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Stabilization of cashew apple juice with chitosan: clarification and microbiological quality

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ABSTRACT

Cashew apple processing could provide a solution to post-harvest losses in cashew production areas. This study aims to add value to the cashew fruit processing chain to improve producers' incomes. The cashew apples were harvested in February 2023 and their juices produced stabilized using chitosan at different concentrations (0%, 0.3%, 0.5% and 1%) then stored at room temperature for 16 days. The physicochemical, phytochemical, and microbiological parameters of the juices were determined using reference methods. The results show an acidic pH for both stabilized (4.56 to 4.66) and unstabilized (4.65 to 4.92) juices. Titratable acidity ranged from 27.46 to 71.66 mg H+ eq/100 g. Brix values ranged from 6.00 to 12.00, but no variation was observed for dry matter content. Vitamin C levels ranged from 19.80 mg/mL to 53.46 mg/mL. Phytochemically, total polyphenols were measured between 127.83 and 331.68 mg GAE/100 mL, and tannins between 26.34 and 81.53 ± 1.50 mg EAT/100 mL. Microbiological analysis revealed a total inhibition of mesophilic aerobic germs (134.104 at 0 CFU/mL), fungi (96.104 at 0 CFU/mL) and coliforms (9.104 at 0 CFU/mL) initially present in the juices without chitosan; whereas an increase in their number was observed in the control juices. Chitosan thus appears to be an effective agent for stabilizing and preserving cashew apple juice at room temperature for more than two weeks.

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INTRODUCTION

Today, like coffee and cocoa, cashew is a cash crop in the northern regions of Côte d'Ivoire. The fruit of this tree consists of the apple, also called the peduncle or false fruit, and a nut. The latter is the main commercial product of the cashew tree (Julia, 2019). Côte d'Ivoire has even become, in the space of a few years, the world's leading producer of cashew nuts (around 20% of world production), in an agricultural sector which contributes 22% of GDP (Juneja *et al.*, 2026). Thus, cashews are one of the main cash crops in the economy and agricultural activities in the savannah regions of the country. This activity concerns more than 400,000 farms and has an impact on the living conditions of more than 1.5 million people (Kouamé, 2021).

The cashew apple, on the other hand, weighs 9 to 10 times the mass of the nut, and is generally discarded at nut harvest sites (Soro *et al.*, 2012) and undervalued due to its astringent taste and certain taboos (Ouattara *et al.*, 2016). In Côte d'Ivoire and some other African countries, consuming cashew apples with milk is considered toxic (Assunção and Mercadante, 2003), despite their very high nutritional value. Also, nearly 30 million tons of cashew apples are produced each year, but they remain largely unknown to the general population (FAO, 2007). They yield juice with 85% carbohydrates, and which has an acidic taste with a significant number of bioactive compounds (6 times richer than sweet orange) (Ouattara *et al.*, 2016). Cashew apple juice makes a significant contribution to the good nutrition of children and adults, as it contains a wide range of vitamins, polyphenols, sugars, minerals (potassium, magnesium, phosphorus), and organic acids. According to some authors, the juice can contain approximately 48 volatile compounds, predominantly esters (48%) and aldehydes (14%) (Garruti *et al.*, 2003; Kouassi, 2022). It also possesses antioxidant properties (Wharta *et al.*, 2004). This juice is also a powerful remedy against chronic dysentery, sore throat, scurvy, diarrhea, and certain cancers. Studies link these properties of the juice to its high tannin content, which are antimutagenic and antioxidant (Morton, 1961).

However, these phenolic compounds, in addition to their astringent effect, contribute to juice instability by promoting the formation of sediment. To address the organoleptic and microbiological stability issues of cashew apple juice, the use of a clarifying agent and an antimicrobial agent is necessary. According to some authors, the use of chitosan in juice production offers several advantages related to its clarifying, stabilizing, and antimicrobial properties (Le Grill, 2018). Chitosan, extracted from the exoskeletons of crustaceans, is a biopolymer that becomes positively charged when dissolved in a weak acid solution. This cationic charge allows chitosan in solution to react with negatively charged biological compounds (such as lipids or proteins) and bind them firmly via ionic bonds (Cissé, 2012). Chitosan films are biodegradable, clear, strong, and flexible, and they exhibit selective permeability to oxygen and carbon dioxide properties that could protect juice against chemical rancidity. Chitosan shows a strong affinity for phenolic compounds, particularly cinnamic acids (Spagna *et al.*, 1996; Spagna and Pifferi, 2000). Furthermore, treatment with chitosan does not affect biochemical parameters and improves organoleptic properties (flavor, appearance, and color), according to Soto-Paralta and Knorr (1989). Its use in treating grape juice yields a highly significant clarifying effect; moreover, the reduction in turbidity is greater with chitosan than with bentonite or gelatin (Chatterjee *et al.*, 2004). It acts as an effective antibacterial and antifungal agent, inhibiting the growth of various pathogens and reducing the development of infections (Shi *et al.*, 2006).

