder)	9.20	US$/h

Classification	Item	Cost/Price	Unit
Product revenues	Juice box (12 items, 1L juice)	24.00	US$/entity
	Clumped solid (pectin-puree)	0.50	US$/kg
	Guava pomace	0.10	US$/kg
Washed Water	Water treatment service charge by IWK	0.13	US$/MT

Operating expenditure (OPEX), or operating costs, encompasses variable, fixed, and plant costs (Heinzle *et al*., 2006). Variable costs include materials, consumables, labour, QC-laboratory expenses, utilities, and waste disposal, with most information sourced from Table 2. In chemical plants, QC-laboratory costs are typically estimated at 15% of the total operating labour cost (Peters *et al*., 2003). Fixed costs, comprising depreciation, maintenance & repair, and insurance & local taxes, are summarised with multiplier values in Table 3 (Heinzle *et al*., 2006; Mokhtar, 2022). Plant overhead costs, such as medical services, safety measures, storage facilities, and cafeteria expenses, are also considered and added to the cost of facilities operation (Heinzle *et al*., 2006).

Table 3. Key assumptions used for the facility-dependent cost and economic evaluation parameters (Heinzle *et al*., 2006; Mokhtar, 2022)

Facility Dependent Cost (FDC)	
Maintenance	0.1 × PC
Depreciation	Straight line
Insurance	0.01 × DFC
Tax	0.02 × DFC
Factory Expense	0.05 × DFC
Economic Evaluation Parameters	
Year of Analysis	2024
Year of Construction Starts	2025
Construction Period	12 months
Start-up Period	4 months
Project Lifetime	10 years
Loan Interest	5%
Depreciation Method / Period	Straight line / 8 years
Salvage Value	0.05 × DFC
Income Taxes	24%

2.10. Economic Evaluation

All economic evaluation parameters are shown in Table 3. The SPD simulator generated CAPEX, OPEX, revenue, gross margin, IRR, ROI, and payback period will be employed as economic indicators (Sánchez *et al*., 2018). NPV based on a 10% discount rate is also an important element to represent the future cash flows related to the project.

2.11. Sensitivity Analysis

The number of batches per year, plant capacity, cost of main raw material, and price of main product were identified as the main factors that influence the feasibility of the proposed project (Mokhtar, 2022). Sensitivity analysis was carried out by changing ±10 to 30 % from their default value. In this section, we will assess how variations in these factors impact ROI and NPV by examining their percentage changes.

3. Results and Discussions

3.1. Pectinase Immobilization

Table 4. Covalent immobilization of pectinase on NDC

Pectinase added		**Unbound pectinase**		**Immobilized pectinase**		**Immobilization yield**	
Protein (μg/g-support), P_i	Activity (U/g-support), A_i	Protein (μg/g-support), P_u	Activity (U/g-support), A_u	Protein (μg/g-support), P_{im}	Activity (U/g-support), A_{im}	Protein binding (%) = $P_{im}\times100/P_i$	Activity (%) = $A_{im}\times100/(A_i - A_u)$
1022.2	0.126	456.8	0.059	565.44	0.042	55.32	62.78

NDC consists of mainly –OH in the carboxyl group, which is possible to involve coupling reaction with $–NH_2$ group located at both ends of the spacer arm. Thus, the amide bond –CONH– was formed between the support and the spacer arm (Cieh *et al*., 2017) as shown in Figure 1a. Then, another end-terminal of the $–NH_2$ group on the spacer arm reacts with the functional group of ligands (GA) and –CHO to form –CH=N– covalent bond as shown in Figure 1b. During pectinase immobilization, the $–NH_2$ group on the enzyme reacts on the other end-terminal of GA to form –CH=N– (Figure 1c). Chemical coupling agents used are very important in covalent interaction because they can improve binding efficiency, provide greater mobility (due to the presence of a spacer arm), and minimise steric hindrance compared to other immobilization methods (Sulaiman *et al*., 2015a). In Table 4, the pectinase immobilized yield is 55.32% of protein with 62.78% residual activity. These results were comparable with previous findings when using covalent immobilization on alginate support (Abdel Wahab *et al*., 2018; Li *et al*., 2007), chitosan support (Ramirez *et al*., 2015), and composites membranes (Lei *et al*., 2007). However, the immobilization yield of enzyme depends on the amount of enzyme added into the solution, surface area and type of support, concentration and type of coupling agents, and immobilization conditions (Cieh *et al*., 2017; Sulaiman *et al*., 2015b).

