



Clarification of cashew apple juice with pectinases extracted from the epicarp of papaya cv. Solo 8

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Abstract:

Cashew apples are often discarded after nut extraction, despite their potential for juice production. This study aimed to clarify cashew apple juice using pectinases extracted from the epicarp of papaya cultivar Solo 8.

These enzymes were previously purified using ammonium sulfate precipitation followed by dialysis and isoelectric focusing. Cashew apples were sourced from a village plantation in Bondoukou, Gontougo region. Clarification tests were conducted using standard methods.

According to the results, the juice clarity improved significantly over 120 minutes: from 44.67% to 83.18% with pectin methylesterase, to 93.32% with polygalacturonase, and to 72.44% with pectin lyase. The browning index and color intensity of the raw juice decreased from 82.98 to 41.69% and from 75.86 to 26.91%, respectively. The vitamin C content declined from 327.82 to 202.94 mg/100 mL (pectin methylesterase), 280.99 mg/100 mL (polygalacturonase), and 208.70 mg/100 mL (pectin lyase). Viscosity reductions were also notable: 67% with pectin methylesterase, 72% with polygalacturonase, and 59% with pectin lyase. Among enzyme combinations, the most effective was a mix of pectin methylesterase (0.087 mg/mL) and polygalacturonase (0.264 mg/mL), which reduced the juice's viscosity by over 76% and increased its clarity by more than 50%. These findings highlight the potential of papaya-derived pectinases in improving cashew apple juice quality and marketability.

Keywords: *Carica papaya* L., cashew apple, juice valorization, epicarp, pectinases, clarification, viscosity

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INTRODUCTION

Coffee and cocoa have always been the mainstay of Ivorian agriculture. However, other crops, although considered minor, have also begun to experience significant development. These crops include the cashew tree (*Anacardium occidentale* L.). The main producers of cashew are Côte d'Ivoire, India, Vietnam, and Nigeria. The cashew tree is primarily cultivated for its nut, which is the main commercial product and represents approximately 8–12% of the total fruit weight, whereas the cashew apple accounts for the remaining 88–92% [1].

Cashew apple has a high water content (85.5 to 90%) and a low protein content. It is rich in sugars and micro-nutrients such as iron, phosphorus, calcium, vitamin C, flavonoids, and polyphenols [2, 3]. Its high water content makes cashew apple a highly perishable food in its fresh state, raising the importance of its transformation into stable products. In Togo, just as in Côte d'Ivoire,

cashew apple valorization trials have enabled the use of cashew apple powder in animal feed [4, 5]. A study in Benin has demonstrated the use of cashew apple powder in the bread-making industry [6]. In Côte d'Ivoire, the work of Sahie *et al.* [7] highlighted the influence of processing steps on the nutritional and organoleptic quality of cashew apples, as well as on clarification, stabilization, concentration, and dehydration methods. Similarly, the study by Adou *et al.* [8] focused on the stabilization and sensory evaluation of cashew apple juices.

The processing of fruits into juice is generally considered the main method of their valorization [9–12]. However, the cashew fruit is usually harvested for the nut, with the apple being left in the fields without proper valorization [13]. Moreover, the juice extracted from cashew apple is astringent due to the presence of condensed tannins and turbid due to the high concentration of pectins [11, 14]. Pectins are complex polysaccharides

found in the cell walls of most higher plants [12]. Their high concentration in cashew apple leads to the formation of colloids [15, 16]. Cloudy juices need to be clarified since most consumers find them unacceptable [17]. Clarified cashew apple juice is clear and has a pale yellow color. Its astringent taste, which dries the mouth, is eliminated by almost 95%. Studies have shown that various enzymes are involved in the clarification of cloudy fruit juices, particularly pectinases. For example, fungal pectinases have been extensively used to clarify fruit and vegetable juices [11, 18].

Cashew apple, a by-product of the cashew tree (*A. occidentale*) has been widely used in juice production [8, 19]. However, the turbidity of cashew apple juice lowers its marketability. Some authors have used different clarification techniques to reduce its viscosity, including tangential microfiltration, membrane coupling, electroflotation, and ultrafiltration [20–22]. However, these techniques require significant financial resources, creating a need for finding more affordable ways of clarifying cashew apple juice. For example, our recent study showed that the epicarp of commercial papaya cultivar Solo 8 contained three pectinases, namely polygalacturonase (acidic and mesophilic), pectin methylesterase (alkaline and thermophilic), and pectin lyase (alkaline and mesophilic) [23]. We purified these pectinases by using three separation steps: ammonium sulfate precipitation, dialysis, and isoelectric focusing. The purification yielded specific activities of 198 U/mg of protein for pectin methylesterase, 3.62 IU/mg of protein for polygalacturonase, and 0.176 IU/mg of protein for pectin lyase. When purified, these enzymes could be used for clarifying cashew apple juice. Therefore, this study aimed to use these pectinolytic enzymes in cashew apple juice clarification trials.

STUDY OBJECTS AND METHODS

Biological material. Cashew apples (Fig. 1) were harvested from a village plantation in Mérékou on the Tanda-Assuéfry axis in the Gontougo region, located about 384 km from the Félix Houphouët-Boigny Airport in Abidjan (Côte d’Ivoire).

Cashew apple sampling. Yellow cashew apples were harvested at the mature stage in April 2016. Harvesting was done manually, and only ripe, undamaged cashew apples were picked. Once in the laboratory, the fruits



Figure 1 Cashew fruit

were pitted, then washed and disinfected. The apples were divided into 10 kg batches to undergo a series of operations detailed in the apple juice extraction section.

Juice extraction. The technique described by Abdullah *et al.* [24] was slightly modified to obtain raw cashew apple juice (Fig. 2). At the end of extraction, 2.5 L of juice was obtained from 10 kg of cashew apples.

Enzymatic clarification. To perform the clarification, the following parameters were previously determined: incubation time (15–120 min), enzyme concentration (0.008 to 0.140 mg/mL), and the concentration of each substrate (0.5–2.0%). The clarification was performed using the modified technique of Anuradha *et al.* [25]. In fact, a determined volume of each enzyme (50 µL of pectin lyase, 100 µL of polygalacturonase, and 100 µL of pectin methylesterase) was added to 100 mL of cashew apple juice. Then, the reaction mixture was incubated in a water bath at the optimal temperature for each enzyme (45°C for polygalacturonase, 50°C for pectin lyase, and 60°C for pectin methylesterase) for 120 min. At regular intervals of 15 min, an aliquot of 10 mL was taken and heated at 85 °C for 5 min to inactivate the pectinases. After cooling, the reaction medium was centrifuged at 10,000 rotations/min for 15 min and the supernatant, which constituted the clarified juice, was collected in tubes for the analyses below.

