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IN VITRO ANTIDIABETIC ACTIVITY OF CASHEW APPLE JUICES, WINE AND VINEGAR

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ABSTRACT

Diabetes affects 589 million adults worldwide, a figure that could reach 853 million by 2050, with Africa experiencing the fastest growth. In this context, valorizing local agro-industrial by-products offers a promising avenue for limiting postprandial hyperglycemia. In Côte d'Ivoire, the world's leading cashew nut producer, the cashew apple represents approximately 90% of the fruit's weight but remains largely underexploited. This study aimed to evaluate and compare, under identical experimental conditions, the *in vitro* antidiabetic potential of five cashew apple-derived products: raw juice, clarified juice, pasteurized juice, wine and vinegar. Following qualitative phytochemical screening, α-amylase inhibitory activity and glucose adsorption capacity were determined. Screening revealed eight families of secondary metabolites, raw juice concentrating six, compared with four for clarified and pasteurized juices, and seven for vinegar. Vinegar showed the lowest IC_{50} (0.88 ± 0.49 mg/mL), statistically equivalent to that of acarbose (0.21 ± 0.08 mg/mL), followed by wine (8.99 ± 3.73 mg/mL) and clarified juice (11.87 ± 2.69 mg/mL), while raw and pasteurized juices recorded the highest IC_{50} values. For glucose adsorption, expressed relative to the dry mass used, vinegar ranked first at the two highest concentrations, and wine at the lowest concentration (0.084 ± 0.009 mmol/g). Vinegar thus stands out as the best-performing product across both mechanisms evaluated, with double fermentation appearing to be the processing method most favorable to the expression of the antidiabetic potential of the cashew apple.

Keywords: Cashew apple; Antidiabetic activity; α-amylase inhibition; Glucose adsorption; Phytochemical screening

1 INTRODUCTION

According to the Diabetes Atlas published in 2025 by the International Diabetes Federation, 589 million adults are affected by diabetes worldwide, a figure that could reach 853 million by 2050. Africa is experiencing the fastest growth, estimated at +142%, and has the highest proportion of undiagnosed cases (International Diabetes Federation, 2025). In the face of this progression, the search for accessible nutritional approaches capable of limiting postprandial hyperglycemia stands out as a public health priority, particularly in low-resource settings.

Two complementary mechanisms help attenuate the glycemic peak following a starch-rich meal: certain dietary fibers physically retain glucose and slow its intestinal absorption, while polyphenols inhibit α-amylase, the key enzyme in starch digestion (Ou *et al.*, 2001; Sun *et al.*, 2019). Acarbose, the reference molecule, exploits this second mechanism, but its digestive side effects and cost limit its use in low-resource settings (Ćorković *et al.*, 2022). This limitation reinforces the interest in seeking alternative natural sources, particularly among agro-industrial by-products already available locally.

In Côte d'Ivoire, the world's leading cashew nut producer since 2015, the nut represents only about 10% of the weight of the fruit. The remaining 90%, made up of the cashew apple, is generally left in the field (Soro *et al.*, 2011). This largely underexploited biomass nevertheless contains bioactive compounds (notably polyphenols and tannins) to which antioxidant properties and an action on carbohydrate metabolism are attributed (Gutiérrez-Paz *et al.*, 2024). Several processing methods have been developed locally: Soro (2012) worked on juice extraction and clarification, while Lowor *et al.* (2016) described the production of wine and vinegar from cashew apple. Each of these processes profoundly modifies the plant matrix and may therefore influence the biological potential of the final product.

To date, few studies have compared, under identical experimental conditions, the various beverages derived from the cashew apple, making it difficult to determine which processing method best enhances the fruit's antidiabetic potential. It is in this context that the present study is set, focusing on five liquid products derived from the cashew apple: raw juice, clarified juice, pasteurized juice, wine and vinegar. After establishing their qualitative phytochemical profile, the study aims to evaluate and compare their *in vitro* antidiabetic activity using two complementary approaches: α-amylase inhibition, assessed by inhibition percentages and half-maximal inhibitory concentrations, and glucose adsorption capacity. The ultimate objective is to identify, among these five processing methods, the one(s) that most effectively preserve or enhance the antidiabetic potential of the cashew apple.

2 MATERIALS AND METHODS

2.1 Plant material

The plant material used in this study consisted of cashew apples (*Anacardium occidentale* L.) freshly harvested in the Yamoussoukro region, in central Côte d'Ivoire. The fruits were supplied, received, sorted and washed at the SODEFORCE plant (Société de Développement des Forces de Côte d'Ivoire), based in Yamoussoukro, during the month of January. Ripe, healthy cashew apples free of any visible damage were selected for the preparation of the cashew apple juices, wine and vinegar.