These findings have sparked significant interest in using chitosan to treat fruit juices and extend their shelf life. This study was initiated with the aim of producing cashew apple juice with high organoleptic and microbiological quality using chitosan as a clarifying agent, thereby adding value to the cashew fruit processing chain and increasing producer incomes.

MATERIALS AND METHODS

Biological material

The material used consists of on the one hand cashew apples, harvested in February 2023 in a field in the town of Korhogo and on the other hand chitosan flakes obtained from the company Vietnam Food (VNF).

Preparation of the chitosan solution

A chitosan stock solution was prepared before starting the various tests. To do this, 1 g of chitosan was dissolved in a mixture of 90 mL of distilled water and 1 mL of acetic acid (Pinotti *et al.*, 1997). The mixture was homogenized using a magnetic stirrer, and 9 mL of distilled water was added to achieve a final chitosan concentration of 1 % (w/v). Continuous magnetic stirring was then maintained for 24 h to ensure complete homogenization.

Cashew apple juice production

Cashew apple juice production began with a pretreatment phase. Apples were sorted to remove damaged or rotten fruits and to separate mature apples from remaining cashew nut residues. Washing and disinfection unit operations were performed in multiple stages. The fruits were first rinsed twice with tap water, followed by disinfection with sodium hypochlorite (0.1° chlorometric). Finally, two subsequent rinses with tap water were carried out to eliminate disinfectant residues. The washed cashew apples were sliced using a sterile knife and then homogenized using a laboratory blender. The resulting pulp was filtered using a 125 µm mesh sieve. Then, the juice was collected in a container and the cakes were discarded as residue. Twelve (12) sterile 500 mL jars were used. Of these, three (3) jars served as a control, each containing cashew apple juice without the addition of chitosan solution. The remaining nine (9) jars, each containing the juice, were divided into three chitosan dosage ranges. Thus, one set of three (3) jars was dedicated to the juice containing 0.3%, 0.5%, and 1% chitosan. For flocculation, the mixture was homogenized for 15 min followed by a 10 min rest before filtration through poplin. The resulting filtrate was stored at room temperature for subsequent analyses.

Analysis of physicochemical parameters

Hydrogen potential (pH)

The pH of the sample was measured using a digital pH meter. Thus, 20 mL of juice were collected in a beaker, then the pH meter reading electrode was immersed in the solution and the pH was read on the pH meter screen after stabilization (Azan-Gnandji and Laurelle, 2009).

Titratable acidity

Titratable acidity is determined by acid-base titration and expressed as the amount of sodium hydroxide required to neutralize the acids in 100 mL of juice. A 10 mL aliquot of juice was taken, and 2 to 3 drops of 1% phenolphthalein were added. The titration was performed by adding sodium hydroxide to the juice until a persistent light pink color appeared, indicating the end of the titration (AFNOR, 1974). The acidity, in mg Eq of acid, was expressed according to equation 1.

$$\text{Titratable Acidity (mg Eq } H^+/100\text{ mL)} = \frac{N(NaOH) \times V(NaOH\ eq) \times 10^3}{V} \quad (1)$$

N (NaOH)= normality of the sodium hydroxide solution= 0.1;

V (NaOH eq) = volume (mL) of the NaOH solution added at the equivalence point;

V= volume of juice collected (mL).

Brix degree

The Brix degree measures the mass of soluble solids contained in 100 g of liquid product (Konfo, 2011). For the measurement, a few drops of each juice were placed on the refractometer prism. The refractometer was then pointed towards a light source, and the reading was taken on the eye scale at the intersection of the light and dark areas and expressed as a percentage.