Physicochemical analyses of clarified juice. To assess the physicochemical and nutritional quality of the clarified juice, we determined its pH, titratable acidity, and vitamin C content using standard and referenced methods. The soluble solids expressed in °Brix were measured with a portable refractometer equipped with a temperature corrector (model FG103/113, Milan, Italy). Clarity, color, and browning index were determined using a

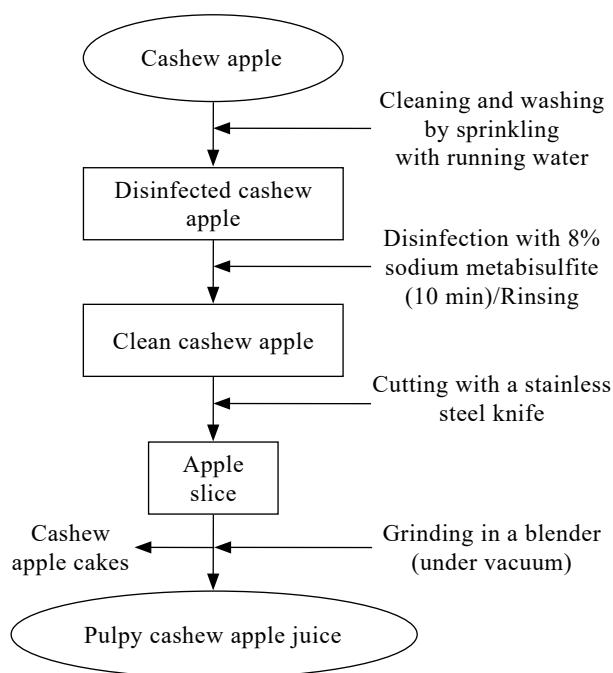


Figure 2 Flow chart of cashew apple juice extraction ([24] modified)

spectrophotometer (NanoDrop® ND-1000, Thermo Fisher Scientific) at the respective wavelengths of 660 nm, 440 nm, and 420 nm, with the use of distilled water as a control [9, 26, 27]. Viscosity was determined using a viscometer (Visco Star R, fungilab.S.A.).

Statistical analyses. All the analyses were performed in triplicate, and the data were expressed as the arithmetic mean with the corresponding statistical standard deviation. One-way analysis of variance (ANOVA) was performed on all the results to determine significant statistical differences between the calculated mean values. Significant statistical differences were demonstrated using Duncan's test at the 95% confidence level. The statistical analysis was performed using Statistica 7.1 software.

RESULTS AND DISCUSSION

This study was conducted in two stages. The first stage consisted of verifying the individual effect of each pectinase on the clarification of cashew apple juice. The second stage involved combining two most effective pectinases for the clarification of cashew apple juice.

Clarification with purified pectinases. Study of the minimum clarifying concentration. Purified pectinases isolated from the epicarp of papaya cultivar Solo 8 were used to clarify cashew apple juice. Their concentrations were 0.008 mg/mL for pectin lyase (0.176 IU/mg),

0.0348 mg/mL for pectin methylesterase (198 U/mg), and 0.0352 mg/mL for polygalacturonase (3.6 IU/mg) (Fig. 3).

Clarification of cashew apple juice with pectin methylesterase. Clarity, color, and browning index. Table 1 shows the changes in clarity, color, and browning index of cashew apple juice after the addition of pectin methylesterase. As can be seen, the clarity of cashew apple juice increased from 44.67 to 83.18% during the 120-min clarification period, stabilizing after 105 min. However, the color and browning decreased throughout clarification, reaching respective values of 43.65 and 28.18% at 120 min. Furthermore, the color intensity of cashew apple juice no longer changed after 105 min.

pH, titratable acidity, soluble solids, and vitamin C. After the addition of pectin methylesterase, the pH and titratable acidity of cashew apple juice changed very little (Table 2). In particular, the pH slightly decreased from 4.87 ± 0.03 to 4.77 ± 0.03 , whereas titratable acidity slightly increased from 0.208 ± 0.030 to 0.240 ± 0.010 g/100 mL at the end of clarification. Statistical analysis revealed that there was no significant difference in the pH values after 15 min of clarification. However, it showed a significant difference in the titratable acidity of the juice clarified with pectin methylesterase between 0 and 120 min. The initial soluble dry extract of the raw juice (12°Brix) decreased during clarification to reach a value of $7.90 \pm 0.01^\circ$ Brix at 105 min.