2.2 Production of juices, wine and vinegar from cashew apple

2.2.1 Production of cashew apple juices

Cashew apple juice was produced from ripe cashew apples (*A. occidentale* L.) sourced from the Yamoussoukro region (central Côte d'Ivoire). Processing of the cashew apples was carried out according to the method described by Soro (2012), at the SODEFORCE agro-industrial production unit, located in Yamoussoukro. The cashew apples were first washed with water, then disinfected for 30 min with 100 ppm active chlorine, in order to remove impurities and foreign matter likely to compromise the quality of the final product. They were then rinsed with water before being crushed and pressed using a screw press, allowing extraction of the raw juice and separation of the solid residues (bagasse).

The raw juice obtained was then subjected to a clarification step using 10% food-grade gelatin powder, previously dissolved in water heated to 70°C. After a settling period, the juice was filtered through clean cotton cloth to obtain a clarified juice.

The clarified juice was then subjected to a pasteurization treatment at 85°C for 20 minutes, in order to eliminate microorganisms; the product thus obtained is the pasteurized cashew apple juice. The three juices (raw, clarified and pasteurized) were packaged in sterile glass bottles and stored at 4°C until use (Couri *et al.*, 2002; Soro, 2012).

2.2.2 Production of cashew apple wine

Cashew apple wine was produced according to the method described by Lowor *et al.* (2016). The juice extracted from the apples was inoculated with a commercial *Saccharomyces cerevisiae* yeast. Fermentation took place in two phases: an initial week under aerobic conditions, followed by four weeks under anaerobic conditions. The resulting wine had an amber color, a dry character, an alcohol content of approximately 12.6% (v/v) and a pH close to 3.8 (Lowor *et al*., 2016; Gnagne *et al*., 2023).

2.2.3 Production of cashew apple vinegar

Vinegar was obtained from the same cashew apple wine through spontaneous acetic fermentation. The naturally present acetic flora ensured the gradual oxidation of ethanol into acetic acid over 29 days. During this acetification, the alcohol content dropped to 0% and the volatile acidity reached approximately 6.85 g acetic acid per 100 mL, yielding a table vinegar (Lowor *et al*., 2016).

2.3 Qualitative phytochemical screening

The phytochemical study of cashew apple fruit (*A. occidentale* L.), based on coloration and/or precipitation tests, was carried out on the juices, wine and vinegar of this fruit (Békro *et al*., 2007; Dohou *et al*., 2003; Harborne, 1998). The families of secondary metabolites targeted in this study were polyphenols, flavonoids, gallic and catechic tannins, saponins, alkaloids, quinones, as well as sterols and polyterpenes.

2.4 *In vitro* antidiabetic study

Although several methods are used to evaluate the *in vitro* antidiabetic activity of plant extracts, two were selected in this study: the α-amylase inhibition test and the glucose adsorption test, in order to assess the antidiabetic potential of cashew apple juices, wine and vinegar.

2.4.1 Study of the inhibitory activity of the juices, wine and vinegar on α-amylase

2.4.1.1 Principle

The principle of the test relies on the fact that α-amylase hydrolyzes polysaccharides, such as starch, into oligosaccharides. Inhibiting this enzyme therefore amounts to slowing glucose release. The test consists of allowing the enzyme to act on starch in the presence of the sample, then measuring the amount of reducing sugars released using the 3,5-dinitrosalicylic acid (DNS) method described by Miller (1959). The fewer sugars released, the more inhibitory the sample. The protocol followed is that described by Wongsa *et al*. (2022).

2.4.1.2 Preparation of solutions

As part of the evaluation of the inhibitory effect of cashew apple juices, wine and vinegar on α-amylase activity, several solutions were prepared at different concentrations.

- Preparation of the 1% starch solution: the starch solution was prepared at a concentration of 1% (w/v), i.e. 1 g of starch dissolved in 100 mL of phosphate buffer at ph 7.
- Preparation of the 0.5 mg/mL α-amylase solution: the enzyme solution was prepared at a concentration of 0.5 mg/mL; thus, 7.5 mg of α-amylase were dissolved in 15 mL of phosphate buffer at ph 7.
- Preparation of the different concentrations of cashew apple juice, wine and vinegar.

In this study, the concentrations of juice, wine and vinegar were prepared using the double dilution method. The dry matter content of the cashew apple juices was first determined in order to establish their concentration. This dry matter was determined by weighing an empty crucible to obtain its tare mass (M0). A volume of 1 mL of pasteurized juice was then introduced into this crucible. The crucible and cashew apple juice assembly was placed in an oven at 60°C for 24 hours. After drying, the crucible containing the dry residue was weighed again to obtain the final mass (M1). The dry matter mass was calculated according to the following formula:

2.4.1.3 Dry matter mass (DM) = M1 (crucible + dry residue) − M0 (empty crucible)

The juice concentration was determined by dividing the dry matter mass obtained by the volume of sample taken. This calculation gave a juice concentration of C1 = 120 mg/mL. From this initial concentration, a series of successive double dilutions was carried out: 2 mL of the initial solution (C1 = 120 mg/mL) were taken and diluted in 2 mL of buffer solution, giving a concentration C2 of 60 mg/mL; then 2 mL of solution C2 were taken and diluted in 2 mL of buffer solution, giving a concentration C3 of 30 mg/mL.

