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Enzymatic Clarification of Cashew Apple Juice using Polygalacturonases Purified from the Digestive Juice of the Snail *Limicolaria flammea*

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Abstract

Background/Objectives: The cashew apple (*Anacardium occidentale L.*) remains largely underutilized. Its processing into juice is limited by its high turbidity, primarily attributed to pectic substances. This study aimed to evaluate and compare the effectiveness of two purified polygalacturonases, PG1 and PG2, isolated from the digestive juice of the land snail *L. flammea*, in clarifying cashew apple juice. **Method**: Aliquots of 25 mL of juice were treated with PG1 and PG2 at different concentrations (0–0.66 mg/mL) and incubation times (0–180 min). The optimal conditions were 0.49 mg/mL for PG1 and 0.57 mg/mL for PG2, with a 90-minute incubation at 50 °C. Efficacy was assessed based on juice yield, transmittance, filtration time, reducing sugars, °Brix, and residue mass. Results were subjected to an ANOVA followed by Duncan's test at (5% significance level). **Findings**: Enzymatic treatment improved several clarification parameters. Juice yield increased from 10.0 mL to 12.7 mL with PG1, and to 14.2 mL with PG2. Transmittance increased from 9.1% to 44.4% with PG1 and to 58.3% with PG2. Reducing sugar rose from 1.042 μmol/mL to 2.351 μmol/mL with PG1 and to 2.978 μmol/mL with PG2. Filtration time decreased from 375 s to 318 s with PG1 and to 282 s with PG2. The °Brix increased from 1.5 to 2.4 and 3.0 with PG1 and PG2, respectively. Residues mass decreased, from 0.41 g to 0.29 g with PG1 and 0.21 g with PG2. **Novelty**: The novelty lies in the applying of two purified polygalacturonases from *L. flammea* to clarify cashew apple juice and comparing their performance. PG2 proved to be more effective than PG1 for all main parameters. These results highlight the potential of these animal-derived enzymes to improve clarification and contribute to better utilization of cashew apple juice.

Keywords: Cashew apple juice; Clarification; *Limicolaria flammea*; Polygalacturonase

1 Introduction

Tropical fruit juices are widely appreciated for their nutritional and organoleptic qualities, due to their high content of vitamins, minerals, dietary fiber, and bioactive compounds that are beneficial to health [1]. Among these, the cashew apple (*Anacardium occidentale L.*), often considered a byproduct of the cashew nut industry, represents a considerable yet still largely underutilized biomass, particularly in tropical-producing regions [2]. While the cashew nut is valued globally, the cashew apple, or pseudo fruit, is frequently left to rot or used only minimally, despite its proven nutritional richness and economic potential [3]. This situation represents a significant loss of resources and contributes to the environmental impact associated with the decomposition of this abundant organic matter. The cashew apple is distinguished by its diverse biochemical composition, which includes sugars, vitamins (particularly vitamin C), minerals, and phenolic compounds with antioxidant properties [4]. This richness in nutrients and bioactive compounds makes it a promising raw material for juices and fermented beverage production [5]. However, the processing of cashew apples into juice still faces several technological challenges. Its juice, like juices extracted from tropical fruits, is frequently characterized by high turbidity and a tendency to form sediment, mainly due to the presence of colloidal substances such as pectins [6]. These characteristics negatively affect juice clarity, filterability, and stability and, consequently, its acceptability and marketability [1], [2], [7]. Improving juice clarity and stability while preserving its nutritional and sensory properties is therefore essential to meeting market demands and ensuring consumer acceptance of the product [2], [8]. Various strategies, ranging from simple sedimentation to the use of chemical agents and membrane processes, have been employed to improve juice clarification [9]. However, these approaches have certain limitations. Chemical agents, while effective, raise concerns regarding food safety and the potential toxicity of residues, which limits their application in the context of growing demand for natural products. Membrane technology offers high-quality clarification, but it is associated with very high investment and maintenance costs and is prone to fouling [9], [10]. These constraints can limit its use in processing facilities with limited technical and financial resources. In light of these challenges, enzymatic clarification has now emerged as a promising, environmentally friendly, and economically viable alternative [11]. This approach relies on the use of enzymes, primarily pectinases, to hydrolyze the pectic substances responsible for the turbidity and viscosity of juices [12], [13]. However, most commercial pectinase preparations are of microbial origin, particularly from genera such as Aspergillus [12]. The search for alternative enzyme sources therefore represents a promising avenue for identifying biocatalysts with properties suited to specific matrices and processing conditions.