Dry matter content

The dry matter content of cashew apple juice is the product of drying cashew apple juice under the conditions described by the AFNOR standard (1974). The dry matter content of cashew apple juice was

determined by weighing 5 mL (M1) of cashew apple juice into an aluminum crucible of known mass. The crucible containing the samples was oven-dried at 105 °C until a constant mass (M2) was reached. The crucible containing the dried sample was then removed, cooled in a desiccator, and weighed. The dry matter content was expressed as a percentage of the sample mass according to Equation 2.

$$\text{Dry matter content (\%)} = \frac{(m2 - m0) \times 100}{m1} \quad (2)$$

m0: mass (g) of the empty crucible;
m1: mass (g) of the assembly (crucible + sample) before baking;
m2: mass (g) of the assembly (crucible + sample) after baking.

Vitamin C content

The dosage of vitamin C is based on the oxidation of ascorbic acid to dehydroascorbic acid by titration with iodine solution (Bogdanski, 1958). It is colored in its oxidized form (blue in a basic medium, red in an acidic medium) and colorless in its reduced form.

$$\text{Vit C content } \left(\frac{mg}{mL}\right) = \frac{Ci\ Vi}{Vs} \quad (3)$$

Vit C: vitamin C (mg/mL);
Ci: concentration of the diiodine solution;
Vi: volume of the diiodine solution added;
Vs: volume of the solution withdrawn.

Analysis of phytochemical parameters

Total polyphenols

The method of Wood *et al.* (2002) was used for the determination of total polyphenols. A volume of 2.5 mL of Folin-Ciocalteu reagent diluted one-tenth was added to 30 µL of cashew apple juice. The mixture was kept for 2 minutes in the dark at room temperature, then 2 mL of sodium carbonate solution (75 g.L-1) was added. Then, the mixture was placed for 15 minutes in a water bath at 50 °C and then cooled quickly. Absorbance was measured at 760 nm against a reagent blank. A calibration curve was established using gallic acid at different concentrations (0 mg/mL, 0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, 0.1 mg/mL, and 0.12 mg/mL) under identical analytical conditions to determine the quantity of polyphenols. Analyses were performed in triplicate, and the total polyphenol concentration was expressed as grams of gallic acid equivalent per liter of juice (g Eq Gal.L^{-1} of juice).

Tannins

5 mL of juice was oven-dried at 50 °C, and then 30 mL of 70% (v/v) methanol was added to the dry extract. After 30 minutes of stirring, the mixture was filtered through Whatman No. 1 filter paper. Two further extractions were performed in the same manner on the residue, and the volume of the extract was made up to 100 mL with distilled water. The tannin content was determined using the vanillin method of Bainbridge *et al.* (1996) with some modifications. The principle of the determination is based on the fact that tannic acid, in the presence of the reagent consisting of vanillin (0.1 mg/mL) in an acidic medium (70% (v/v) sulfuric acid), gives a red color with a maximum absorption at 500 nm. In a tube containing 1 mL of juice, 5 mL of vanillin reagent were added and then the whole was incubated for 30 minutes in the dark. Then, the absorbance was read at 500 nm against a reagent blank prepared under the same conditions. A range of standards was prepared, in 98% methanol, from a standard solution of tannic acid at concentrations between 0 and 166 µg/mL for the establishment of the calibration curve. The results were expressed as milligrams of tannic acid equivalent per 100 mL of juice (mg EAT/100 mL of juice).

Enumeration of mesophilic aerobic bacteria (MAB)

The medium used for the enumeration of mesophilic aerobic germs (GAM) is PCA (Plate Count Agar) agar (oxoid LTD, Basingstore, Hamsphire, England). Seeding was done by incorporating 1 mL of the dilutions into sterile Petri dishes. Then, 12 to 15 mL of the PCA agar previously melted, cooled and kept supercooled at 45 °C was poured into the box. The mixture was homogenized by slow stirring and then

left on the bench to cool to room temperature. After solidification, a second layer of 4 to 5 mL of PCA agar was poured over the first layer to prevent the plates from being invaded by certain microorganisms. The inoculated Petri dishes were incubated at 30 °C, and readings were taken every 24 hours up to 72 hours. Colony counts were performed by counting all colonies in Petri dishes containing between 30 and 300 colonies (ISO 4833-1: 2013).