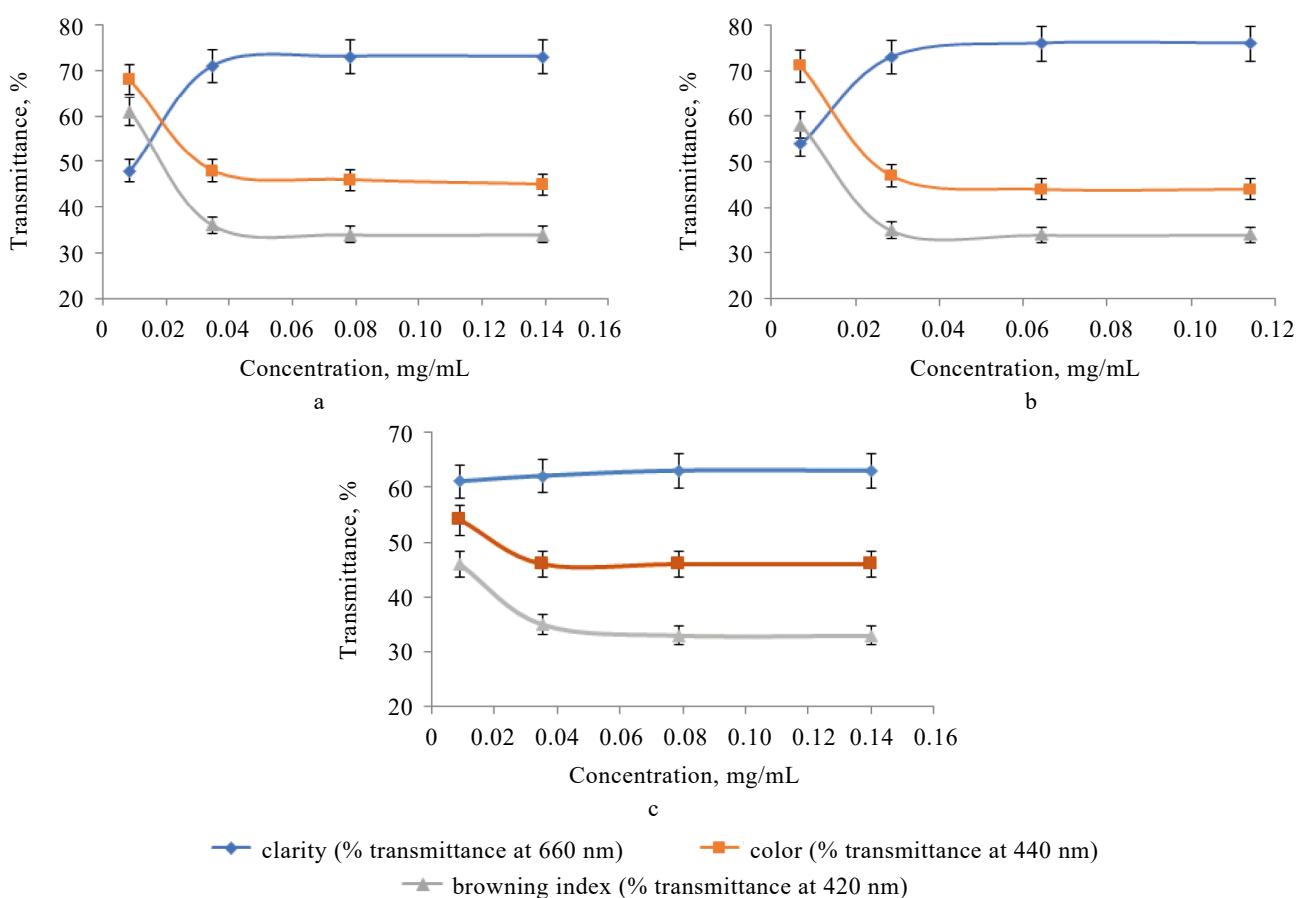


Figure 3 Influence of pectinase concentration on the appearance of cashew apple juice: (a) pectin methylesterase, PMI; (b) polygalacturonase, PG; and (c) pectin lyase, PL

Table 1 Changes in clarity, color, and browning index of cashew apple juice after addition of pectin methylesterase

Clarification time, min	Clarity (660 nm), %	Color (440 nm), %	Browning (420 nm), %
0	44.67 ± 0.01 ^h	82.98 ± 0.03 ^a	75.86 ± 0.01 ^a
15	50.12 ± 0.04 ^g	72.44 ± 0.01 ^b	58.88 ± 0.00 ^b
30	52.48 ± 0.03 ^f	56.23 ± 0.03 ^c	43.65 ± 0.01 ^c
45	63.09 ± 0.06 ^c	50.12 ± 0.01 ^d	38.02 ± 0.01 ^d
60	72.44 ± 0.01 ^d	46.77 ± 0.01 ^c	35.48 ± 0.01 ^c
75	75.86 ± 0.01 ^c	45.71 ± 0.01 ^f	33.11 ± 0.01 ^f
90	81.28 ± 0.03 ^b	44.67 ± 0.03 ^e	30.20 ± 0.03 ^e
105	83.18 ± 0.03 ^a	43.65 ± 0.01 ^h	28.84 ± 0.03 ^h
120	83.18 ± 0.01 ^a	43.65 ± 0.00 ^h	28.18 ± 0.01 ⁱ

Means ± standard deviations followed by the same lowercase letter in a column for a parameter are not significantly different at the 5% threshold according to Duncan’s test

Table 2 Changes in pH, titratable acidity, soluble solids, and vitamin C during clarification of cashew apple juice using pectin methylesterase

Clarification time, min	Parameter			
	pH	Titratable acidity, g/100 mL	Refractometric dry extract, °Brix	Vitamin C, mg/100 mL
0	4.87 ± 0.03 ^a	0.208 ± 0.000 ^c	12.00 ± 0.00 ^a	327.82 ± 0.00 ^a
15	4.80 ± 0.03 ^b	0.212 ± 0.000 ^c	11.40 ± 0.01 ^b	304.41 ± 0.03 ^b
30	4.79 ± 0.01 ^b	0.220 ± 0.010 ^{b,c}	10.60 ± 0.00 ^c	280.99 ± 0.03 ^c
45	4.79 ± 0.03 ^b	0.224 ± 0.000 ^{a,b,c}	10.10 ± 0.03 ^d	257.56 ± 0.03 ^d
60	4.79 ± 0.01 ^b	0.224 ± 0.000 ^{a,b,c}	9.30 ± 0.03 ^e	249.76 ± 0.03 ^c
75	4.79 ± 0.00 ^b	0.232 ± 0.000 ^{a,b}	8.60 ± 0.01 ^f	234.15 ± 0.01 ^f
90	4.78 ± 0.01 ^b	0.232 ± 0.000 ^{a,b}	8.20 ± 0.03 ^e	218.54 ± 0.00 ^g
105	4.77 ± 0.01 ^b	0.240 ± 0.000 ^a	7.90 ± 0.01 ^h	218.54 ± 0.01 ^g
120	4.77 ± 0.03 ^b	0.240 ± 0.010 ^a	7.76 ± 0.00 ⁱ	202.94 ± 0.03 ^h

Means ± standard deviations followed by the same lowercase letter on a line for a parameter are not significantly different at the 5% threshold according to Duncan’s test

Table 3 Changes in clarity, color, and browning index of cashew apple juice after addition of polygalacturonase

Clarification time, min	Clarity (660 nm), %	Color (440 nm), %	Browning (420 nm), %
0	44.67 ± 0.01 ^h	82.98 ± 0.03 ^a	75.86 ± 0.01 ^a
15	50.12 ± 0.03 ^g	74.13 ± 0.01 ^b	57.28 ± 0.00 ^b
30	54.95 ± 0.03 ^f	51.29 ± 0.01 ^c	48.98 ± 0.01 ^c
45	66.07 ± 0.04 ^c	46.77 ± 0.01 ^d	39.81 ± 0.01 ^d
60	75.86 ± 0.03 ^d	46.77 ± 0.00 ^e	34.67 ± 0.01 ^e
75	81.28 ± 0.03 ^c	45.71 ± 0.01 ^f	32.16 ± 0.01 ^f
90	87.09 ± 0.01 ^b	45.71 ± 0.00 ^f	30.90 ± 0.01 ^g
105	93.32 ± 0.01 ^a	44.67 ± 0.01 ^g	30.20 ± 0.01 ^h
120	93.32 ± 0.00 ^a	43.65 ± 0.01 ^h	29.51 ± 0.01 ⁱ

Means ± standard deviations followed by the same lowercase letter in a column for a parameter are not significantly different at the 5% threshold according to Duncan’s test

Furthermore, the initial vitamin C content (327.82 ± 0.00 mg/100 mL) of cashew apple juice decreased to 218.54 ± 0.01 mg/100 mL after 90 min of clarification and remained at this level after 105 min of the treatment. Statistical analysis also showed a significant difference between the soluble dry extract and the vitamin C content of the juice clarified with pectin methylesterase.