1.1 Research Gaps and Rationale for the Study

Despite significant progress in the enzymatic clarification of fruit juices, several knowledge gaps remain. First, research has focused primarily on microbial enzyme preparations, whereas unconventional animal sources remain largely understudied [14]. Second, although gastropods are known for their enzymatic diversity and polygalacturonases have been identified in Limicolaria flammea, their potential for cashew apple juice clarification remains poorly documented. Kanga et al. [15] isolated, purified, and characterized two polygalacturonases, PG1 and PG2, from the digestive juice of *L. flammea*; however, their comparative efficacy in a real-world application for clarifying cashew apple juice has not yet been demonstrated. Finally, previous studies have reported improvements in clarification using various pectinolytic preparations, but performance depends heavily on the enzyme source, its degree of purification, its concentration, the reaction time, and the composition of the matrix. A comparative evaluation of two polygalacturonases derived from the same biological source and applied to the same juice can therefore provide valuable insights into their technological potential. Therefore, this study aimed to evaluate and compare the efficacy of PG1 and PG2, purified from *L. flammea*, for the clarification of cashew apple juice. The novelty of this study lies in the experimental evaluation of two purified polygalacturonases of gastropod origin for a specific application in the processing of cashew apple juice, as well as in the quantitative comparison of their performance.

2 Materials and Methods

Clarification tests were conducted according to the method described by Ladeira Azar et al. [16], with slight modifications. In this study, fresh, ripe cashew apples were collected naturally falling from a village plantation in Didiévi, Côte d'Ivoire (7°7′45″ N; 4°53′48″ W). After harvest, the cashew nuts were removed, and the fruits were sanitized by three successive washes with distilled water at room temperature. The sanitized fruits were then blended at 50% (w/v) in distilled water for 3 min at medium speed using a household blender (Electronic Filter, China) to extract the juice. The resulting juice was pasteurized at 80 °C for 30 min in a water bath and then rapidly cooled to 4 °C. The PG1 and PG2 enzymes used for clarifying cashew apple juice were isolated, purified, and characterized by Kanga *et al.* [15]. Preliminary experiments were conducted to determine the optimal concentration and incubation time for each enzyme. Initially, the effect of enzyme concentration on clarification efficiency was investigated

by varying the concentrations of PG1 and PG2 separately over a range of 0 to 0.66 mg/mL. For each concentration tested, the same volume of cashew apple juice (25 mL) was used, and the experiments were conducted at a controlled temperature of 50 °C.

The physicochemical and technological parameters including filtration time, juice yield, residue mass, reducing sugar content, transmittance, and total soluble solids (°Brix), were selected as indicators of clarification efficiency, which were selected to evaluate clarification efficiency, were determined for each concentration tested. This approach allowed the optimal concentration to be identified for each enzyme. Next, the effect of incubation time was evaluated at the optimal concentration determined for each enzyme by varying the reaction time from 0 to 180 minutes, at the optimal concentration determined for each enzyme and at a constant temperature of 50 °C. As in the concentration experiments, the same volume of cashew apple juice (25 mL) wase used, and the same physicochemical and technological parameters were evaluated. Thus, the optimal time was determined based on the same evaluation criteria as those used to determine the optimal concentration. Following these two optimization steps, the conditions corresponding to the best performance of each enzyme were selected for enzymatic clarification. The enzyme concentrations ultimately used were 0.49 mg/mL for PG1 and 0.57 mg/mL for PG2, with an incubation time of 90 minutes. Enzymatic hydrolysis was therefore performed by incubating 25 mL of cashew apple juice separately with PG1 and PG2 at their respective optimal concentrations. A control without enzyme was prepared under the same conditions. The same parameters used in the preliminary experiments were then evaluated under these optimal conditions, allowing for a direct comparison of the performance obtained during optimization and that obtained under the final clarification conditions. The samples were incubated at 50 °C for 90 min with constant stirring at 225 rpm. The temperature of 50 °C corresponds to the optimal temperature for the activity of both enzymes, as characterized by Kanga *et al.*[15]. After the incubation period, the enzymatic reaction was stopped by heat treatment. The reaction mixture was immersed in a boiling water bath for 5 minutes. The treated cashew apple juice was then filtered using Whatman No. 1 filter paper and a vacuum pump. Juice yield was determined from the final volume of juice collected after filtration and was used as an indicator of extraction efficiency. Transmittance, measured at 660 nm, is a key parameter for assessing the clarity of the juice. The filtration time was timed, providing an objective measure of the reduction in juice viscosity and the improvement in its filterability. Residual dry matter was determined by drying the residues on filter paper at 70 °C until a constant weight was reached, allows for the evaluation of the number of insoluble solids removed. The reducing sugar content was quantified using the 3,5-dinitrosalicylic acid (DNS) colorimetric method described by Miller[17], which serves as an indicator of the hydrolysis of polysaccharides into simpler sugars. Total soluble solids (°Brix) were measured using a digital refractometer. All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation. The data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine significant differences among treatments at $p < 0.05$. Statistical analyses were performed using Statistica software, version 7.1.