Enumeration of yeast and mold

The medium used for yeast enumeration was Sabouraud Chloramphenicol agar (Fluka, Bochemica 89579, Sigma-Aldrich ChemieGmbH, India). 0.1 mL of the dilutions was applied to the surface of the solidified agar in a Petri dish and then spread. The Petri dishes were incubated at 30 °C for 24 to 72 hours. The colonies appear as downy growths in the case of molds and as whitish, smooth, convex growths in the case of yeasts, with a characteristic bakery odor and a diameter of 0.5 to 2 mm. Colony counts were performed by counting the colonies present in Petri dishes containing 30 to 300 colonies (ISO 21527-1: 2008).

Enumeration of total coliform

The medium used for coliform enumeration was bile lactose agar with crystal violet and neutral red staining. Inoculation was performed by adding 1 mL of the dilutions to sterile Petri dishes. Then, 12 to 15 mL of the medium were poured while still molten at 45 °C into the Petri dishes containing the inoculum, and the mixture was homogenized by gentle stirring. After solidification, a second layer of 4 to 5 mL of the medium was poured over the first layer. Incubation was carried out anaerobically at 30 °C for 48 to 72 hours. The coliforms appeared purplish-red, round, with a diameter of 0.5 mm. All colonies present in Petri dishes containing 15 to 300 colonies were counted (ISO 4832: 2006).

Expression of the microorganism load results

The enumeration results were expressed in CFU/ml (Colony Forming Units per milliliter) using the following formula:

$$N\left(\frac{UFC}{ml}\right) = \frac{\sum C}{(n1 + 0{,}1n2)\, dV} \quad (4)$$

V: Volume of inoculum or juice deposited (0.1 ml or 1 ml);

n1: Number of Petri dishes retained at the 1st dilution;

n2: Number of dishes retained at the 2nd dilution;

d: Dilution retained or factor of the first dilution retained;

N: Number of colonies in CFU per mL of juice;

C: Number of colonies counted on the Petri dishes retained from two successive dilutions.

Statistical analyses

All trials were conducted in triplicate. Means were calculated and presented, followed by their standard deviations, to assess the physicochemical, phytochemical, and microbiological parameters of cashew apple juice. Statistical analysis of the data was performed using Statistical Program for Social Sciences (SPSS version 20.0). A two-way analysis of variance (ANOVA-2) was performed. The factors were chitosan concentration and storage time. The significance level of the statistical test was set at 95% confidence (5% error). The mean parameters were classified using the Student-Newman-Keuls post-ANOVA test.

RESULTS

pH, titratable acidity, and vitamin C of cashew apple juice

Table 1 presents the pH, titratable acidity, and vitamin C values of cashew apple juice at different chitosan concentrations (0%, 0.30%, 0.50%, 1%) over 16 days of storage at room temperature (Day 0, Day 4, Day 8, Day 12, and Day 16).

The measured pH values ranged from 4.56 ± 0.005 to 4.92 ± 0.025. Statistical analysis showed a significant difference ($p < 0.001$) in juice pH according to chitosan concentration and storage duration. Higher pH values were obtained with juice control (0%) for all storage durations except Day 0. With the juice containing chitosan, the pH decreased from 4.66 to

4.62 (0.30%), from 4.66 to 4.58 (0.50%), and from 4.65 to 4.56 (1%); while that of the control juice (0%) increased from 4.65 to 4.92. Over the days of storage, no significant difference in pH was observed on day 0. However, on days 4, 8, 12, and 16, the pH remained significantly higher in the juice without chitosan.

Regarding the titratable acidity values of the juices, a significant decrease was observed in the juices without chitosan from day 0 to day 4, followed by a significant increase from day 8 to day 16. They decreased from 71.66 ± 1.66 to 54.53 ± 0.50 meq H+/100 g and from 54.53 ± 0.92 to 71.66 ± 1.52 meq H+/100 g. For the juices with 0.30% chitosan, the titratable acidity was higher on day 0 (28 meq H+/100 g) and decreased significantly until day 16 (27.46 ± 0.23). With 0.5% and 1% chitosan, the shelf life does not influence the titratable acidity values, which range from 29.26 to 30.66 meq H+/100 g and 29.66 to 30.33 meq H+/100 g respectively.