For vinegar and wine, the solvent was first evaporated in an oven at 60°C. The dry matter was then weighed and dissolved in phosphate buffer at pH 7, so as to obtain a stock solution concentration of C1 = 50 mg/mL. From this stock solution, a series of double dilutions was carried out as for the cashew apple juices, giving concentrations C2 = 25 mg/mL and C3 = 12.5 mg/mL.

Acarbose, used as the reference, was dissolved in phosphate buffer to obtain concentrations of C1 = 4 mg/mL, C2 = 2 mg/mL and C3 = 1 mg/mL.

2.4.2 Study of the effect of the juices, wine and vinegar on starch hydrolysis by α-amylase

Evaluation of the inhibitory effect of the cashew apple products on α-amylase activity was carried out in test tubes. In each tube, 500 µL of sample solution were mixed with 500 µL of 0.5 mg/mL α-amylase solution, and the mixture was then incubated at 37°C for 10 minutes. The reaction was then triggered by the addition of 500 µL of starch solution, followed by a second 10-minute incubation at 37°C. This reaction mixture brought the juice, wine, vinegar and acarbose solutions to the following concentrations:

- Juices: C1 = 40 mg/mL, C2 = 20 mg/mL, C3 = 10 mg/mL
- Vinegar and wine: C1 = 16.67 mg/mL, C2 = 8.33 mg/mL, C3 = 4.17 mg/mL
- Acarbose: C1 = 1.33 mg/mL, C2 = 0.67 mg/mL, C3 = 0.33 mg/mL

Enzyme activity was stopped by adding 1 mL of DNS reagent. The tubes were then placed in a boiling water bath at 100°C for 5 minutes, then cooled in an ice-water bath. After shaking, 10 mL of distilled water were added to dilute the mixture, and absorbance was read at 540 nm using a spectrophotometer. Three controls accompanied the assays: the negative control, consisting of enzyme and starch supplemented with buffer instead of sample, corresponds to the maximal α-amylase activity; the matrix blank, consisting of sample and starch supplemented with buffer instead of enzyme, allows subtraction of the sample's own color as well as the reducing sugars it already contains; the positive control is acarbose, tested under the same conditions.

2.4.3 Determination of reducing sugar concentrations

After reading the absorbance, or optical density (OD), the actual reducing sugar concentration was determined using the calibration curve equation. In this study, the calibration curve equation is Y = 1.3682X, where:

- Y = absorbance (OD) measured by spectrophotometer
- X = reducing sugar concentration (mg/mL)
- R^2 = 0.999

Thus, to determine the sugar concentration, the formula is rearranged as follows:

X = Y / 1.3682

This relationship allows absorbance values to be converted into concentrations usable for the analysis of enzymatic activity.

2.4.4 Determination of the percentage of enzymatic inhibition

The purpose of this calculation is to measure the effect of the cashew apple products on α-amylase activity, i.e. their ability to reduce sugar production. The percentage inhibition is determined from the absorbance values obtained, using the following formula:

$$\% \text{ Inhibition} = [Ac - (At - Ab)] / Ac \times 100$$

with:

- Ac = absorbance of the control (starch + enzyme)
- At = absorbance of the tested sample (product + starch + enzyme)
- Ab = absorbance of the blank (starch + product)

2.4.5 Determination of half-maximal inhibitory concentrations (IC_{50})

IC_{50} values were estimated by nonlinear regression using GraphPad Prism version 10 software. The fit was performed on three concentrations in a geometric progression with a ratio of 1:2, each measured in three independent replicates. The model used was “log(inhibitor) versus normalized response” with a fixed slope (Hill = 1): the number of available

data points does not allow an identifiable fit of a variable-slope model, which involves four parameters. IC_{50} values thus constitute estimated values, to be interpreted together with the percentage inhibition values measured at the three concentrations (Sebaugh, 2011). The lower the estimated IC_{50}, the more active the sample is considered under the assay conditions.

2.5 Determination of glucose adsorption capacity

2.5.1 Principle

This test evaluates the ability of the extract's constituents to retain glucose molecules at their surface, thereby making them less available in the surrounding medium, a mechanism demonstrated *in vitro* by Ou *et al*. (2001) and Chau et al. (2003). In practice, a known amount of glucose is brought into contact with the sample. After a contact period, the fraction that has retained the glucose is separated by centrifugation, and the glucose remaining free in the supernatant is assayed by the DNS method.