3 Results and Discussion

Enzymatic clarification is an important step in improving the quality and stability of fruit juices, particularly tropical fruit juices such a cashew apple juice. Comparison with an untreated control allowed the effectiveness of these biocatalysts to be assessed based on juice yield transmittance, filtration time, and reducing sugars content, and total soluble solids.

Increasing the enzyme concentration gradually improved the clarification parameters up to 0.49 mg/mL for PG1 and 0.57 mg/mL for PG2 (Figure 1). Beyond these concentrations, no significant improvement in the parameters studied was observed. This trend may be explained by the increased hydrolysis of pectic components as enzyme concentration increases, facilitating matrix degradation and juice release. Similar observations have been reported when using pectinases for cashew apple juice extraction[18]. However, the optimal concentration depends on several factors, including the enzyme's source, its activity, its degree of purification, the matrix composition, and the operating conditions. Beyond the optimal concentration, the lack of further improvement may be related to a gradual decrease in the amount of readily accessible substrate.

A similar trend was observed for incubation time (Figure 2). The improvement in the measured parameters continued up to 90 min, after which it plateaued. This behavior suggests that a large portion of the readily accessible pectic substances is hydrolyzed during this period. Extending the treatment beyond 90 min would therefore increase processing time without significantly improving clarification. Similar trends have been observed during the enzymatic clarification of various juices[19],[16],[18]. Thus, concentrations of 0.49 mg/mL for PG1 and 0.57 mg/mL for PG2, combined with an incubation time of 90 min, were selected as the optimal conditions within the experimental range studied.

Under optimal conditions, both polygalacturonases improved several parameters associated with cashew apple juice clarification. The increase in juice yield was particularly pronounced. The control yielded 10 mL of juice, compared to 12.7 mL after treatment with PG1 and 14.2 mL with PG2, corresponding to increases of 27% and 42%, respectively. These values were higher than the 10.65% increase reported by Ladeira-Ázar *et al.*[16] using a purified polygalacturonase from *Calonectria*