Clarification of cashew apple juice with polygalacturonase. Clarity, color, and browning index. The addition of polygalacturonase caused an increase in clarity and a decrease in color and browning index (Table 3). In particular, the clarity of the raw juice increased signifi-

cantly from 44.67 ± 0.01 to 93.32 ± 0.00% between 0 and 120 min, with no significant difference between 105 and 120 min. The color and browning index decreased to 43.65 ± 0.01 and 29.51 ± 0.01%, respectively.

pH, titratable acidity, soluble solids, and vitamin C. The treatment of cashew apple juice with polygalacturonase caused a decrease in its pH, refractometric solids, °Brix, and vitamin C, as well as an increase in its titratable acidity (Table 4). In particular, the raw juice’s pH of 4.87 ± 0.03 and vitamin C content of 327.82 ± 0.00 mg/100 mL decreased to 4.77 ± 0.00 and 280.99 ± 0.03 mg/100 mL, respectively, after 120 min of clarifica-

Table 4 Changes in pH, titratable acidity, soluble solids, and vitamin C during clarification of cashew apple juice by polygalacturonase

Clarification time, min	Parameter			
	pH	Titratable acidity, g/100 mL	Refractometric dry extract, °Brix	Vitamin C, mg/100 mL
0	4.87 ± 0.03 ^a	0.208 ± 0.000 ^c	12.00 ± 0.00 ^a	327.82 ± 0.00 ^a
15	4.79 ± 0.03 ^b	0.212 ± 0.000 ^c	11.60 ± 0.01 ^b	320.01 ± 0.03 ^b
30	4.78 ± 0.01 ^b	0.224 ± 0.000 ^b	10.80 ± 0.03 ^c	312.21 ± 0.04 ^c
45	4.78 ± 0.03 ^b	0.232 ± 0.000 ^a	9.90 ± 0.03 ^d	312.21 ± 0.01 ^c
60	4.77 ± 0.00 ^b	0.236 ± 0.000 ^a	9.50 ± 0.03 ^e	304.41 ± 0.01 ^d
75	4.77 ± 0.01 ^b	0.236 ± 0.000 ^a	9.30 ± 0.04 ^f	296.60 ± 0.01 ^c
90	4.77 ± 0.03 ^b	0.236 ± 0.000 ^a	8.20 ± 0.03 ^g	296.60 ± 0.00 ^c
105	4.77 ± 0.01 ^b	0.236 ± 0.000 ^a	7.90 ± 0.01 ^h	288.80 ± 0.01 ^f
120	4.77 ± 0.00 ^b	0.236 ± 0.000 ^a	7.79 ± 0.01 ⁱ	280.99 ± 0.03 ^g

Means ± standard deviations followed by the same lowercase letter on a line for a parameter are not significantly different at the 5% threshold according to Duncan's test

Table 5 Changes in clarity, color, and browning index of cashew apple juice after addition of pectin lyase

Clarification time, min	Clarity (660 nm), %	Color (440 nm), %	Browning (420 nm), %
0	44.67 ± 0.01 ^g	82.98 ± 0.03 ^a	75.86 ± 0.01 ^a
15	48.98 ± 0.01 ^f	82.98 ± 0.00 ^b	61.66 ± 0.02 ^b
30	52.48 ± 0.02 ^e	54.95 ± 0.01 ^c	43.65 ± 0.01 ^c
45	53.70 ± 0.01 ^d	50.12 ± 0.01 ^d	38.90 ± 0.05 ^d
60	63.10 ± 0.04 ^c	45.71 ± 0.02 ^c	33.11 ± 0.01 ^c
75	70.80 ± 0.01 ^b	43.65 ± 0.01 ^f	32.36 ± 0.03 ^f
90	72.44 ± 0.03 ^a	41.69 ± 0.01 ^g	28.18 ± 0.01 ^g
105	72.44 ± 0.01 ^a	41.69 ± 0.00 ^g	26.91 ± 0.01 ^h
120	72.44 ± 0.03 ^a	41.69 ± 0.01 ^g	26.91 ± 0.00 ^h

Means ± standard deviations followed by the same lowercase letter in a column for a parameter are not significantly different at the 5% threshold according to Duncan's test

tion. Furthermore, its soluble solids ($12.00 \pm 0.00^\circ\text{Brix}$) decreased to $7.79 \pm 0.01^\circ\text{Brix}$ in 120 min. On the other hand, the titratable acidity of the juice increased from 0.208 ± 0.000 to 0.236 ± 0.000 g/100 mL after 120 min. Statistical analysis showed no significant difference between the pH values of the clarified juice from 15 min of the treatment. It found no significant difference between the acidity values of the juice from 45 min of clarification. However, the analysis revealed a significant difference for the soluble solids and vitamin C values during the treatment.

Clarification of cashew apple juice with pectin lyase. Clarity, color, and browning index. Table 5 shows changes in the clarity, color, and browning index of cashew apple juice after adding pectin lyase. In particular, the clarity increased from 44.67 to 72.44% and no longer changed after 90 min of preincubation with pectin lyase. The color intensity decreased from 82.98 to 41.70% at the end of clarification, stabilizing after 90 min. Furthermore, the initial browning index (75.86%) of cashew apple juice decreased by half (38.90%) after 45 min of hydrolysis.

pH, titratable acidity, soluble solids, and vitamin C. The physicochemical parameters of cashew apple juice measured after the addition of pectin lyase are given in Table 6. During the clarification process, the initial

pH decreased from 4.87 to 4.78 after 120 min, while the soluble solids and vitamin C decreased from 12 to 7.76°Brix and from 327.82 to 208.70 mg/100 mL, respectively, after 120 min. In contrast, the titratable acidity increased from 0.208 to 0.232 g/100 mL after 120 min. Furthermore, statistical analysis revealed no significant difference in titratable acidity during clarification. However, it indicated a significant difference in pH values after 15 min of the treatment. The analysis of soluble solids, °Brix, and vitamin C results showed significant differences during the clarification of cashew apple juice with pectin lyase.