2.5.2 Preparation of solutions

A 20 mmol/L glucose solution (i.e. approximately 3.60 g/L) was used. The concentrations of juice, wine and vinegar were prepared as in the study of their effect on starch hydrolysis by α-amylase, using the same concentrations. For the juices (raw, clarified, pasteurized), the starting solutions were C1 = 120 mg/mL, C2 = 60 mg/mL and C3 = 30 mg/mL; for vinegar and wine, C1 = 50 mg/mL, C2 = 25 mg/mL and C3 = 12.5 mg/mL.

2.5.3 Procedure

In a test tube, 1 mL of sample was mixed with 1 mL of a 20 mmol/L glucose solution. The mixture was vigorously shaken, then incubated at 35°C for one hour, with shaking every 15 minutes to promote contact between the extract's constituents and the glucose. In the reaction medium, the new concentrations were:

- Juices: C1 = 60 mg/mL, C2 = 30 mg/mL, C3 = 15 mg/mL
- Vinegar and wine: C1 = 25 mg/mL, C2 = 12.5 mg/mL, C3 = 6.25 mg/mL
- Glucose: 10 mmol/L, i.e. 1.80 g/L

The tubes were then centrifuged at 4,000 rpm for 20 minutes. A 1 mL aliquot of the supernatant was carefully collected and supplemented with 1 mL of DNS reagent. The mixture was placed in a water bath at 100°C for 5 minutes to develop the coloration, immediately cooled, then diluted with 10 mL of distilled water. Absorbance was finally measured at 540 nm. A control tube containing only the glucose solution and distilled water was used to establish the reference concentration in the absence of sample. A blank without glucose was also prepared for each sample, in order to subtract the contribution of the sugars already present in the product.

Two parameters were determined from the blank-corrected absorbances. The first corresponds to the concentration of free glucose in the supernatant; absorbances were converted to concentration using the previously established glucose calibration curve (Y = 1.4071X − 0.0006; X in g/L). This parameter reflects the fraction of glucose that remained available after adsorption by the extract: the lower the value, the more glucose the product has retained.

The second parameter is the amount of glucose adsorbed, expressed relative to the dry mass of product used, calculated according to the relation:

$$\textit{Adsorbed glucose (mmol/g)} = [(G_1 - G_2) / M] \times (V / W)$$

where G_1 is the glucose concentration of the control tube (0.185 g/L in the reading medium), G_2 is that remaining free in the supernatant, M is the molar mass of glucose expressed in mg/mmol, V is the total volume of the reaction medium (2 mL), and W is the dry mass of sample actually used in the tube (in g), derived from the stock solution concentration and the 1 mL test aliquot volume.

2.6 Statistical analysis

Results are expressed as mean ± standard deviation, calculated from three independent replicates. Comparisons between samples were performed using two-way analysis of variance (ANOVA), to simultaneously test the effect of the derived product, the effect of concentration and their possible interaction, followed by Tukey's post-hoc test at the 5% significance threshold ($p < 0.05$). The standard deviation associated with each IC_{50} comes from three independent fits performed on three distinct experimental series (n = 3). All calculations and fits were performed using GraphPad Prism version 10 software (GraphPad Software, San Diego, USA).

3 RESULTS

3.1 Secondary metabolite composition

Qualitative phytochemical screening performed on the cashew apple-derived products revealed eight families of secondary metabolites: polyphenols, flavonoids, gallic tannins, catechic tannins, saponins, alkaloids, quinones, sterols and polyterpenes (Table 1).

Polyphenols are present in all derived products; sterols and polyterpenes are likewise present in all five products. Flavonoids appear in the three juices and the vinegar, wine remaining negative. Gallic tannins are found in the three juices and the vinegar, but are absent from the wine. Catechic tannins and saponins, present in the raw juice, disappear after clarification and pasteurization; catechic tannins do not reappear in any of the other liquid products, whereas saponins are again detected in the vinegar and the wine. Alkaloids and quinones, absent from the three juices, are detected in the vinegar and the wine.

Table 1: Secondary metabolites detected in cashew apple-derived products

Metabolites	RJ	CJ	PJ	Vg	Wn
Polyphenols	+	+	+	+	+
Flavonoids	+	+	+	+	−
Gallic tannins	+	+	+	+	−
Catechic tannins	+	−	−	−	−
Saponins	+	−	−	+	+
Alkaloids	−	−	−	+	+
Quinones	−	−	−	+	+
Sterols and polyterpenes	+	+	+	+	+

RJ: raw juice; CJ: clarified juice; PJ: pasteurized juice; Vg: cashew apple vinegar; Wn: cashew apple wine. (+): Present; (−): Absent.