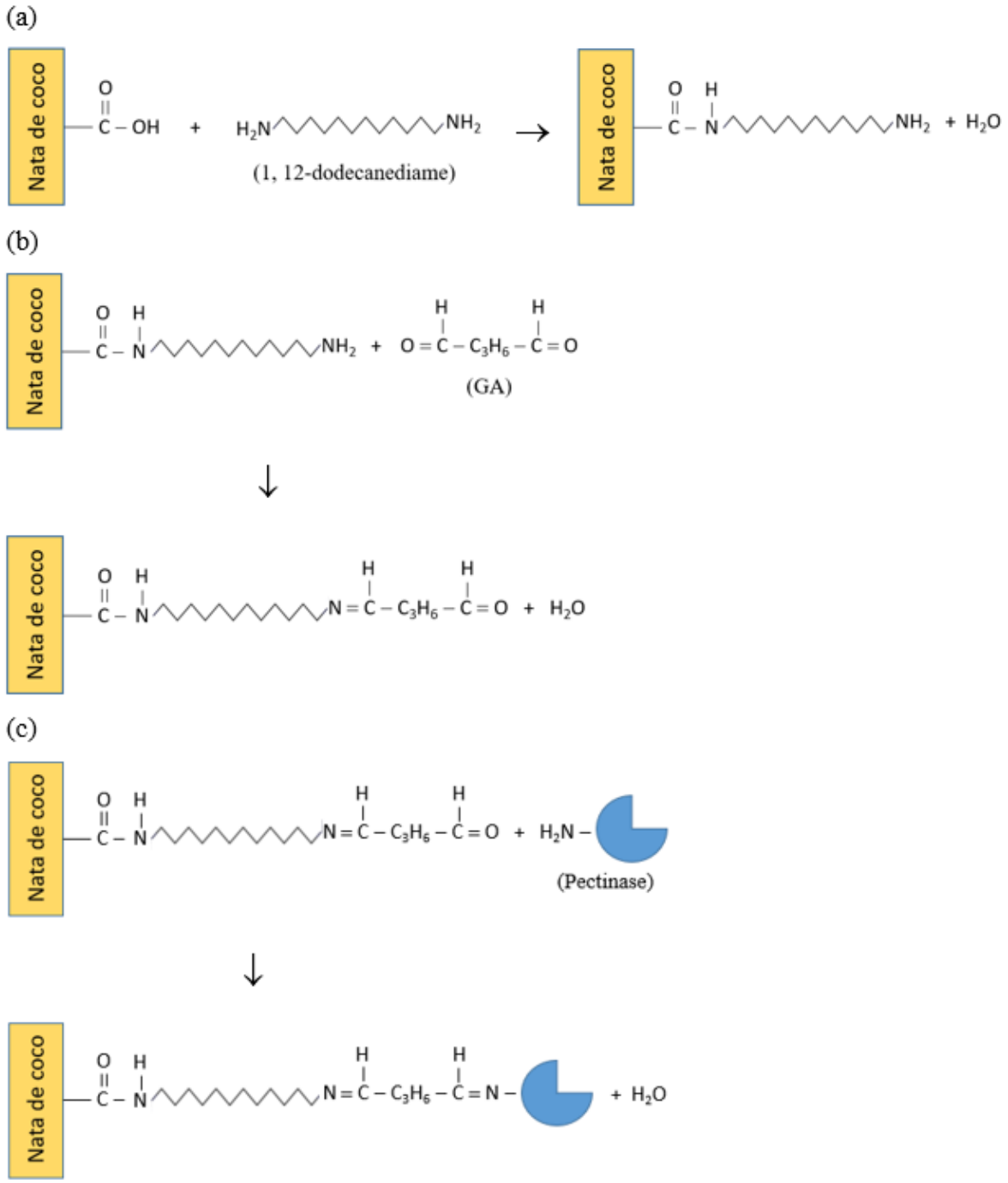


Figure 1. Reaction mechanisms for a) spacer arm preparation, b) ligand preparation, and c) immobilized pectinase preparation

3.2. Morphology Analysis

Figure 2 shows changes in the surface morphology of NDC observed in SEM analysis following different treatment steps. Figure 2a displays the NDC surface without any treatment, which exhibits a smooth surface. After coupling 1,12-dodecanediame on NDC,

considerable alterations were observable due to the formation of a granular layer on the NDC surface (Figure 2b). Figure 2c depicts that the effect of the use of GA on NDC—1,12-dodecanediame led to a significant covering of the rough waxy layer. Subsequently, upon immobilising pectinase on the activated NDC (NDC—1,12-dodecanediame—GA), the surface of NDC exhibited agglomerations, as depicted in Figure 2d. The covalent binding of pectinase to activated NDC is shown by this observation.

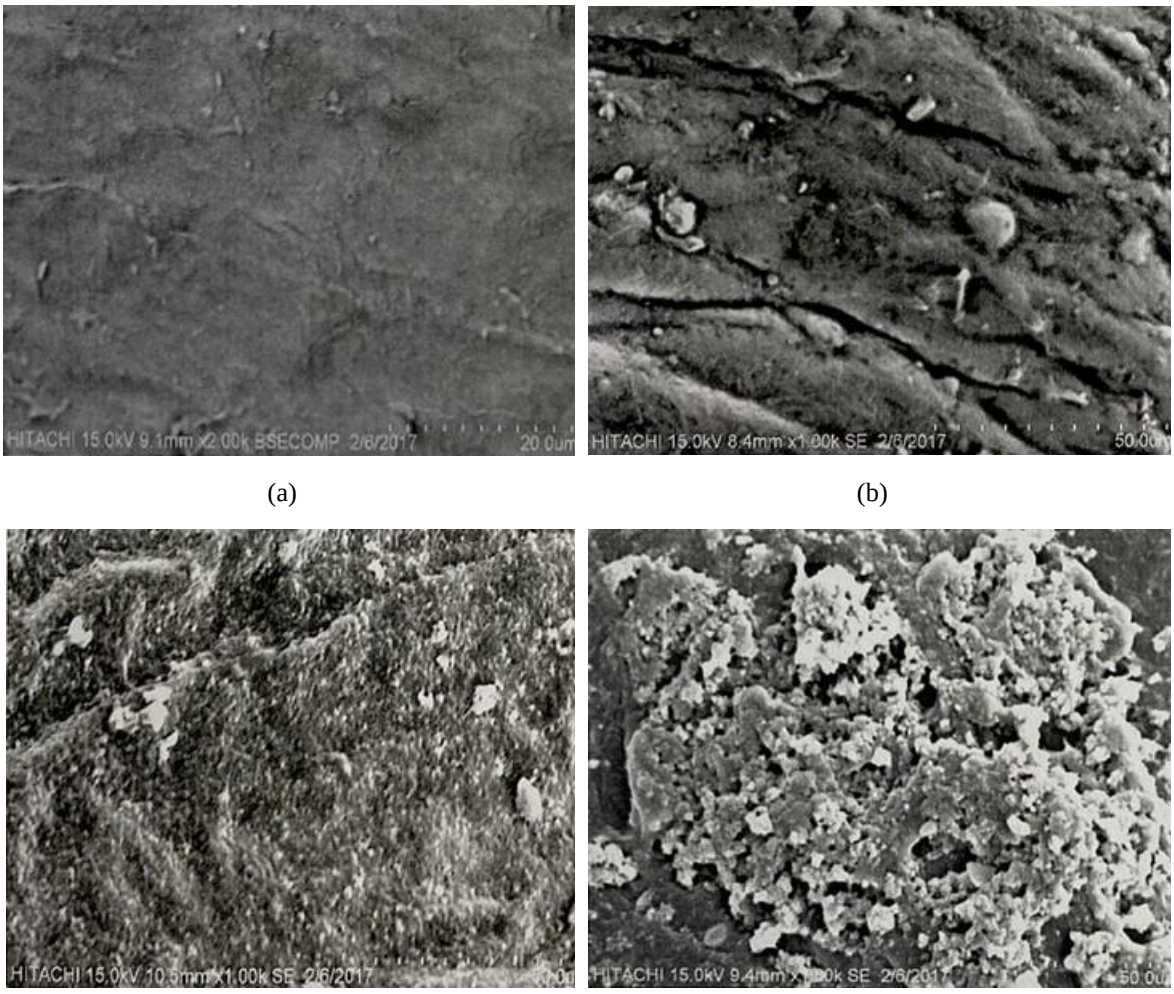
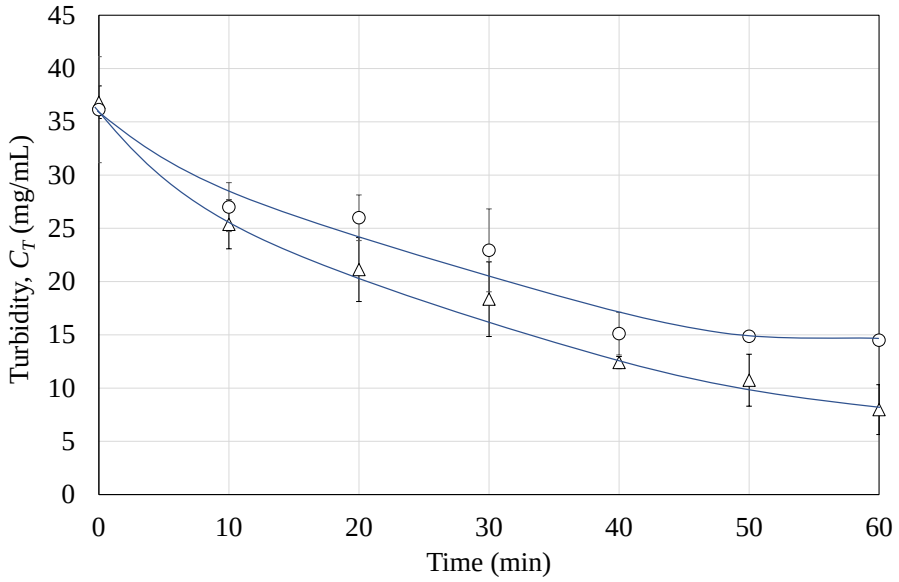
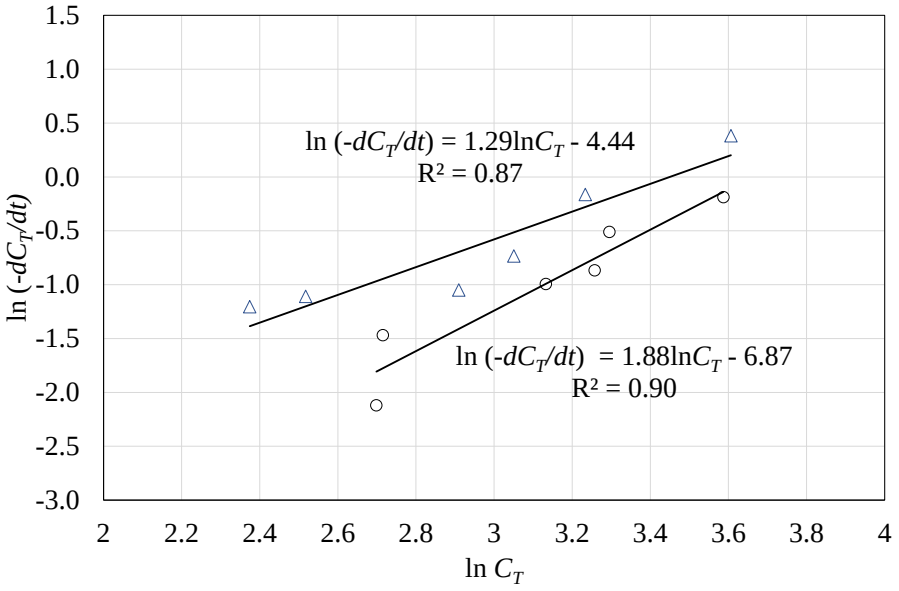