Clarification of cashew apple juice with pectin methylesterase and polygalacturonase. Clarity, color, and browning index. Table 7 shows changes in the clarity, color, and browning index of cashew apple juice after the addition of different mixtures of pectin methylesterase and polygalacturonase, namely $0.087 + 0.264$ mg/mL (mixture 1), $0.174 + 0.176$ mg/mL (mixture 2), and $0.261 + 0.088$ mg/mL (mixture 3), respectively. As can be seen, mixture 1 provided greater clarity than the other two mixtures during the clarification period. In particular, it increased the clarity to 83.18% after six minutes, while mixtures 2 and 3 provided a clarity of 81.28 and 79.43%, respectively, after 75 min of clarification. After 105 min of treatment with mix-

Table 6 Changes in pH, titratable acidity, soluble solids, and vitamin C during clarification of cashew apple juice by pectin lyase

Clarification time, min	Parameter			
	pH	Titratable acidity, g/100 mL	Refractometric dry extract, °Brix	Vitamin C, mg/100 mL
0	4.87 ± 0.03 ^a	0.208 ± 0.000 ^a	12.00 ± 0.00 ^a	327.82 ± 0.00 ^a
15	4.80 ± 0.00 ^b	0.210 ± 0.000 ^a	11.40 ± 0.04 ^b	304.41 ± 0.03 ^b
30	4.79 ± 0.04 ^b	0.220 ± 0.000 ^a	10.16 ± 0.03 ^c	288.80 ± 0.01 ^c
45	4.79 ± 0.03 ^b	0.228 ± 0.000 ^a	10.12 ± 0.03 ^d	280.99 ± 0.03 ^d
60	4.78 ± 0.01 ^b	0.232 ± 0.000 ^a	9.30 ± 0.03 ^e	265.36 ± 0.03 ^c
75	4.78 ± 0.01 ^b	0.232 ± 0.000 ^a	8.60 ± 0.04 ^f	248.76 ± 0.01 ^f
90	4.78 ± 0.00 ^b	0.232 ± 0.000 ^a	8.20 ± 0.04 ^g	234.15 ± 0.01 ^g
105	4.78 ± 0.03 ^b	0.232 ± 0.000 ^a	7.90 ± 0.05 ^h	218.54 ± 0.03 ^h
120	4.78 ± 0.01 ^b	0.232 ± 0.000 ^a	7.76 ± 0.03 ⁱ	208.70 ± 0.01 ⁱ

Means ± standard deviations followed by the same lowercase letter on a line for a parameter are not significantly different at the 5% threshold according to Duncan’s test

Table 7 Changes in clarity, color, and browning index of cashew apple juice after addition of mixtures of pectinases (pectin methylesterase and polygalacturonase)

Clarification time, min	Enzymes concentration	Clarity (660 nm), %	Color (440 nm), %	Browning (420 nm), %
0	Mixture 1	44.67 ± 0.01	82.98 ± 0.01	75.86 ± 0.01
	Mixture 2	44.67 ± 0.01	82.98 ± 0.01	75.86 ± 0.01
	Mixture 3	44.67 ± 0.01	82.98 ± 0.01	75.86 ± 0.01
15	Mixture 1	52.48 ± 0.06	54.95 ± 0.04	51.29 ± 0.01
	Mixture 2	50.12 ± 0.01	67.61 ± 0.03	54.95 ± 0.03
	Mixture 3	50.12 ± 0.03	67.61 ± 0.03	61.66 ± 0.04
30	Mixture 1	66.07 ± 0.09	48.98 ± 0.01	46.77 ± 0.03
	Mixture 2	54.95 ± 0.06	52.48 ± 0.04	50.12 ± 0.04
	Mixture 3	54.95 ± 0.01	54.95 ± 0.03	46.77 ± 0.03
45	Mixture 1	79.43 ± 0.03	46.77 ± 0.03	35.48 ± 0.01
	Mixture 2	66.07 ± 0.06	48.98 ± 0.01	38.02 ± 0.03
	Mixture 3	67.61 ± 0.03	50.12 ± 0.01	43.65 ± 0.03
60	Mixture 1	83.18 ± 0.01	45.71 ± 0.03	35.48 ± 0.03
	Mixture 2	72.44 ± 0.01	46.77 ± 0.03	34.67 ± 0.01
	Mixture 3	74.13 ± 0.03	46.77 ± 0.03	39.81 ± 0.03
75	Mixture 1	91.20 ± 0.04	44.67 ± 0.03	31.62 ± 0.01
	Mixture 2	81.28 ± 0.06	46.77 ± 0.04	30.90 ± 0.04
	Mixture 3	79.43 ± 0.03	45.71 ± 0.01	33.88 ± 0.04
90	Mixture 1	93.32 ± 0.06	44.67 ± 0.01	30.20 ± 0.01
	Mixture 2	89.12 ± 0.03	45.71 ± 0.03	29.51 ± 0.03
	Mixture 3	83.18 ± 0.03	44.67 ± 0.03	31.62 ± 0.03
105	Mixture 1	95.50 ± 0.03	43.65 ± 0.03	29.51 ± 0.03
	Mixture 2	91.20 ± 0.03	43.65 ± 0.03	29.51 ± 0.01
	Mixture 3	87.10 ± 0.01	43.65 ± 0.01	29.51 ± 0.03
120	Mixture 1	95.50 ± 0.01	43.65 ± 0.01	28.84 ± 0.04
	Mixture 2	91.20 ± 0.06	43.65 ± 0.01	28.18 ± 0.03
	Mixture 3	87.10 ± 0.03	43.65 ± 0.03	28.18 ± 0.03

Mixture 1: pectin methylesterase (0.087 mg/mL) + polygalacturonase (0.264 mg/mL); Mixture 2: pectin methylesterase (0.174 mg/mL) + polygalacturonase (0.176 mg/mL); and Mixture 3: pectin methylesterase (0.261 mg/mL) + polygalacturonase (0.088 mg/mL)

ture 1, the juice showed a maximum clarity of 95.50%, compared to 91.20 and 87.10% for mixtures 2 and 3, respectively. Furthermore, the three mixtures decreased the color intensity of cashew apple juice from 82.98 to 43.65%. The color intensity stabilized after 105 min. Finally, the browning index of cashew apple juice decreased by half after 45 min of treatment with the three mixtures.