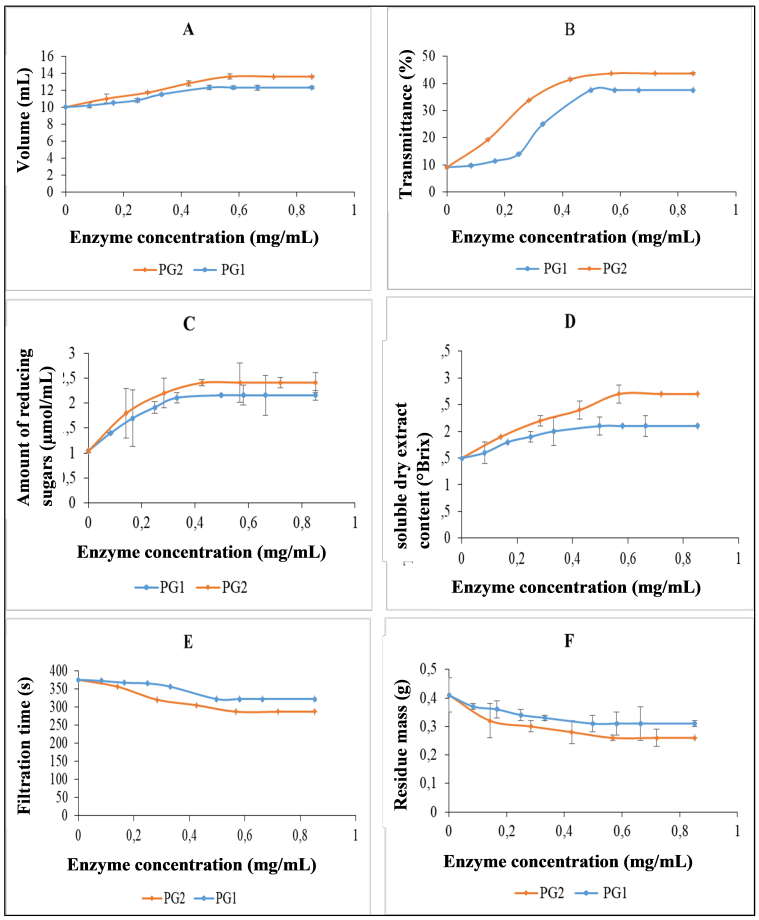


Fig 1. Effect of enzyme concentration on clarification parameters: A: Volume of juice collected; B: Transmittance (clarity); C: Amount of reducing sugars; D: Soluble dry matter content (°Brix); E: Juice filtration time; F: Mass of residue.

pteridis and the 2% increase reported by Dey et al.[19] in a study on apple juice. In contrast, Abdullah *et al.*[18] reported an extraction yield exceeding 80% when using a commercial pectinase to extract cashew apple juice under optimized conditions. These differences may be related to the source of the enzymes, the concentrations used, the extraction conditions, the plant matrix, and the method used to calculate the yield. Nevertheless, the results indicate that, under the conditions tested, PG1 and particulary PG2, effectively improved cashew apple juice recovery. This increase in yield is directly attributable to the enzymatic degradation of pectic substances, which form a colloidal matrix that retains water and insoluble solids in the raw juice[7]. The lysis of this matrix by polygalacturonases releases the trapped juice, thereby facilitating its extraction and separation from the solid residues.

In addition to juice yield, transmittance, a direct indicator of juice, also increased significantly after enzymatic treatment. The control juice had a transmittance of 9.1%, whereas transmittance reached 44.4% with PG1 and 58.3% with PG2. These results demonstrate the effectiveness of polygalacturonases in reducing juice turbidity. By depolymerizing galacturonan chains, polygalacturonases may reduce the size of colloidal particles and promote their aggregation, thereby facilitating their removal by filtration[2]. Dey *et al.*[19] achieved a transmittance of 97% in apple juice treated with a combination of polygalacturonase and α-amylase. The value obtained in the present study is therefore lower than that reported by these authors. However, the comparison involves different enzymatic systems and matrices. In contrast, Adingra *et al.*[20] reported an increase in transmittance from 44.67% to 93.32% after treating the juice with a polygalacturonase extracted from the pericarp of *Carica papaya cv. Solo 8*, representing an improvement of 48.65%, which is slightly lower than that obtained with PG2. More recently, Potkule and Annapure[21] also reported an improvement in apple juice clarity following polygalacturonase treatment. The authors observed an increase in clarity from 16.96% to 19.53% after atmospheric cold plasma treatment and from 11.32% to 16.30% following treatment with microwave-treated polygalacturonase. Although the treatment conditions and fruit matrix differed from those