Figure 2. SEM image of a) NDC (2000x), b) NDC—1,12-dodecanediame (1000x), c) NDC—1,12-dodecanediame—GA (1000x), and d) NDC—1,12-dodecanediame—GA—Pectinase (1000x)

3.3. Reaction Kinetics



(a)



(b)

Figure 3. Profile of a) turbidity reduction and b) reaction kinetics study, (Δ native pectinase, o immobilized pectinase)

Figure 3a visually depicts the impact of utilising both native pectinase and immobilized pectinase (NDC—1,12-dodecanediame—GA—Pectinase) on reducing turbidity in guava juice. The graph demonstrates that the turbidity reduction rate achieved with native pectinase was significantly higher than that of immobilized pectinase, with reductions of 81% and 61%, respectively. This observation was further validated by the

findings presented in Table 4, which elucidates the decline in pectinase activity after the process of immobilization.

Figure 3b depicts graphical results from a non-elementary kinetics model to estimate the n-reaction order and reaction rate constant (*k*) for native and immobilized pectinase. The k-values for native and immobilized pectinase were 0.0118 and 0.0014, respectively. The data also estimated the n-reaction order for native and immobilized pectinase were 1.29 and 1.88, respectively

3.4. Reusability

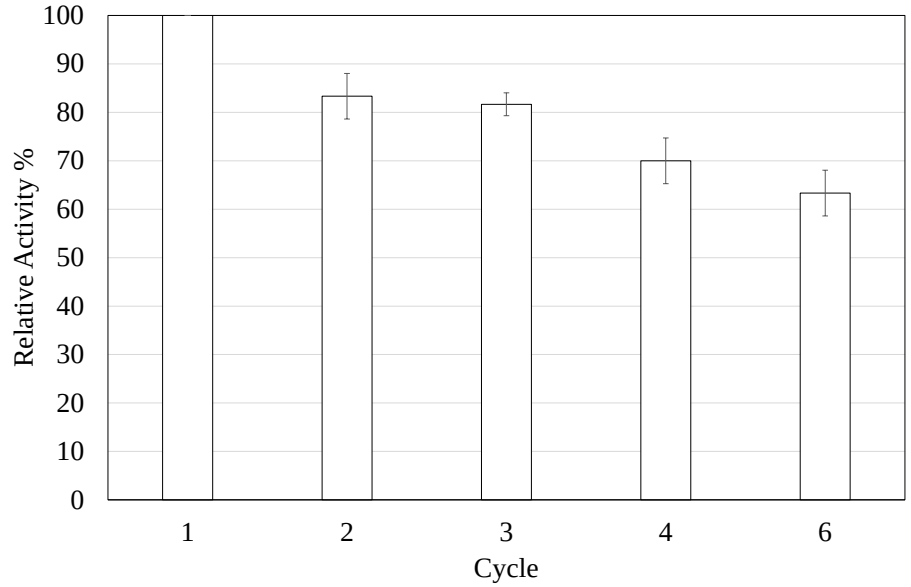


Figure 4. Reusability of immobilized pectinase (NDC—1,12-dodecanediame—GA—Pectinase) in guava juice clarification process