3.2 α-Amylase inhibition

3.2.1 Percentage inhibition of α-amylase

The percentages of α-amylase inhibition obtained for the cashew apple-derived products and for acarbose are presented in Figure 1.

At 40 mg/mL for the juices and 16.67 mg/mL for vinegar and wine, vinegar gave the highest percentage inhibition among the cashew apple-derived products, at 90.48 ± 2.38%. Raw juice reached 73.81 ± 3.64% and wine 72.33 ± 8.18%. Clarified juice gave 64.28 ± 4.96%. Pasteurized juice showed the lowest inhibition at this level, at 57.14 ± 4.13%. Acarbose, tested at 1.33 mg/mL, reached 95.23 ± 0.23%.

At 20 mg/mL for the juices and 8.33 mg/mL for vinegar and wine, vinegar retained first place with 85.71 ± 2.75%. Raw juice gave 59.52 ± 4.96% and clarified juice 52.38 ± 6.30%. Wine gave 48.58 ± 6.99%. Pasteurized juice reached 45.24 ± 4.95%. Acarbose, at 0.67 mg/mL, maintained a high inhibition of 92.87 ± 0.23%.

At 10 mg/mL for the juices and 4.17 mg/mL for vinegar and wine, vinegar again showed the highest percentage, at 78.57 ± 4.12%. Clarified juice reached 38.09 ± 4.96%, raw juice 35.71 ± 6.30% and pasteurized juice 33.33 ± 5.50%. Wine gave 20.68 ± 2.91%. Acarbose, tested at 0.33 mg/mL, reached 76.20 ± 0.46%.

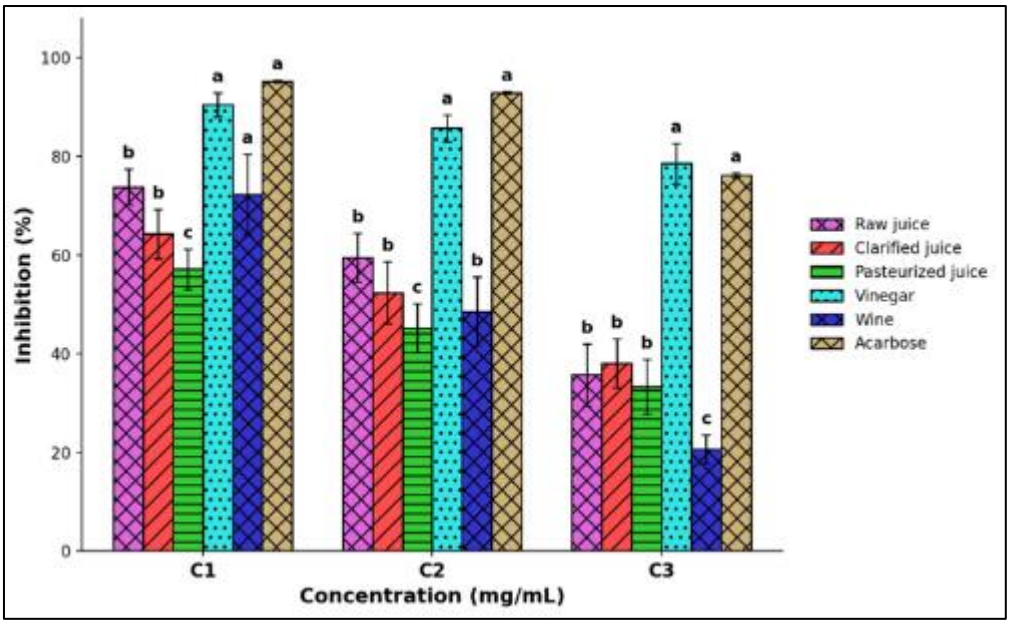

Figure 1: Percentage α-amylase inhibition of cashew apple-derived products

Values are expressed as mean ± standard deviation (n = 3). Means bearing different letters (a, b, c) indicate a statistically significant difference ($p < 0.05$), whereas those sharing at least one letter do not differ significantly ($p \geq 0.05$). C1: juices 40 mg/mL, vinegar and wine 16.67 mg/mL, acarbose 1.33 mg/mL. C2: juices 20 mg/mL, vinegar and wine 8.33 mg/mL, acarbose 0.67 mg/mL. C3: juices 10 mg/mL, vinegar and wine 4.17 mg/mL, acarbose 0.33 mg/mL.

3.2.2 Half-maximal inhibitory concentrations (IC_{50})

The α-amylase half-maximal inhibitory concentrations determined for the cashew apple-derived products and for acarbose are gathered in Table 2.