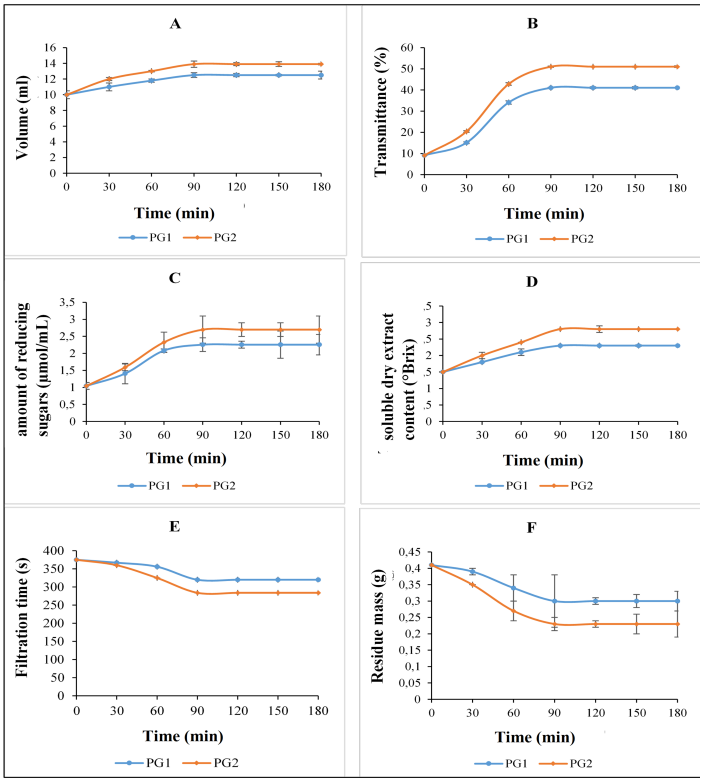


Fig 2. Effect of reaction time on clarification parameters. A: Volume of juice collected; B: Transmittance (clarity); C: Amount of reducing sugars (l); D: Soluble dry matter content (°Brix); E: Juice filtration time; F: Mass of residue

used in the present study, these findings further support the ability of polygalacturonase-based treatments to improve juice clarity. The importance of these findings lies primarily in the fact that the use of polygalacturonases from *L. flammea* led to a marked improvement in the clarity of cashew apple juice that was initially very cloudy. The combined impact on yield and transmittance highlights the effectiveness of *L. flammea* enzymes in transforming an initially cloudy and difficult-to-extract cashew apple juice into a clearer and more abundant product. The influence of the fruit matrix on clarification efficiency has also been highlighted by Glienke *et al.*[22], who reported different clarification performances of polygalacturonases when applied to plantain and Haden mango pulps. Their results showed clarification rates ranging from 45.8% to 63.4%, depending on the enzyme and fruit matrix. Such differences suggest that the effectiveness of polygalacturonases may depend not only on their catalytic properties but also on their interaction with the composition of the substrate. In the present study, the higher performance observed with PG2 may therefore reflect, at least in part, differences in the interaction of the two enzymes with the cashew apple juice matrix. This dual improvement is crucial for the commercialization of fruit juices, as clarity is an organoleptic attribute highly valued by consumers[5]. PG2's performance across the various parameters evaluated highlights its potential as a biocatalyst of interest for the value-added processing of cashew apple juice.

Enzymatic treatment also improved juice filterability. A reduction in filtration time is an important indicator of clarification efficiency and may improve processing productivity. In this study, filtration time decreased from 375 s for the control to 318 s with PG1 and 282 s with PG2, representing reductions of 15.2% and 24.8%, respectively. The decrease in filtration time may be related to the degradation of pectic substances, which reduces viscosity and facilitates the passage of juice through the filter[8]. Similarly, the mass of solid residues retained after filtration also decreased. It fell from 0.41 g in the control to 0.29 g with PG1 and 0.21 g with PG2, corresponding to reductions of 29.26% and 48.78%. This decrease accompanies the improvement in filterability and demonstrates that the degradation of pectic components facilitates the separation of juice from insoluble matter[23].

The reduction in residue mass may provide an operational advantage by reducing filter clogging and potentially extending filter service life, thereby lowering maintenance costs and minimizing production downtime. These improvements are consistent with and more significant than those observed by Ladeira-Ázar *et al.*[16]. Indeed, those authors observed a 7-second reduction

in filtration time following enzymatic treatment of apple juice and a 25% decrease in residue mass. The results obtained with *L. flammea* enzymes demonstrate that polygalacturonases derived from an unconventional animal source can be highly effective for clarifying a complex fruit matrix such as cashew apple juice. These results confirm that the enzymatic degradation of pectocellulosic substances is an effective means of improving the filterability and overall quality of the juice[24].