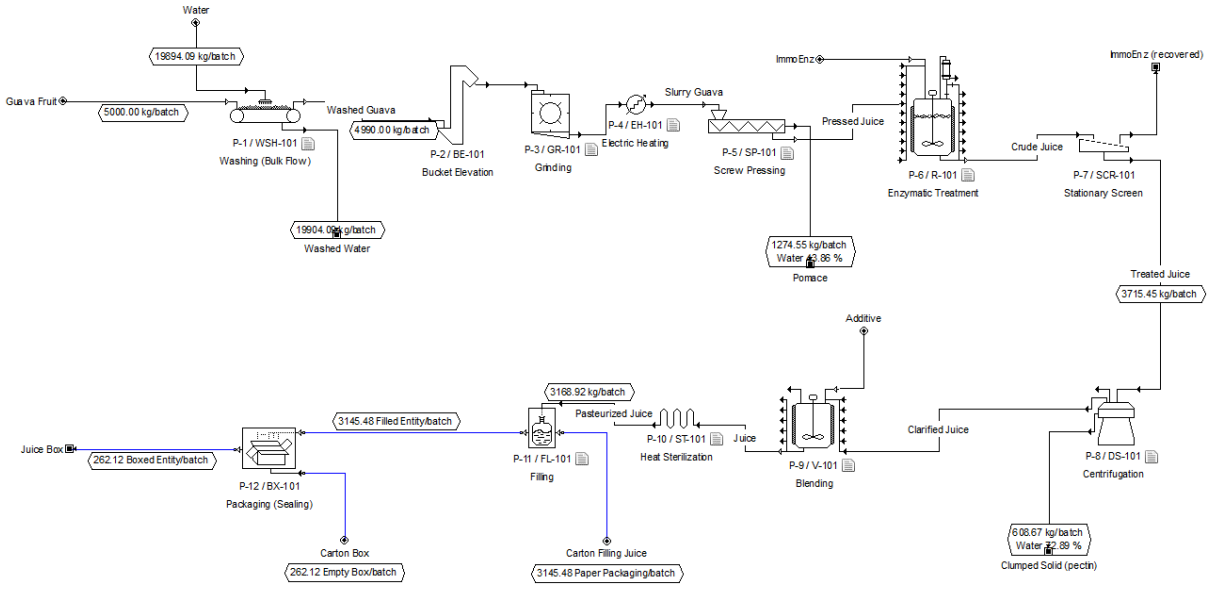
Figure 4 shows the reusability results for NDC—1,12-dodecanediame—GA—Pectinase, showcasing its performance over 6 consecutive cycles of batch reaction. After the 6th cycle, NDC—1,12-dodecanediame—GA—Pectinase exhibited an impressive above 60.0% residual activity. The main factor contributing to the reduction in pectinase activity is the denaturation of the enzyme during the reuse experiment (Mohammadi *et al*., 2020). However, a previous study by Chauhan *et al*. (2015) found that commercial pectinase immobilized on celite by adsorption retained only 13.33% of its residual enzyme activity after the 6th reaction cycle. This significant drop is likely due to the leaching out of enzyme molecules from the support, as physical adsorption involves weaker interactions compared to covalent binding. The notable reusability of NDC—1,12-dodecanediame—GA—Pectinase underscores the efficacy of activation through appropriate coupling agents,

suggesting the possibility of applying the NDA as support for pectinase in the juice clarification process.

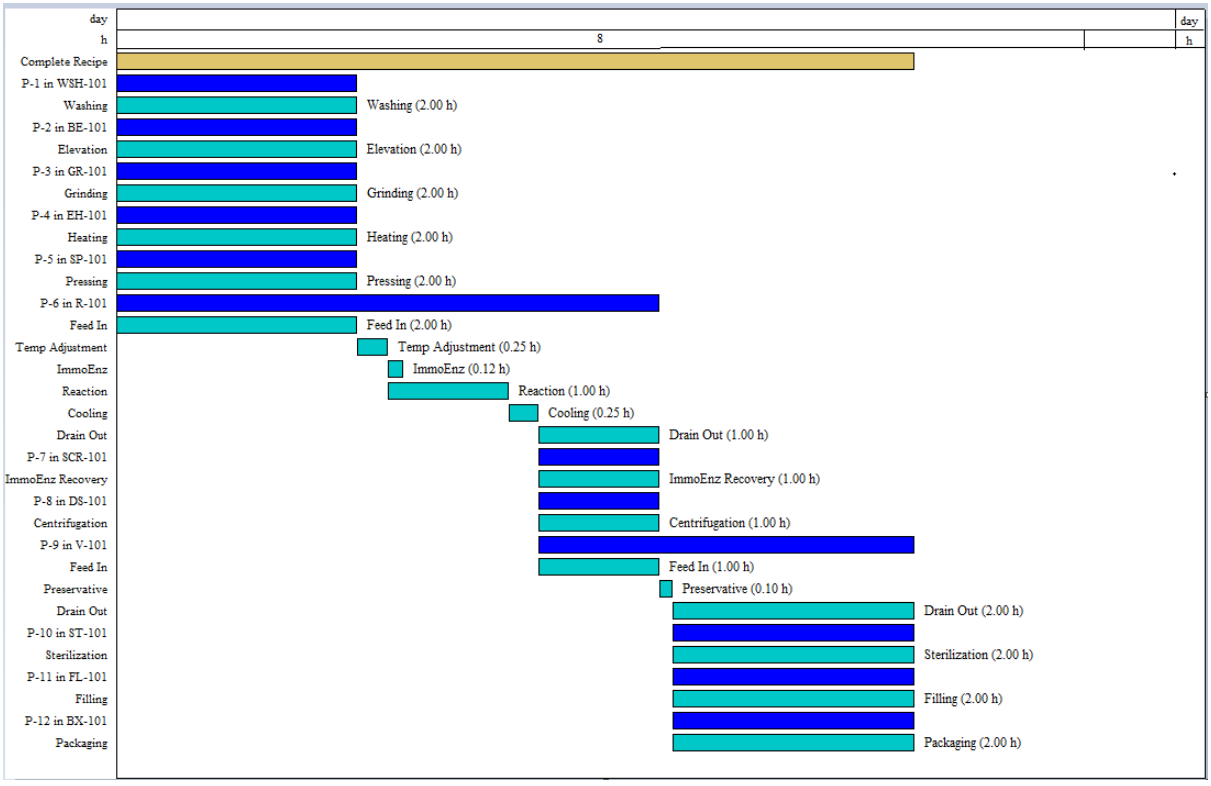
3.5. Process Description and Mass Balance

The washing of guava fruit is essential to eliminate foreign materials like dirt and impurities. This step not only prolongs the lifespan of processing equipment and machinery in subsequent sections but also ensures the maintenance of product quality (Mokhtar, 2022). Table 5 summarised the composition of the selected streams. Heating after grinding is required to help deactivate the native enzymes present in the fruit (e.g., peroxidase, polyphenase, etc.) to prevent excessive oxidation. High temperatures also will help more juice to be extracted during screw pressing. In this study, 50% residual solid was set and remained in the pomace. In the clarification tank, the temperature of extracted crude juice must be maintained at an optimum pectinase temperature of 50^{o}C. The use of immobilized pectinase in the clarification step resulted in 60 min reaction time of at least 60% turbidity reduction. When the treated juice is drained out, the immobilized pectinase can be easily separated by static sieving, and it can be reused for the next batch. In SPD simulation, the immobilized pectinase was set as 6 cycles of batch, and the SPD flowsheet is shown in Figure 5a.