pH, titratable acidity, soluble solids, and vitamin C of cashew apple juice clarified with mixture 1. Mixture 1 (0.087 mg/mL pectin methylesterase + 0.264 mg/mL polygalacturonase) resulted in a reduction in pH, soluble solids, and vitamin C values (Table 8). Statistical analysis revealed a significant difference in soluble solids during the enzymatic clarification process ($P < 5\%$). This analysis also showed a significant difference in

Table 8 Changes in pH, titratable acidity, soluble solids, and vitamin C during clarification of cashew apple juice with mixture 1

Clarification time, min	Parameter			
	pH	Titratable acidity, g/100 mL	Refractometric dry extract, °Brix	Vitamin C, mg/100 mL
0	4.87 ± 0.03 ^a	0.208 ± 0.000 ^c	12.00 ± 0.00 ^a	327.82 ± 0.00 ^a
15	4.82 ± 0.03 ^b	0.210 ± 0.000 ^c	11.60 ± 0.08 ^a	320.01 ± 0.03 ^b
30	4.80 ± 0.01 ^{b,c}	0.212 ± 0.000 ^c	11.00 ± 0.04 ^b	312.21 ± 0.03 ^c
45	4.80 ± 0.00 ^{b,c}	0.220 ± 0.000 ^b	10.40 ± 0.06 ^c	296.60 ± 0.01 ^d
60	4.79 ± 0.01 ^{b,c}	0.228 ± 0.000 ^a	9.50 ± 0.00 ^d	288.80 ± 0.03 ^c
75	4.79 ± 0.00 ^{b,c}	0.232 ± 0.000 ^a	8.90 ± 0.14 ^c	280.97 ± 0.03 ^f
90	4.78 ± 0.00 ^c	0.232 ± 0.000 ^a	8.40 ± 0.00 ^f	265.36 ± 0.01 ^g
105	4.78 ± 0.00 ^c	0.232 ± 0.000 ^a	7.80 ± 0.07 ^g	249.75 ± 0.03 ^h
120	4.78 ± 0.00 ^c	0.232 ± 0.000 ^a	7.77 ± 0.01 ^g	241.95 ± 0.01 ⁱ

Means ± standard deviations followed by the same lowercase letter on a line for a parameter are not significantly different at the 5% threshold according to Duncan's test

Table 9 Changes in pH, titratable acidity, soluble solids, and vitamin C during clarification of cashew apple juice with mixture 2

Clarification time, min	Parameter			
	pH	Titratable acidity, g/100 mL	Refractometric dry extract, °Brix	Vitamin C, mg/100 mL
0	4.87 ± 0.03 ^a	0.208 ± 0.000 ^c	12.00 ± 0.00 ^a	327.82 ± 0.00 ^a
15	4.80 ± 0.03 ^b	0.212 ± 0.000 ^{d,c}	11.60 ± 0.03 ^b	320.01 ± 0.03 ^b
30	4.80 ± 0.01 ^b	0.216 ± 0.000 ^d	11.00 ± 0.03 ^c	312.21 ± 0.03 ^c
45	4.79 ± 0.01 ^b	0.224 ± 0.000 ^c	10.20 ± 0.01 ^d	294.55 ± 0.01 ^d
60	4.79 ± 0.00 ^b	0.232 ± 0.000 ^b	9.00 ± 0.03 ^e	286.66 ± 0.03 ^c
75	4.78 ± 0.01 ^b	0.240 ± 0.000 ^a	8.40 ± 0.03 ^f	279.47 ± 0.01 ^f
90	4.77 ± 0.00 ^b	0.240 ± 0.000 ^a	8.00 ± 0.00 ^g	273.17 ± 0.03 ^g
105	4.77 ± 0.00 ^b	0.244 ± 0.000 ^a	7.79 ± 0.01 ^h	257.56 ± 0.01 ^h
120	4.77 ± 0.00 ^b	0.244 ± 0.000 ^a	7.77 ± 0.00 ^h	241.74 ± 0.03 ⁱ

Means ± standard deviations followed by the same lowercase letter on a line for a parameter are not significantly different at the 5% threshold according to Duncan's test

vitamin C levels ($P < 5\%$). However, there was no significant difference in the pH values during the clarification time intervals of 30 to 75 min, or 90 to 120 min of treatment. In particular, the initial pH of 4.87 ± 0.03 decreased to the value of 4.82 ± 0.03 after 15 min of treatment. This value hardly varied, reaching 4.79 ± 0.00 after 75 min of treatment. From 90 to 120 min, the pH value stabilized at 4.78. Furthermore, the soluble solids of the raw juice varied very little during the first half hour of clarification. However, after 45 min of treatment, the soluble solids decreased from 10.4 ± 0.28 to $7.77 \pm 0.01^\circ\text{Brix}$ at 120 min. Finally, the vitamin C content decreased according to the clarification time. After half an hour of clarification, it decreased from 327.82 ± 0.00 to 312.21 ± 0.03 mg/100 mL. It then continued to decrease to reach 241.95 ± 0.01 mg/100 mL. On the other hand, the titratable acidity of cashew apple juice (0.208 ± 0.000 g/100 mL) increased during the treatment (Table 8) to reach 0.232 ± 0.000 g/100 mL after 75 min. This value remained stable until the end of the treatment.

pH, titratable acidity, soluble solids, and vitamin C of cashew apple juice clarified with mixture 2. Changes in the parameters of cashew apple juice clarified with mixture 2 (0.174 mg/mL pectin methylesterase + 0.176 mg/mL polygalacturonase) are presented in Table 9.