Vinegar showed the lowest IC_{50} among the derived products, at 0.88 ± 0.49 mg/mL. This value is not statistically different from that of acarbose (0.21 ± 0.08 mg/mL), placing vinegar at the same level as the reference molecule for this parameter. Wine followed with 8.99 ± 3.73 mg/mL and clarified juice with 11.87 ± 2.69 mg/mL, these two products forming a homogeneous intermediate group. Raw juice (19.07 ± 3.52 mg/mL) and pasteurized juice (19.97 ± 2.38 mg/mL) recorded the highest values and are not distinguishable from one another. The six preparations are thus distributed into three homogeneous groups: vinegar and acarbose; wine and clarified juice; and raw juice and pasteurized juice.

The coefficients of determination (R^2) ranged between 0.921 and 0.999, reflecting a good fit of the dose-response curves.

Table 2: α-Amylase half-maximal inhibitory concentrations (IC_{50})

Derived products	IC_{50} (mg/mL)	R^2
Raw juice	19.07 ± 3.52^a	0.948
Clarified juice	11.87 ± 2.69^b	0.929
Pasteurized juice	19.97 ± 2.38^a	0.926
Wine	8.99 ± 3.73^b	0.921
Vinegar	0.88 ± 0.49^c	0.989
Acarbose	0.21 ± 0.08^c	0.999

Values are expressed as mean ± standard deviation (n = 3). R^2 = coefficient of determination of the nonlinear fit. Means bearing different letters (a, b, c) indicate a statistically significant difference ($p < 0.05$), whereas those sharing at least one letter do not differ significantly ($p \geq 0.05$).

3.3 Glucose adsorption

3.3.1 Free glucose concentration remaining in the supernatant

The concentrations of glucose remaining free in the supernatant are presented in Figure 2.

At 60 mg/mL for the juices and 25 mg/mL for vinegar and wine, pasteurized juice left the lowest free glucose concentration, at 0.02 ± 0.01 g/L. Vinegar gave 0.03 ± 0.00 g/L and raw juice 0.04 ± 0.03 g/L. Clarified juice reached 0.06 ± 0.00 g/L. Wine gave 0.16 ± 0.01 g/L.

At 30 mg/mL for the juices and 12.5 mg/mL for vinegar and wine, vinegar showed the lowest free glucose concentration, at 0.03 ± 0.01 g/L. Raw juice gave 0.06 ± 0.02 g/L. Clarified juice reached 0.10 ± 0.01 g/L. Pasteurized juice and wine gave 0.12 ± 0.02 g/L and 0.12 ± 0.01 g/L, respectively.

At 15 mg/mL for the juices and 6.25 mg/mL for vinegar and wine, raw juice gave 0.07 ± 0.02 g/L of free glucose. Clarified juice and wine gave 0.09 ± 0.02 g/L and 0.09 ± 0.01 g/L, respectively. Pasteurized juice reached 0.10 ± 0.03 g/L. Vinegar showed 0.11 ± 0.02 g/L.