Traditional clarification techniques such as filtration, centrifugation, or decantation can be notably enhanced by the degradation of pectin, resulting in improved efficiency of these processes (Sandri *et al*., 2011; Pasha *et al*., 2013). From an industrial and processing perspective, viscosity reduction is particularly significant, as it simplifies pumping, filtering, stirring, and packaging operations. Numerous studies have highlighted the correlation between decreased viscosity, attributed to reduced total solids, and improved filtration flow rates, as well as overall enhancement of membrane filtration processes (Jiao *et al*., 2004). Sugar and a small number of additives such as citric acid, natural colouring, flavourings, and preservatives (e.g., potassium sorbate) can also be added to the juice (da Silva *et al*., 2016). The overall process scheduling was suggested as depicted in Figure 5b, indicating each batch of process will take approximately 6.6 hr.



(a)



(b)

Figure 5. Proposed (a) SPD process flowsheet and b) operations Gantt chart of guava juice processing using immobilized pectinase (5000 kg guava fruit/batch)

Table 5. Estimated component balances in selected streams by SPD simulation

Component balances	Stream				
	Ground guava	Pressed juice	Crude juice	Treated juice	Clarified juice
Total soluble solid (%)	7.6	8.7	8.5	8.7	9.0
Clumped solid (%)	-	-	2.1	2.1	< 0.1
Guava pomace (%)	12.9	0.2	0.2	0.2	< 0.1
Suspended solid (%)	3.1	3.6	1.4	1.4	0.5
Immo. pectinase (%)	-	-	2.0	-	-
Water (%)	76.4	87.6	85.9	87.6	90.4
Total steam (kg/batch)	4990	3715	3790	3715	3107

Table 5 provides a comprehensive summary of the outcomes derived from a selected stream within the SPD simulation process. As previously explained, the grinding of guava fruit initiates the release of its constituents, including pomace (primarily starch and fibre), suspended solids (predominantly pectin), and total soluble solids (comprising sugars and other nutrients). Following the screw pressing stage, a significant portion of the pomace, categorised as particulate solid, was separated, resulting in the extraction of juice, which primarily consists of water (over 87%). The extracted juice undergoes treatment with immobilized pectinase, resulting in a reduction of suspended solids by approximately 60%, lowering the content from 3.6% to 1.4%. These values are almost validated with experimental data using the same amount of immobilized pectinase. Subsequently, the immobilized pectinase was recovered for reuse across multiple cycles. The treated juice then undergoes clarification via centrifugation to eliminate clumped solids, which accumulate due to the pectinase reaction, and other residual insoluble solids. As a result, the final clarified juice content exhibits significantly reduced levels of clumped solids and pomace, predicting less than 0.1%.

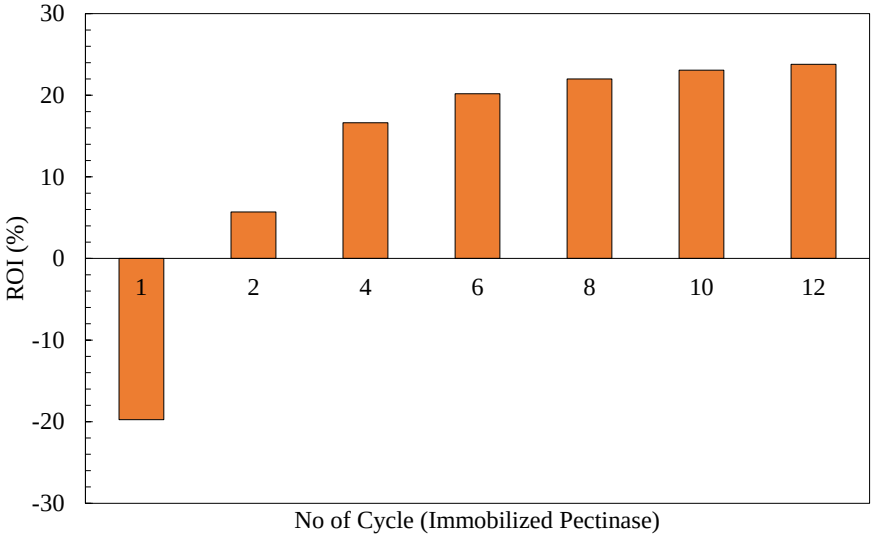
3.6. CAPEX, OPEX, Revenues, and Project Indices

PC constitutes a critical aspect of CAPEX, as it directly impacts the valuation of all capital investment components, including TPDC, TPIC, and TPC, among others. As depicted in Table 6, the outcomes derived from processing 5000 kg/batch of guava fruit encompass PC, DFC, WC, and SC. Utilising the SPD simulator allows for flexible adjustment of DFC parameters for individual sections, improving CAPEX estimation accuracy across different process scenarios (Heinzle *et al*., 2006), with a typical simulation precision of about ±30% (Peters *et al*., 2003). Overall, DFC covers 87.5% of CAPEX, of which US$ 2,125,000 is from TPDC alone.

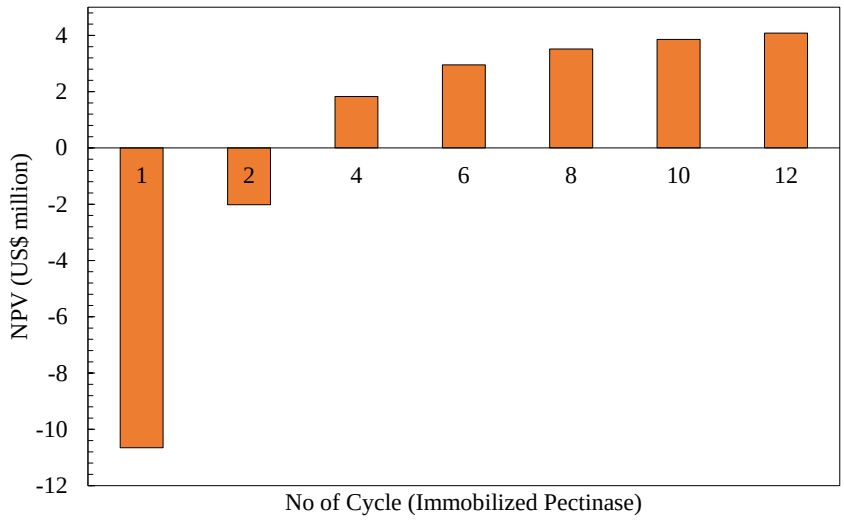
Table 6. Capital cost, operating cost, and revenues for 5000 kg/batch guava fruit (543 batches/year)