As can be seen, the pH, soluble solids, and vitamin C decreased during the clarification process, while the titratable acidity increased. In particular, the initial pH of 4.87 ± 0.03 decreased to 4.77 ± 0.00 after 90 min of clarification, where it remained stable for up to 120 min. Furthermore, the vitamin C content of the raw juice (327.82 ± 0.03 mg/100 mL) decreased with the duration of the clarification treatment to reach 241.74 ± 0.03 mg/100 mL after 120 min. The soluble dry extract of the raw juice decreased from 12.00 ± 0.00 to $7.77 \pm 0.00^\circ\text{Brix}$ at the end of clarification. Statistical analysis revealed no significant difference between the pH values after 15 min of the treatment but it showed a significant difference between the pH values at different clarification times and the control juice. The analysis also showed a significant difference between the contents of soluble solids and vitamin C. However, no significant difference was found for titratable acidity after 75 min of clarification.

pH, titratable acidity, soluble solids, and vitamin C of cashew apple juice clarified with mixture 3 consisting of pectin methylesterase (0.261 mg/mL) and polygalacturonase (0.088 mg/mL). The clarification of cashew apple juice with mixture 3 (0.261 mg/mL pectin methylesterase + 0.088 mg/mL polygalacturonase) caused

a reduction in the initial physicochemical parameters, except for titratable acidity (Table 10). This reduction was accentuated over the clarification time. Indeed, the initial vitamin C content of 327.82 ± 0.00 mg/100 mL decreased to 312.21 ± 0.03 mg/100 mL after 15 min. It then continued to decrease reaching 234.15 ± 0.03 mg/100 mL after 120 min. The pH values decreased to stabilize at 4.77 after 75 min of clarification. Statistical analysis revealed no significant difference between the pH values after 15 min of clarification. The content of soluble solids in the juice also decreased with clarification time. At the beginning of the treatment, its variation was low but after 30 min, it became more noticeable. The titratable acidity, however, increased from 0.208 to 0.24 g/100 mL after 75 min of the treatment. This value remained stable until the end of clarification. Statistical analysis showed a significant difference between the contents of vitamin C and soluble solids during the clarification process.

Viscosity. The raw cashew apple juice had a viscosity of 18 cP (18 mPa·s), which decreased after its clarification with the enzymes (pectin methylesterase, polygalacturonase, and pectin lyase) (Fig. 4). In particular, polygalacturonase produced a lower viscosity than the other

two enzymes. Furthermore, mixture 1 (0.264 mg/mL polygalacturonase and 0.087 mg/mL pectin methylesterase) reduced the viscosity to 4.3 mPa·s. This viscosity value was lower than that produced by the other two mixtures. Indeed, the clarification treatment using this mixture resulted in a 76% reduction in the initial viscosity.

Clarification is a very important process in the juice industry as it improves the product’s acceptability. In this study, we tested the effect of pectinases isolated, and partially purified, from the epicarp of papaya cultivar Solo 8 on the turbidity of cashew apple juice. We found that the use of pectin methylesterase, polygalacturonase, and pectin lyase reduced the viscosity of the juice by 67, 72, and 59%, respectively. In modern fruit juice manufacturing, enzymes (pectinases, hemicellulases, cellulases, amylases) should be used to optimize the process. The main objectives are to increase the extraction of juice from raw materials; improve the processing efficiency; and generate a final product that is clear and visually attractive to the consumer [9]. The transmittances at 660, 440, and 420, which are excellent indices of clarity, color, and browning, made it possible to conduct the enzymatic clarification of cashew apple juice [9, 26, 27].

Table 10 Changes in pH, titratable acidity, soluble solids, and vitamin C during clarification of cashew apple juice with mixture 3

Clarification time, min	Parameter			
	pH	Titratable acidity, g/100 mL	Refractometric dry extract, °Brix	Vitamin C, mg/100 mL
0	4.87 ± 0.03^a	0.208 ± 0.000^c	12.00 ± 0.00^a	327.82 ± 0.00^a
15	4.80 ± 0.03^b	$0.212 \pm 0.000^{d,e}$	11.60 ± 0.03^b	320.01 ± 0.03^b
30	4.80 ± 0.01^b	0.216 ± 0.000^d	11.00 ± 0.03^c	312.21 ± 0.03^c
45	4.79 ± 0.01^b	0.224 ± 0.000^c	10.20 ± 0.01^d	294.55 ± 0.01^d
60	4.79 ± 0.00^b	0.232 ± 0.000^b	9.00 ± 0.03^c	286.66 ± 0.03^c
75	4.78 ± 0.01^b	0.240 ± 0.000^a	8.40 ± 0.03^f	279.47 ± 0.01^f
90	4.77 ± 0.00^b	0.240 ± 0.000^a	8.00 ± 0.00^g	273.17 ± 0.03^g
105	4.77 ± 0.00^b	0.244 ± 0.000^a	7.79 ± 0.01^h	257.56 ± 0.01^h
120	4.77 ± 0.00^b	0.244 ± 0.000^a	7.77 ± 0.00^h	241.74 ± 0.03^i

Means ± standard deviations followed by the same lowercase letter on a line for a parameter are not significantly different at the 5% threshold according to Duncan’s test

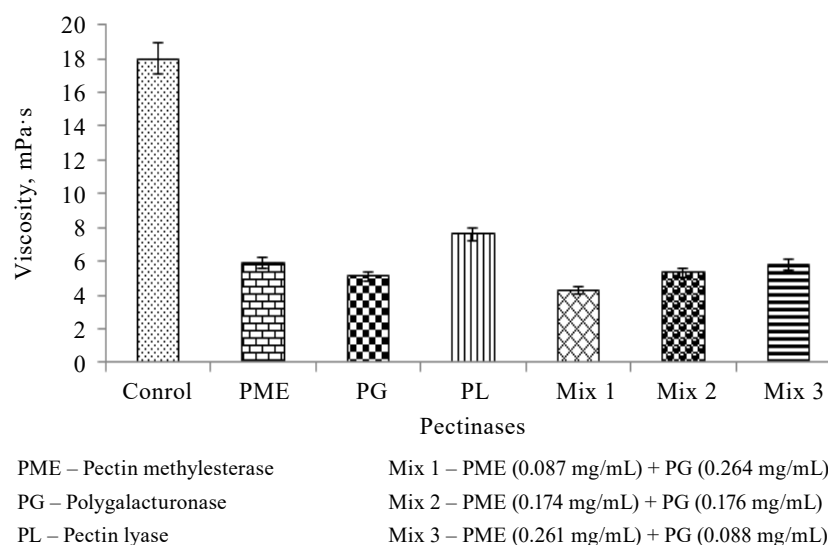


Figure 4 Viscosity of cashew apple juice clarified with pectinases

To obtain visual clarity, the juice needs to be completely depectinized by adding pectinases. In particular, partially purified pectin methylesterase, polygalacturonase, and pectin lyase required concentrations of 0.034, 0.028, and 0.008 mg/mL, respectively, to initiate clarification. The use of these enzymes could be explained by their reaction mechanism. Indeed, pectin lyases require a lower concentration because they act on pectins, oligomers, and polymers of galacturonic acid by catalyzing the breakage between two galacturonic acid units by a β -elimination mechanism [28]. In addition, only pectin lyases are able to degrade pectins without the prior action of other enzymes [29, 30]. This low concentration required for pectin lyase could also be due to the satisfactory purity of this enzyme compared to the other two enzymes (pectin methylesterase and polygalacturonase), which were partially purified.