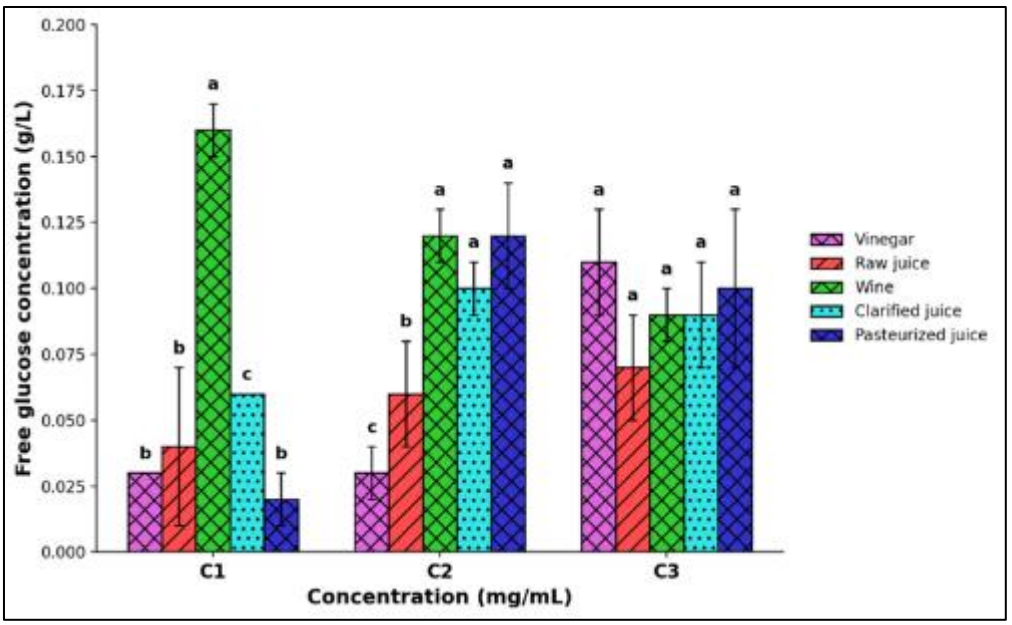

Figure 2: Glucose concentration in the supernatant

Values are expressed as mean ± standard deviation (n = 3). Means bearing different letters (a, b, c) indicate a statistically significant difference ($p < 0.05$), whereas those sharing at least one letter do not differ significantly ($p \geq 0.05$). Acarbose was not tested in this assay. C1: juices 60 mg/mL, vinegar and wine 25 mg/mL. C2: juices 30 mg/mL, vinegar and wine 12.5 mg/mL. C3: juices 15 mg/mL, vinegar and wine 6.25 mg/mL.

3.3.2 Amount of glucose adsorbed

The amounts of glucose adsorbed are presented in Table 3.

At 60 mg/mL for the juices and 25 mg/mL for vinegar and wine, vinegar adsorbed the highest amount relative to the mass used, at 0.034 ± 0.000 mmol/g. Pasteurized juice gave 0.015 ± 0.001 mmol/g, raw juice 0.013 ± 0.003 mmol/g and clarified juice 0.012 ± 0.000 mmol/g. Wine showed the lowest value, at 0.006 ± 0.002 mmol/g.

At 30 mg/mL for the juices and 12.5 mg/mL for vinegar and wine, vinegar remained in the lead with 0.069 ± 0.004 mmol/g, ahead of wine (0.029 ± 0.004 mmol/g) and raw juice (0.023 ± 0.004 mmol/g). Clarified juice reached 0.016 ± 0.002 mmol/g and pasteurized juice 0.012 ± 0.004 mmol/g.

At 15 mg/mL for the juices and 6.25 mg/mL for vinegar and wine, wine showed the highest adsorbed amount of the study, at 0.084 ± 0.009 mmol/g, followed by vinegar (0.067 ± 0.018 mmol/g). Raw juice reached 0.043 ± 0.007 mmol/g, clarified juice 0.035 ± 0.007 mmol/g and pasteurized juice 0.031 ± 0.011 mmol/g. All four products thus adsorbed more at this concentration than at the two higher levels.

Table 3: Amount of glucose adsorbed (mmol/g)

Sample	C1	C2	C3
Pasteurized juice	0.015 ± 0.001^{b}	0.012 ± 0.004^{c}	0.031 ± 0.011^{b}
Vinegar	0.034 ± 0.000^{a}	0.069 ± 0.004^{a}	0.067 ± 0.018^{a}
Raw juice	0.013 ± 0.003^{b}	0.023 ± 0.004^{b}	0.043 ± 0.007^{b}
Clarified juice	0.012 ± 0.000^{b}	0.016 ± 0.002^{c}	0.035 ± 0.007^{b}
Wine	0.006 ± 0.002^{c}	0.029 ± 0.004^{b}	0.084 ± 0.009^{a}

Values are expressed as mean ± standard deviation (n = 3). Means bearing different letters (a, b, c) indicate a statistically significant difference ($p < 0.05$), whereas those sharing at least one letter do not differ significantly ($p \geq 0.05$); letters are assigned independently within each column. C1: juices 60 mg/mL, vinegar and wine 25 mg/mL. C2: juices 30 mg/mL, vinegar and wine 12.5 mg/mL. C3: juices 15 mg/mL, vinegar and wine 6.25 mg/mL.

4 DISCUSSION

The three approaches adopted do not explore the same mechanism: the first describes the content of each cashew apple-derived product, the second measures enzymatic blockade, and the third measures physical retention of glucose. Reading them together makes it possible to identify which process preserves, degrades or shifts the antidiabetic potential of the cashew apple.

Polyphenols are present in all five derived products, as are sterols and polyterpenes: the phenolic background of the cashew apple therefore withstands the processing methods tested. The flavonols of this fruit are predominantly glycosylated and water-soluble (de Brito *et al.*, 2007). Raw juice concentrates six of the eight families, compared with four for clarified juice and pasteurized juice. Catechic tannins and saponins disappear with clarification, an operation intended to remove astringency (Couri *et al*., 2002; Abreu et al., 2005; Dedehou *et al*., 2015). Pasteurized juice shows the same qualitative profile as clarified juice: pasteurization does not remove any additional family, even though it may quantitatively degrade the phenols (Chen *et al*., 2013). Vinegar and wine show the appearance of alkaloids and quinones, absent from the three juices. Vinegar, however, retains flavonoids and gallic tannins, whereas wine is devoid of them: the conduct of fermentation therefore strongly influences the final profile (Ousaaid *et al*., 2022).