Project Evaluation	Estimated value
Capital Investment/CAPEX	
Equipment purchases cost (PC) (US$ million)	0.71
Direct fixed capital (DFC) (US$ million)	3.91
Working capital (WC) (US$ million)	0.36
Start-up & validation cost (SC) (US$ million)	0.20
Total Capital Investment (US$ million)	4.47
Operating Cost/OPEX	
Materials (US$/year)	1,920,619
Facility-dependent (US$/year)	740,060
Labour-dependent (US$/year)	229,571
Laboratory/QC/QA (US$/year)	34,436
Utilities (US$/year)	26,670
Waste treatment/disposal (US$/year)	1,405
Total Annual Operating Cost (US$/year)	2,952,761
Total Revenue (US$/year)	3,650,458
Project Indices	
Gross margin (%)	19.11
ROI (%)	20.19
Payback time (year)	4.95
IRR (%)	35.55
NPV at 10% (US$)	2,953,865

Variable (materials, labour, lab, *etc.*) and facility-dependent costs are all included in OPEX as also depicted in Table 6. Materials cost is considered the highest involved cost, estimating around 65% of OPEX. Its cost mainly comes from the price of guava fruit (74.3% of materials cost) and immobilized pectinase (22.1% of materials cost). Facility-dependent cost is the second highest, 25% from OPEX, followed by labour-dependent cost.



(a)



(b)

Figure 6. Effect of number of cycles of immobilized pectinase on a) ROI and b) NPV (5000 kg/batch & 543 batches/year)

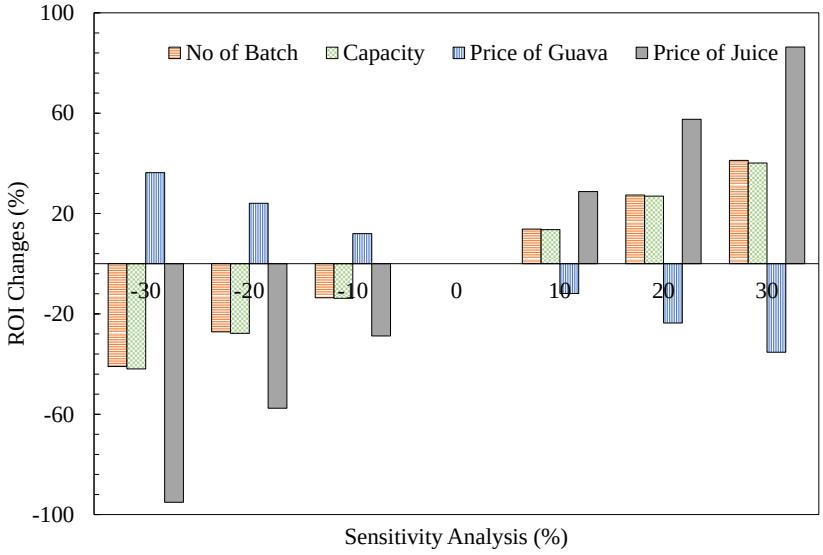
The total revenue is also shown in Table 6, estimating US 3,650,458 per year of which about 94% comes from guava juice, and the remaining comes from pomace and pectin (clumped solid). ROI is a measure of a project's profitability, calculated as the ratio of net profit to CAPEX. In this study, the ROI is determined to be 20.19%, with a payback period of 4.95 years. IRR represents the interest rate at which the project's NPV equals zero. A higher IRR signifies a more appealing project with a greater investment yield. NPV is the difference between the present values of cash inflows and outflows. In this analysis, the NPV amounts to US$ 2,953,865, indicating the potential for enhanced shareholder wealth through the proposed process scenario.

Due to the high cost of immobilized pectinase, as described in the previous statement, the investigation into the impact of multiple cycles of immobilized pectinase reveals crucial insights into process viability (Bedzo *et al*., 2019). Analysis indicates that a sustainable and economically feasible operation can be achieved, notably evident by the sixth cycle or higher, as depicted in Figure 6. This observation is substantiated by favourable ROI and NPV metrics. Conversely, employing a single cycle of immobilized pectinase yields starkly negative ROI and NPV values. This highlights a significant opportunity for further enhancement through an in-depth study aimed at augmenting the number of cycles of immobilized pectinase. Such a refinement endeavour holds promise for enhancing process efficiency and economic viability, potentially unlocking substantial benefits for the overall operation.

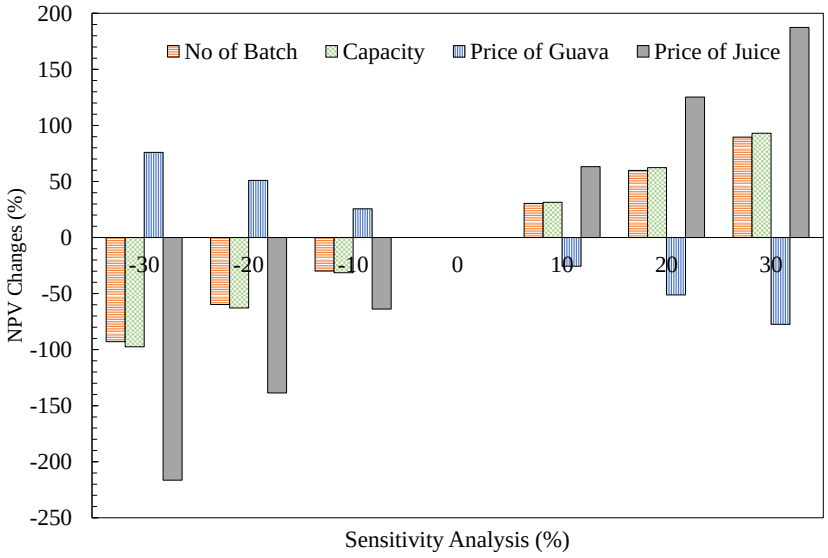
3.7. Sensitivity Analysis

The investigation into price variation is imperative to gauge its impact on the project's profitability. ROI is a pivotal tool for assessing the potential growth rate of investments, while NPV provides a comprehensive evaluation by factoring in the time value of earned money. With a positive NPV and ROI, the proposed project demonstrates its viability for approval. To delve deeper into the dynamics, a sensitivity analysis was conducted, as depicted in Figure 7, exploring the effects of varying parameters such as the number of batches per year, guava fruit price, and guava juice price within a range of ±30% on both ROI and NPV. The results indicate a direct correlation between an increase in the number of batches per year and a subsequent rise in ROI and NPV. For instance, a 30% increase in batches per year leads to a notable 41% surge in ROI and a substantial 90% increase in NPV.