Table 1. pH, titratable acidity and vitamin C of cashew apple juices during storage as a function of their chitosan concentration

Shelf life	Physicochemical parameters	0 %	0.30 %	0.50 %	1 %
D0	pH	4.65±0.015aD	4.66±0.015aA	4.66±0.002aA	4.65±0.005aA
	Titratable acidity (%)	71.33±1.54aA	28.00±0cA	30.66±1.15bA	30.33±0.57bA
	Vitamin C (mg/mL)	53.46±5.40aA	27.27±0.41cA	29.48±4.03cA	41.36±1.06bA
D4	pH	4.68±0.015aD	4.65±0.005bA	4.62±0.005cB	4.63±0.005cB
	Titratable acidity (%)	54.53±0.50aC	27.93±0.11cA	30.13±0.24bA	30.00±0.00bA
	Vitamin C (mg/mL)	51.92±5.33aA	27.50±1.90bB	27.72±1.32bAB	34.76±3.04bB
D8	pH	4.74±0.011aC	4.63±0.005bB	4.61±0.011cB	4.61±0.010cC
	Titratable acidity (%)	54.53±0.92aC	27.73±0.11cAB	29.86±0.11bA	29.66±0.11bA
	Vitamin C (mg/mL)	50.60±4.03aA	24.64±2.01cBC	26.62±1.00cAB	32.12±2.01cBC
D12	pH	4.87±0.025aB	4.63±0.005bB	4.59±0.011cC	4.57±0.010cD
	Titratable acidity (%)	62.00±1.24aB	27.53±0.11cB	29.53±0.11bA	29.73±0.11bA
	Vitamin C (mg/mL)	47.52±4.72aA	21.56±2.01cBC	25.52±0.76bcAB	29.92±2.74bBC
D16	pH	4.92±0.025aA	4.62±0.005bB	4.58±0.005cC	4.56±0.005cD
	Titratable acidity (%)	71.66±1.52aA	27.46±0.23cB	29.26±0.11bA	29.66±0.11bA
	Vitamin C (mg/mL)	45.54±5.14aA	19.80±1.32cC	23.54±1.90bcB	27.28±1.52bC

Means within the same row or column, each containing the same lowercase letter, do not show a significant difference (p = 0.05). D0: Day 0; D4: Day 4; D8: Day 8; D12: Day 12; D16: Day 16.

The average vitamin C content of cashew apple juice varies significantly at different concentrations. The highest values are observed in juices without chitosan (45.54 to 53.46 mg/mL). Different chitosan concentrations lead to a significant decrease in vitamin C content. However, this decrease is more pronounced at chitosan concentrations of 0.30% and 0.50%. A non-significant decrease in vitamin C content is also observed in the control juices without chitosan, but a significant decrease is observed in the juices containing chitosan over time.

Brix degree and dry matter

Table 2 reports the Brix degree and dry matter values of cashew apple juice, at different concentrations of chitosan (0%, 0.30%, 0.50%, 1%) stored for 16 days at room temperature.

Statistical analysis shows a significant difference (p <0.001) according to storage durations (days 8, 12, and 16) and no significant difference (p>0.001) in the Brix level of the juices according to chitosan concentrations, except on day 0. High Brix levels were obtained with juices containing 0.30%, 0.50%, and 1% chitosan for all storage durations. The Brix level of juices without chitosan decreased significantly from 11.00 (day 0) to 6.00 (day 16); while that of juices containing chitosan remained stable until day 16 (11.90–12.00) with no significant difference.

Regarding dry matter content, it ranged from 9.33 ± 0.98% to 13.66 ± 3.20%. Chitosan concentration and storage time did not have a significant effect on thedry matter content of the juices.