Reducing sugar content, an indicator of polysaccharides hydrolysis, increased significantly after enzymatic treatment. The reducing sugar contents are 2.351 and 2.978 μmol/mL for PG1 and PG2, respectively, compared to 1.042 μmol/mL for the control, representing increases of 125.62% and 185.79% for PG1 and PG2, respectively. This increase may be attributed to the hydrolysis of polysaccharides particularly pectins into smaller molecules. Polygalacturonases, by cleaving the glycosidic bonds in pectin chains, release oligosaccharides and monosaccharides that contribute to the increase in reducing sugars. This release of reducing sugars could potentially influence the sensory characteristics of the juice, although sensory evaluation was not performed in the present study[5]. Similarly, the total soluble solids content, measured in °Brix, showed a significant increase. The control juice had a °Brix of 1.5. After treatment with PG1, this figure rose to 2.4, representing a 60% increase. PG2 once again outperformed PG1, with a °Brix of 3.0, representing a 100% increase compared to the control. The increase in total soluble solids may be associated with the release of soluble compounds from the fruit matrix, including sugars, organic acids, and other water-soluble substances, which were previously trapped within the fruit's cellular matrix[4]. The action of enzymes on the cell walls facilitates the diffusion of these components into the juice, thereby enriching its composition. Aluko *et al.*[2], studied the clarification of cashew apple juice using gelatin. Contrary to their findings in which the clarification of cashew apple juice with gelatin did not result in a significant change in total soluble solids and was accompanied by a slight decrease in sugars treatment with the polygalacturonases PG1 and PG2 in the present study resulted in a marked increase. This difference can be explained by the mode of action of the clarifying agents: gelatin acts primarily by interacting with and precipitating certain components of the juice, whereas polygalacturonases hydrolyze pectic substances and can thus release soluble compounds. Similar increases in °Brix have been reported for other enzymatically treated fruit juices, such as mango, apple, and citrus juices, confirming the effectiveness of this approach in improving nutritional and organoleptic quality[16],[18],[24]. These data highlight not only the effectiveness of *L. flammea* polygalacturonases for clarification but also their ability to enrich the juice with soluble compounds. Taken together, these results highlight the different performances of the two enzymes. PG2 outperformed PG1 across all key parameters evaluated. Yield increased by 42% with PG2 compared to 27% for PG1; transmittance reached 58.3% compared to 44.4%; reducing sugars increased by 185.79% compared to 125.62%; Brix reached 100% versus 60%, filtration time decreased by 24.8% versus 15.2%, and residue mass decreased by 48.78% versus 29.26% (Figure 3). The superior performance of PG2 is consistent with the findings of Kanga et al.[15], who reported higher catalytic activity of PG2 toward polygalacturonic acid. The results suggest that this difference in catalytic activity is also reflected in a complex food matrix such as cashew apple juice. Thus, compared to previous work, the significance of this study lies not only in improving juice clarity. It also lies in the evaluation of two purified polygalacturonases from *L. flammea*, in determining their optimal conditions of use, and in comparing their effects on several technological parameters of the juice. The results obtained show, in particular, that the polygalacturonases PG1 and PG2 especially PG2 can simultaneously improve the yield, clarity, and filterability of the juice. This characteristic may be of interest for the value-added processing of cashew apples and provides additional data on the potential of enzymes derived from unconventional sources.

4 Conclusion

The results of this study show that the polygalacturonases PG1 and PG2, purified from the digestive juice of *L. flammea*, can improve the clarification of cashew apple juice. The optimal conditions were 0.49 mg/mL for PG1 and 0.57 mg/mL for PG2, with an incubation time of 90 min at 50 °C. This superiority of PG2, previously observed at the biochemical level, was also reflected in its better technological performance in a real food matrix. PG2 resulted in a 42% increase in juice yield, compared with 27% for PG1, and increased transmittance from 9.1% in the control to 58.3%. Filtration time was reduced by 24.8%, while residue mass decreased by 48.78%. The reducing sugar content increased from 1.042 μmol/mL to 2.978 μmol/mL. The °Brix value also increased from 1.5 in the control to 3.0 following treatment with PG2. These results show that PG2 performed better than PG1 for the main clarification parameters evaluated. They also indicate that polygalacturonases derived from *L. flammea* may represent a promising enzymatic source for the treatment of cashew apple juice. The main contribution of this study is therefore to provide experimental data on the use of these gastropod-derived enzymes in juice clarification and to demonstrate their potential to improve juice recovery, clarity, and filterability. Overall, these results reinforce the value of exploring unconventional enzyme sources for the value-added processing of tropical products, particularly cashew apples, whose processing potential remains underutilized.