The duration and temperature of heat treatments affect the clarity, color, browning index, brix, pH, titratable acidity, viscosity, and vitamin C content of the juice. The use of pectinases increases the clarity of juices during processing. Generally, pectinases are added to cashew apple juice to hydrolyze colloids. It is well known that pectin is the main cause of opalescence of the juice [31, 32]. Indeed, interactions between the negatively charged sites of demethylated pectin and divalent ions (Ca^{2+} and Mg^{2+}) lead to the formation of insoluble pectic salts.

In the clarification experiments using partially purified pectinases extracted from papaya epicarp, polygalacturonase (PG) showed a greater effect on the reduction of cashew apple juice turbidity than the other enzymatic fractions. This higher efficiency can be explained by the structural characteristics of pectic polysaccharides present in cashew apple, which are mainly composed of homogalacturonan regions susceptible to hydrolysis by polygalacturonase. Recent studies have highlighted that the degradation of pectic substances by polygalacturonase promotes the breakdown of the pectin network, facilitating the aggregation and sedimentation of suspended particles and consequently improving juice clarity [33, 34].

Furthermore, the pectinases added for clarification caused the hydrolysis of pectins, which are mainly responsible for the cloudiness of cashew apple juice. Also, we found that the clarity of the juice increased over time and stabilized thereafter. This finding is consistent with the results of Sharma *et al.* [35]. According to them, the increase in juice clarity with incubation time reflects the progressive hydrolysis of pectic polysaccharides by pectinases. As the enzyme–substrate interaction proceeds, the pectin network is disrupted, reducing colloidal stability and facilitating the sedimentation of suspended particles. Once most of the accessible pectin has been hydrolyzed, further incubation has little effect on juice clarity, resulting in a stabilization phase.

During the clarification of cashew apple juice, its color intensity decreased by almost half after 60 min. Thus, the juice was neither too pale nor too dark. To be more competitive on the market, the color of the juice must

be an effective means of attracting the consumer [36]. The decrease in the yellow color intensity of cashew apple juice could be explained by the destruction of carotenoids responsible for the yellow color. Indeed, carotenoids are molecules sensitive to oxygen, light, acidity, metals, and heat [37]. However, the enzymatic clarification tests of cashew apple juice were carried out in a lit laboratory in the presence of oxygen from the air. In contrast, high-temperature processing has been widely reported to promote non-enzymatic browning in fruit juices through ascorbic acid degradation, 5-hydroxymethylfurfural (5-HMF) formation, and Maillard-type reactions, leading to increased color intensity. Therefore, the discrepancy between our findings and those reported for thermally processed cashew apple juice is most likely due to the absence of severe heat treatment in the present study [38].

The low viscosity of the juice clarified with polygalacturonase is a good indicator of this enzyme's effectiveness. This decrease in viscosity has also been highlighted by Ninga *et al.* [39] and Falcão *et al.* [40]. For these authors, the depolymerization of pectin reduces its ability to keep particles in suspension by causing their precipitation. This process causes a reduction in the viscosity of the juice [41]. However, the three enzymes used in our study showed different results in clarifying cashew apple juice. In particular, pectin methylesterase, polygalacturonase, and pectin lyase decreased the viscosity of the raw juice by 67, 72, and 59%, respectively. Although reducing viscosity, pectin methylesterase should be used with caution because its action generates methanol, which is toxic.

The decrease in pH and the increase in titratable acidity during the enzymatic clarification of cashew apple juice could be explained by the presence of organic acids in the medium. Indeed, the hydrolysis of pectin by pectinases leads to the formation of galacturonic acids. Our results corroborate those of Nguyen *et al.* [42]. These authors also showed that the pH of clarified apple juice decreases, while its acidity increases during clarification.

The decrease in soluble solids from 12 to 7.76°Brix in all the treatments was due to the loss of suspended particles. Indeed, after the action of pectinases the juice is centrifuged to remove its pellet. Our results are contrary to those of Sharma *et al.* [35], who reported an increase in soluble solids during the clarification of cloudy fruit juices. This difference could be explained by the juice extraction protocol and the state of maturity of the fruit used for juice production.

Finally, the vitamin C content also decreased during the clarification. This decrease was more pronounced with higher heating time and the loss of vitamin C was due to its thermolability. Our results were consistent with those of Nguyen *et al.* [42], who studied the clarification of apple juice by pectinases.

Of all the combinations tested, the most effective was mixture 1, consisting of pectin methylesterase (0.087 mg/mL) and polygalacturonase (0.264 mg/mL). It re-

duced the viscosity of raw cashew apple juice (18 mPa·s) by more than 76%, while increasing its clarity by more than half. This performance of mixture 1 could be explained by the synergistic action of the two enzymes on natural pectins. Indeed, pectin methylesterase creates demethoxylated sequences allowing hydrolysis by polygalacturonase. Our findings agree with those Flores-Fernández *et al.* [43], Padma *et al.* [44] and Lhamo *et al.* [45]. However, the use of pectin methylesterase in this combination could be a limiting factor for its application because its action generates methanol, which is a toxic product.

CONCLUSION

The partially purified pectinases from the crude epicarp extract of papaya cultivar Solo 8 were effective in reducing the viscosity of cashew apple juice. Of the three enzymes, polygalacturonase resulted in the greatest reduction of the juice's viscosity (72%). The combination of pectin methylesterase (0.087 mg/mL) and polygalacturonase (0.264 mg/mL) led to an even greater viscosity reduction of 76%, while also increasing the clarity of cloudy juice by half. Thus, these pectinases can be used to clarify local cloudy juices.

Our results open up new research avenues for a better use of papaya and cashew nuts, which are widely

produced in Côte d'Ivoire. The scientific rigor of this study could be strengthened by measuring the residual concentration of sulfur dioxide and quantifying protopectin before and after enzymatic treatment. A preliminary mechanical clarification would also help optimize enzymatic efficiency and enhance the clarity of the results.

CONTRIBUTION

K.M.-D. Adingra and K. Tano participated in the original research and writing of the manuscript. N.J.P. Kouadio and K.M.-D. Adingra conducted the clarification tests. H.D. Gnahe and S. Soro performed the physicochemical evaluation. All the authors were involved in editing, revising, and proofreading of the manuscript. All the authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest which could hinder the publication of this article.

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