Plant capacity significantly influences changes in ROI and NPV within a business context. Specifically, augmenting the capacity tends to enhance both ROI and NPV. Increasing or decreasing plant capacity may influence CAPEX and OPEX. The impact of such capacity modifications can be visualised through Figure 7. For instance, deviations of approximately ±30% from the base capacity value of 5000 kg per batch correlate with shifts of approximately ±40-42% and ±93-97% in ROI and NPV, respectively. These findings highlight the critical role of plant capacity adjustments in driving financial performance metrics, illustrating the potential gains achievable through strategic capacity planning.



(a)



(b)

Figure 7. Sensitivity analysis of the number of batches per year, plant capacity, cost of guava fruit and price of guava juice on a) ROI changes and b) NPV changes

Furthermore, the analysis underscores the impact of changes in guava juice pricing on ROI and NPV, revealing a greater influence on project profitability. Generally, an uptick in guava juice price yields augmented ROI and NPV (up to 86% and 187% changes, respectively when +30% from default juice price), while an escalation in guava fruit cost is associated with a decline in these metrics. These findings highlight the intricate interplay between pricing dynamics and project profitability, emphasising the need for strategic decision-making informed by comprehensive sensitivity analyses.

4. Conclusions

The successful immobilization of pectinase through covalent binding using 1,12-dodecanediame—GA to form NDC—1,12-dodecanediame—GA—Pectinase represents a significant advancement in enzyme technology. Through testing, the immobilized pectinase exhibited good performance, maintaining effective operation for up to 6 cycles in the guava juice clarification process. Utilising data from these laboratory trials, a thorough techno-economic analysis was performed to evaluate the feasibility of integrating immobilized pectinase into the commercial production of guava juice. The results of this analysis yielded encouraging outcomes, showcasing positive ROI, NPV, and IRR. These metrics underscore the proposed project's economic viability and potential profitability, marking it as an attractive candidate for commercialisation. The sensitivity analysis highlighted those fluctuations in the selling price of guava juice had the most significant impact on project viability, emphasising the critical influence of market dynamics on economic outcomes. In conclusion, the successful immobilization of pectinase coupled with favourable techno-

economic indicators highlights the potential of this innovation to revolutionise guava juice manufacturing, paving the way for further exploration and eventual commercial deployment of this enzymatic process.

Author Contributions: Conceptualisation, N.A.Z. and M.N.M.; methodology, N.A.Z..; software, M.N.M.; validation, N.A.Z. and M.N.M.; formal analysis, N.A.Z.; investigation, N.A.Z.; resources, N.A.Z. and M.N.M.; data curation, N.A.Z.; writing—original draft preparation, N.A.Z.; writing—review and editing, M.N.M.

Funding: No external funding was provided for this research.

Acknowledgements: We would like to thank the Department of Process and Food Engineering for their kind support in providing access to the SuperPro Designer license.

Conflicts of Interest: The authors declare no conflict of interest.

References

Abdel Wahab, W. A., Karam, E. A., Hassan, M. E., *et al*. (2018). Optimization of pectinase immobilization on grafted alginate-agar gel beads by 2(4) full factorial CCD and thermodynamic profiling for evaluating of operational covalent immobilization. *International Journal of Biological Macromolecules*, *113*, 159–170. https://doi.org/10.1016/j.ijbiomac.2018.02.086

Bayazidi, P., Almasi, H., & Asl, A. K. (2018). Immobilization of lysozyme on bacterial cellulose nanofibers: Characteristics, antimicrobial activity and morphological properties. *International Journal of Biological Macromolecules*, *107*. 2544–2551. https://doi.org/10.1016/j.ijbiomac.2017.10.137

Bedzo, O.K.K., Mandegari, M., Görgens, J.F. (2019). Comparison of immobilized and free enzyme systems in industrial production of short-chain fructooligosaccharides from sucrose using a techno-economic approach. *Biofuels, Bioproducts and Biorefining*, *13*(5), 1274–1288. doi: https://doi.org/10.1002/bbb.2025

Bhattacharjee, C., Saxena, V. K., & Dutta, S. (2017). Fruit juice processing using membrane technology: A review. *Innovative Food Science and Emerging Technologies*, *43*, 136–153. https://doi.org/10.1016/j.ifset.2017.08.002

Chauhan, S., Vohra, A., Lakhanpal, A., *et al*. (2015). Immobilization of commercial pectinase (polygalacturonase) on celite and its application in juice clarification. *Journal of Food Processing and Preservation*, 39, 2135–2141. https://doi.org/10.1111/jfpp.12457

Cieh, N. L., Sulaiman, S., Mokhtar, M. N., *et al.* (2017). Bleached kenaf microfiber as a support matrix for cyclodextrin glucanotransferase immobilization via covalent binding by different coupling agents. *Process Biochemistry*, *56*, 81–89. https://doi.org/10.1016/j.procbio.2017.02.025

da Silva, N.K.V., Sabino, L.B.S., de Oliveira, L.S., *et al*. (2016). Effect of food additives on the antioxidant properties and microbiological quality of red guava juice. *Revista Ciência Agronômica*, *47*(1), 77–85. https://doi.org/10.5935/1806-6690.20160009

Deng, Z., Wang, F., Zhou, B., *et al.* (2019). Immobilization of pectinases into calcium alginate microspheres for fruit juice application. *Food Hydrocolloids*, *89*, 691–699. https://doi.org/10.1016/j.foodhyd.2018.11.031

Dikshit, P. K., & Kim, B. S. (2020). Bacterial cellulose production from biodiesel–derived crude glycerol, magnetic functionalization, and its application as carrier for lipase immobilization. *International Journal of Biological Macromolecules*, *153*, 902–911. https://doi.org/10.1016/j.ijbiomac.2020.03.047

Do, T. X., Lim, Y.-I., & Yeo, H. (2014). Techno-economic analysis of biooil production process from palm empty fruit bunches. *Energy Conversion and Management*, *80*, 525–534. https://doi.org/10.1016/j.enconman.2014.01.024

dos Santos, J. C. S., Barbosa, O., Ortiz, C., *et al.* (2015). Importance of the Support Properties for Immobilization or Purification of Enzymes. *ChemCatChem*, *7*(16), 2413–2432. https://doi.org/10.1002/cctc.201500310

Frazão, C. J. R., Silva, N. H. C., Freire, C. S. R., *et al.* (2014). Bacterial cellulose as carrier for immobilization of laccase: Optimization and characterization. *Engineering in Life Sciences*, *14*(5), 500–508. https://doi.org/10.1002/elsc.201400054

Garcia-Galan, C., Berenguer-Murcia, Á., Fernandez-Lafuente, R., *et al.* (2011). Potential of different enzyme immobilization strategies to improve enzyme performance. *Advanced Synthesis and Catalysis*, *353*(16), 2885–2904. https://doi.org/10.1002/adsc.201100534

García, C. Á. (2018). *Application of enzymes for fruit juice processing*. In Rajauria, G. & B. K. Tiwari (Ed.), Fruit Juices - Extraction, Composition, Quality and Analysis (pp. 201–216). London: Academic Press.