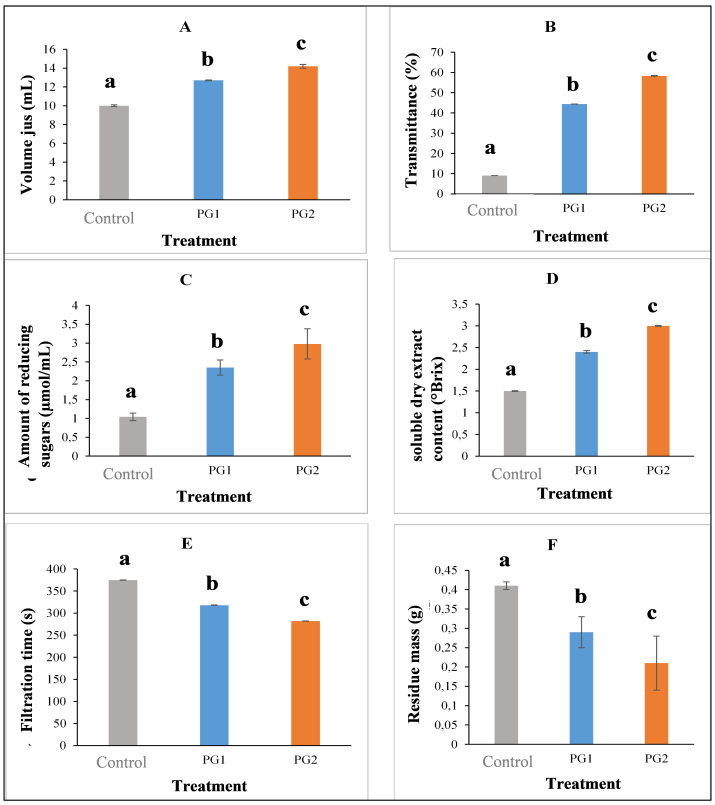


Fig 3. Determination of clarification parameters under optimal conditions. A: Volume of juice collected; B: Transmittance (clarity); C: Amount of reducing sugars; D: Soluble dry matter content (°Brix); E: Juice filtration time; F: Mass of residues. *Bars representing the averages of the trials labeled with the same lowercase letters are not statistically different at a significance level of* $\alpha = 0.05$.

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References

1) Toy JYH, Lu Y, Huang D, Matsumura K, Liu SQ. Enzymatic treatment, unfermented and fermented fruit-based products: Current state of knowledge. *Food Chemistry*. 2022 Jun;385:132628. https://doi.org/10.1016/j.foodchem.2022.132628.
2) Aluko A, Makule E, Kassim N. Effect of clarification on physicochemical properties and nutrient retention of pressed and blended cashew apple juice. *Food Science & Nutrition*. 2023 Jan;11(4):1891–1903. https://doi.org/10.1002/fsn3.3222.
3) Gutiérrez-Paz C, Rodríguez-Moreno MC, Hernández-Gómez MS, Fernández-Trujillo JP. The Cashew Pseudofruit (Anacardium occidentale): Composition, Processing Effects on Bioactive Compounds and Potential Benefits for Human Health. *Foods*. 2024 Jul;13(15):2357. https://doi.org/10.3390/foods13152357.
4) Fonteles TV, Barroso MKA, Filho EGA, Fernandes FAN, Rodrigues S. Ultrasound and Ozone Processing of Cashew Apple Juice: Effects of Single and Combined Processing on the Juice Quality and Microbial Stability. *Processes*. 2021 Dec;9(12):2243. https://doi.org/10.3390/pr9122243.
5) Codjia FS, Dabadé DS, Agbobatinkpo P, Collombel I, Achir N, Azokpota P, et al. Fermented Cashew Apple Beverages: Current State of Knowledge and Prospects. *Beverages*. 2025;11(2):49. https://doi.org/10.3390/beverages11020049.
6) Shirvani A, Mirzaaghaei M, Goli SAH. Application of natural fining agents to clarify fruit juices. *Comprehensive Reviews in Food Science and Food Safety*. 2023 Nov;22(6):4190–4216.
7) Nehmé L, Tekle ME, Barakat N, Khoury AE, Azzi-Achkouty S, Rayess YE. Alternative Processes for Apple Juice Stabilization and Clarification: A Bibliometric and Comprehensive Review. *Processes*. 2024 Feb;12(2):296. https://doi.org/10.3390/pr12020296.
8) Detoni B, Sanches ACC, Maller A. Use of Pectinases in Juice Clarification Processes: A Scoping Review. *Novel Techniques in Nutrition and Food Science*. 2025;8(2). https://doi.org/10.31031/NTNF.2025.08.000685.
9) Sarbatly R, Sariau J, Krishnaiah D. Recent Developments of Membrane Technology in the Clarification and Concentration of Fruit Juices. *Food Engineering Reviews*. 2023 Dec;15:420–437. https://doi.org/10.1007/s12393-023-09346-2.
10) Katibi KK, Nor MZM, Yunos KFM, Jaafar J, Show PL. Strategies to Enhance the Membrane-Based Processing Performance for Fruit Juice Production: A Review. *Membranes*. 2023 Jul;13(7):679. https://doi.org/10.3390/membranes13070679.