All five derived products inhibit α-amylase in a dose-dependent manner. Acarbose remains superior in percentage terms, but at markedly lower concentrations; only the IC_{50} allows a genuine comparison. Vinegar ranks first with 0.88 ± 0.49 mg/mL, a value not statistically different from that of acarbose (0.21 ± 0.08 mg/mL); it still retains 78.57% inhibition at 4.17 mg/mL. Three factors could explain this performance: organic acids (Noh *et al*., 2020), the lowering of pH below 4 (Santos *et al*., 2019), and the retained phenolic component. Wine (8.99 ± 3.73 mg/mL) and clarified juice (11.87 ± 2.69 mg/mL) form an intermediate group, ahead of raw juice (19.07 ± 3.52 mg/mL) and pasteurized juice (19.97 ± 2.38 mg/mL), which are not statistically distinguishable from one another. Clarification removes catechic tannins but retains gallic tannins, whose galloyl groups are determinant for enzyme binding (Sun *et al*., 2019), which may explain why clarified juice is more active than raw juice; pasteurization adds thermal degradation of the phenols; without being sufficient to significantly separate pasteurized juice from raw juice on this parameter. Wine inhibits strongly at 16.67 mg/mL but drops markedly at 4.17 mg/mL, and the absence of flavonoids and gallic tannins suggests the loss of high-affinity compounds.

All derived products lower the free glucose concentration of the supernatant. Expressed relative to the dry mass used, adsorption capacity places vinegar first at the two highest concentrations (0.034 then 0.069 mmol/g), wine taking first place at the lowest concentration (0.084 mmol/g). The three juices remain behind at all levels, which is primarily due to their dry matter load: at equal volume, a juice involves two to four times more matter than a vinegar, so that the bound amount is spread over a larger mass. All four products adsorb more when diluted, a pattern already described for fibrous matrices: Chau *et al.* (2003) showed that adsorption capacity depends mainly on the structure of the material and its accessible surface area, rather than on the amount of matter involved, while Ahmed *et al.* (2011) observed that glucose binding followed neither fiber content nor the dose tested. Aggregation of the matrix under concentrated conditions, likely to reduce the exchange surface, offers a plausible explanation for this behavior. In any case, the two assays measure distinct mechanisms: inhibition relies on a polyphenol-enzyme bond (Sun *et al*., 2019), adsorption on physical retention by the polysaccharide network (Ou *et al*., 2001). Pasteurized juice illustrates this dissociation well, since it ranks last for inhibition while outperforming the other juices for adsorption at the highest concentration. Heat indeed solubilizes the pectins of the cell walls (Sila *et al*., 2009), which could shift part of the potential from one mechanism to the other. These values reflect a capacity measured under the experimental conditions of the assay, not an actual reduction in intestinal glucose absorption.

Vinegar proves to be the best-performing derived product on both assays: it shows the lowest IC_{50}, statistically equivalent to that of acarbose, as well as the highest adsorption capacity at the two higher concentrations. Double fermentation provides it with a preserved phenolic material, new compounds and additional acidity. The juices lose inhibitory potential as processing intensifies, but pasteurization redistributes this potential rather than cancelling it out. Under the experimental conditions studied, a matrix acting through two pathways is thus of greater interest than a matrix mobilizing only one.

5 CONCLUSION

The present study aimed to identify, among raw juice, clarified juice, pasteurized juice, wine and vinegar derived from the cashew apple, the processing method(s) most favorable to preserving or expressing the antidiabetic potential of this biomass. Phytochemical screening shows that raw juice concentrates six families of secondary metabolites. Clarification and pasteurization remove two of them, mainly catechic tannins and saponins. Vinegar appears the most diverse, with seven families, while wine groups five. Regarding α-amylase inhibition, vinegar shows the lowest IC_{50} (0.88 ± 0.49 mg/mL), statistically equivalent to that of acarbose; wine follows, then the three juices, whose activity decreases progressively from raw juice to pasteurized juice. Regarding glucose adsorption, expressed relative to the dry mass used, vinegar ranks first at the two highest concentrations, wine taking first place at the lowest concentration; the three juices remain behind, owing to their high dry matter load. Overall, vinegar stands out as the best-performing product on both mechanisms simultaneously. The juices lose inhibitory power as processing intensifies, while pasteurization redistributes part of this potential toward adsorption. Under the experimental conditions studied, double fermentation thus appears to be the processing method most favorable to the expression of the antidiabetic potential of the cashew apple. Further work will help clarify the scope of these observations: identification of individual compounds by HPLC-DAD or LC-MS/MS, kinetic study of the type of inhibition, and subsequent evaluation in a digestive model and *in vivo*.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflictas of interest regarding this manuscript.

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