Halib, N., Amin, M. C. I. M., & Ahmad, I. (2012). Physicochemical properties and characterization of nata de coco from local food industries as a source of cellulose. *Sains Malaysiana*, *41*(2), 205–211.

Harsh, P. S., Patel, H., & Sharma, S. (2014). Enzymatic extraction and clarification of juice from various fruits-A review. *Trends in Post Harvest Technology*, *2*(1), 1–14.

Heinzle, E., Biwer, A. & Cooney, C. (2006). *Development of sustainable bioprocess; Modeling and assessment*. Chichester: Wiley.

ITA. (2024, 5 January 2024). Malaysia - Country Commercial Guide. Retrieved from https://www.trade.gov/knowledge-product/malaysia-import-tariffs

Jiang, X., Chen, P., Yin, M., *et al.* (2013). Constitutive expression, purification and characterisation of pectin methylesterase from Aspergillus niger in Pichia pastoris for potential application in the fruit juice industry. *Journal of the Science of Food and Agriculture*, *93*(2), 375–381. https://doi.org/10.1002/jsfa.5771

Jiao, B., Cassano, A., Drioli, E. (2004). Recent advances on membrane processes for the concentration of fruit juices: a review. *Journal of Food Engineering*, *63*(3), 303–324. https://doi.org/10.1016/j.jfoodeng.2003.08.003

Lei, Z., Bi, S., Hu, B., *et al.* (2007). Combined magnetic and chemical covalent immobilization of pectinase on composites membranes improves stability and activity. *Food Chemistry*, *105*, 889–896. https://doi.org/10.1016/j.foodchem.2007.04.045

Li, T., Wang, N., Li, S., *et al.* (2007). Optimization of covalent immobilization of pectinase on sodium alginate support. *Biotechnology Letter*, *29*(9), 1413–1416. https://doi.org/10.1007/s10529-007-9409-3

Magro, L.D., de Moura, K. S., Backes, B. E., *et al.* (2019). Immobilization of pectinase on chitosan-magnetic particles: Influence of particle preparation protocol on enzyme properties for fruit juice clarification. *Biotechnology Report*, *24*, e00373. https://doi.org/10.1016/j.btre.2019.e00373

Mercadante, A. Z., Steck, A., & Pfander, H. (1999). Carotenoids from guava (Psidium guajava l.): isolation and structure elucidation. *Journal of Agricultural and Food Chemistry*, *47*(1), 145–151. https://doi.org/10.1021/jf980405r

Mohammadi, M., Mokarram, R.R., Shahvalizadeh, R., *et al*. (2020). Immobilization and stabilization of pectinase on an activated montmorillonite support and its application in pineapple juice clarification. *Food Bioscience*, *36*, 100625

Mokhtar, M. N. (2022). Techno-economic evaluation of a process for the transformation of VitAto sweet potato into value-added products. *Journal of Food Process Engineering*, *45*(8), e14043. https://doi.org/10.1111/jfpe.14043

Mousa, R. M. A. (2020). Simultaneous development of cloud stability and antioxidant preservation in cloudy guava juice using hydrocolloid combinations. *International Food Research Journal*, *27*(4), 762–774.

Ninga, K. A., Carly Desobgo, Z. S., De, S., *et al*. (2021). Pectinase hydrolysis of guava pulp: effect on the physicochemical characteristics of its juice. *Heliyon*, *7*(10), e08141. https://doi.org/10.1016/j.heliyon.2021.e08141

Ninga, K. A., Sengupta, S., Jain, A., *et al.* (2018). Kinetics of enzymatic hydrolysis of pectinaceous matter in guava juice. *Journal of Food Engineering*, *221*, 158–166. https://doi.org/10.1016/j.jfoodeng.2017.10.022

Pasha, K.M., Anuradha, P., Subbarao, D. (2013). Applications of Pectinases in Industrial Sector. *International Journal of Pure and Applied Sciences and Technology*, *16*(1), 89–95.

Peters, M., Timmerhaus, K., & West, R. (2003). *Plant Design and Economics for Chemical Engineers* (5th ed.). New York: Mc Graw Hill.

Ramirez, H. L., Brizuela, L. G., Iranzo, J. U., *et al*. (2015). Pectinase Immobilization on a Chitosan-Coated Chitin Support. *Journal of Food Process Engineering*, *39*(1), 97–104. https://doi.org/10.1111/jfpe.12203

Sandri, I.G., Fontana, R. C., Barfknecht, D.M., *et al.* (2011). Clarification of fruit juices by fungal pectinases. *LWT - Food Science and Technology*, *44*(10), 2217–2222. https://doi.org/10.1016/j.lwt.2011.02.008

Sánchez, A., Singh, S., Sánchez, E., *et al.* (2018). Techno-economic evaluation and conceptual design of a liquid biofertilizer plant. *Revista Colombiana de Biotecnología*, *20*(2), 6–18. https://doi.org/10.15446/rev.colomb.biote.v20n2.77053

Sikder, J., Roy, M., Dey, P., *et al.* (2012). Techno-economic analysis of a membrane-integrated bioreactor system for production of lactic acid from sugarcane juice. *Biochemical Engineering Journal*, *63*, 81–87. https://doi.org/10.1016/j.bej.2011.11.004

Sulaiman, S., Mokhtar, M.N., Naim, M.N., *et al.* (2015a). A review: potential usage of cellulose nanofibers (CNF) for enzyme immobilization via covalent interactions. *Applied Biochemistry and Biotechnology*, *175*(4), 1817–1842. https://doi.org/10.1007/s12010-014-1417-x

Sulaiman, S., Mokhtar, M.N., Naim, M.N., *et al.* (2015b). Study on the preparation of cellulose nanofibre (CNF) from kenaf bast fibre for enzyme immobilization application. *Sains Malaysiana*, *44*(11), 1541–1550.

Suwanwong, Y., & Boonpangrak, S. (2021). Phytochemical contents, antioxidant activity, and anticancer activity of three common guava cultivars in Thailand. *European Journal of Integrative Medicine*, *42*. https://doi.org/10.1016/j.eujim.2021.101290

Verma, A. K., Rajkumar, V., Banerjee, R., *et al.* (2013). Guava (*Psidium guajava L.*) powder as an antioxidant dietary fibre in sheep meat nuggets. *Asian-Australasian Journal of Animal Sciences*, *26*(6), 886–895. https://doi.org/10.5713/ajas.2012.12671

Wu, S.-C., & Lia, Y.-K. (2008). Application of bacterial cellulose pellets in enzyme immobilization. *Journal of Molecular Catalysis B: Enzymatic*, *45*(3–4), 103–108. https://doi.org/10.1016/j.molcatb.2007.12.021