11) Wang F, Xu H, Wang M, Yu X, Cui Y, Xu L, et al. Application of Immobilized Enzymes in Juice Clarification. *Foods*. 2023 Nov;12(23):4258. https://doi.org/10.3390/foods12234258.
12) Roy K, Dey S, Uddin MK, Barua R, Hossain MT. Extracellular Pectinase from a Novel Bacterium Chryseobacterium indologenes Strain SD and Its Application in Fruit Juice Clarification. *Enzyme Research*. 2018;2018:3859752. https://doi.org/10.1155/2018/3859752.
13) Ahmed A, Sohail M. Characterization of pectinase from Geotrichum candidum AA15 and its potential application in orange juice clarification. *Journal of King Saud University - Science*. 2020 Jan;32(1):955–961. https://doi.org/10.1016/j.jksus.2019.07.002.
14) Ozyilmaz G, Gunay E. Clarification of apple, grape and pear juices by co-immobilized amylase, pectinase and cellulase. *Food Chemistry*. 2023 Jan;398:133900. https://doi.org/10.1016/j.foodchem.2022.133900.
15) Kanga AK, Koffi DM, Sea BT, Gbotognon JO, Kouamé LP. Biochemical Characterization of Two Polygalacturonases Purified from the Digestive Juice of the Snail Limicolaria flammea. *Biotechnology Journal International*. 2021;25(1):41–54. https://doi.org/10.9734/bji/2021/v25i130133.
16) Ázar RISL, da Luz Morales M, Maitan-Alfenas GP, Falkoski DL, Alfenas RF, Guimarães VM. Apple juice clarification by a purified polygalacturonase from Calonectria pteridis. *Food and Bioproducts Processing*. 2020 Jan;119:238–245. https://doi.org/10.1016/j.fbp.2019.11.013.
17) Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*. 1959;31(3):426–428. https://doi.org/10.1021/ac60147a030.
18) Abdullah S, Pradhan RC, Pradhan D, Mishra S. Modeling and optimization of pectinase-assisted low-temperature extraction of cashew apple juice using artificial neural network coupled with genetic algorithm. *Food Chemistry*. 2021 Mar;339:127862. https://doi.org/10.1016/j.foodchem.2020.127862.
19) Dey TB, Banerjee R. Application of decolorized and partially purified polygalacturonase and a-amylase in apple juice clarification. *Brazilian Journal of Microbiology*. 2014 Mar;45(1):97–104. https://doi.org/10.1590/S1517-83822014000100014.
20) Adingra KMD, Gnahe HD, Soro S, Kouadio NJP, Tano K. Clarification of cashew apple juice with pectinases extracted from the pericarp of papaya cv. Solo 8. *Foods and Raw Materials*. 2026;15(2):358–369. https://doi.org/10.21603/2308-4057-2027-2-715.
21) Potkule JB, Annapure US. Clarification of apple juice using atmospheric cold plasma-treated and microwave-treated polygalacturonase. *Food Bioscience*. 2026;80:109028. https://doi.org/10.1016/j.fbio.2026.109028.
22) Glienke NN, de Alencar Guimarães NC, de Oliveira Simas AL, Galeano RMS, Santana QM, Masui DC, et al. Production, characterization, and application of polygalacturonases in fruit juice clarification. *Journal of Food Measurement and Characterization*. 2025;19:9193–9204. https://doi.org/10.1007/s11694-025-03633-8.
23) Ghag SS, Gokhale JS, Lele SS. Effect of chemical pretreatment on quality attributes of the cashew apple. *Journal of Food Science*. 2023 Jun;88(6):2353–2367. https://doi.org/10.1111/1750-3841.16577.
24) Hassan SS, Williams GA, Jaiswal AK. Computational modelling approach for the optimization of apple juice clarification using immobilized pectinase and xylanase enzymes. *Current Research in Food Science*. 2020;3:243–255. https://doi.org/10.1016/j.crfs.2020.09.003.