Table 2. Brix degree and dry matter content of cashew apple juice during storage as a function of chitosan concentration

Shelf life	Physicochemical parameters	0%	0.30 %	0.50 %	1 %
D0	Brix degree	11.00±0.87bA	12.00±1.00aA	12.00±0.50aA	12.00±0.87aA
	Dry matter (%)	9.33±0.98aA	10.13±2.24aA	10.20±1.56aA	10.26±1.52aA
D4	Brix degree	10.01±0.01bA	12.00±0.05aA	12.00±0.87aA	12.00±0.50aA
	Dry matter (%)	9.60±2.95aA	10.13±1.60aA	10.46±1.52aA	10.80±1.50aA
D8	Brix degree	8.00±0.50bB	12.00±0.36aA	11.90±0.05aA	11.90±0.05aA
	Dry matter (%)	13.00±3.11aA	10.20±1.80aA	10.86±1.90aA	10.86±1.13aA
D12	Brix degree	7.46±0.42bB	12.00±0.26aA	11.90±0.04aA	11.90±0.06aA
	Dry matter (%)	13.06±1.13aA	10.26±0.75aA	11.00±2.00aA	11.33±1.50aA
D16	Brix degree	6.00±0.70bB	12.00±0.43aA	11.90±0.87aA	11.90±0.08aA
	Dry matter (%)	13.66±3.20aA	10.46±0.92aA	11.33±1.50aA	11.46±0.75aA

Means within the same row or column. each containing the same lowercase letter. do not show a significant difference (p = 0.05). D0: Day 0; D4: Day 4; D8: Day 8; D12: Day 12; D16: Day 16.

Table 3. Total polyphenol and tannin contents of cashew apple juice during storage as a function of chitosan concentration

Shelf life	Phytochemical parameters	0%	0.30%	0.50%	1%
D0	Polyphenols (mg EqAcGal/100 mL)	218.61±5.27aC	158.22±15.48bC	149.29±8.43bC	127.83±8.13cE
	Tannins (mg EAT /100 mL)	72.44±4.63aB	36.30±1.60cA	46.04±3.30bA	46.81±2.43bA
D4	Polyphenols (mg EqAcGal/100 mL)	215.48±5.54aC	158.22±2.94bC	155.34±4.56cC	210.68±2.60aD
	Tannins (mg EAT /100 mL)	76.79±1.12aAB	35.85±2.76cA	41.59±2.08bB	42.34±1.86bB
D8	Polyphenols (mg EqAcGal/100 mL)	231.84±6.70aC	194.90±8.71bB	207.42±25.27aB	220.54±6.09aC
	Tannins (mg EAT /100 mL)	77.51±0.61aAB	33.25±4.05cAB	39.45±1.58bB	40.71±1.45bB
D12	Polyphenols (mg EqAcGal/100 mL)	259.68±4.77aB	228.29±12.69bA	249.04±0.82aA	246.20±5.53aB
	Tannins (mg EAT /100 mL)	79.13±0.68aA	27.93±3.14dB	34.16±2.39cC	38.73±1.10bBC
D16	Polyphenols (mg EqAcGal/100 mL)	331.68±12.43aA	241.52±4.18cA	268.65±13.15bA	267.57±8.27bA
	Tannins (mg EAT /100 mL)	81.53±1.50aA	26.34±4.02cB	30.10±0.73bcD	34.52±3.8bC

Means within the same row or column. each containing the same lowercase letter. do not show a significant difference (p = 0.05). D0: Day 0; D4: Day 4; D8: Day 8; D12: Day 12; D16: Day 16.

Total polyphenol and tannin contents of cashew apple juice as a function of shelf life and chitosan concentration

The total polyphenol and tannin contents of cashew apple juice (0%. 0.30%. 0.50%. 1% chitosan) stored for 16 days at room temperature are recorded in Table 3. Polyphenol and tannin content ranged from 127.83 ± 8.13 to 331.68 ± 12.43 mg Eq Ac Gal/100 mL and from 26.35 to 81.53 mg EAT/100 mL. respectively (day 16). The highest polyphenol and tannin content was observed in juices without chitosan throughout the entire storage period. A significant increase in polyphenol and tannin content was observed over time in all juices. This increase also increased with increasing chitosan concentration.

Microbiological parameters of cashew apple juice as a function of its chitosan concentration and shelf life

These results relate to the number of mesophilic aerobic bacteria, yeasts and molds and coliforms in cashew apple juices at different concentrations of chitosan (0%. 0.30%. 0.50% and 1%) during their storage.

Fig. 1. Mesophilic aerobic bacteria in cashew apple juice during storage as a function of chitosan concentration (CFU/mL)

Means within the same row or column. each containing the same lowercase letter. do not show a significant difference (p = 0.05). J0: Day 0; J4: Day 4; J8: Day 8; J12: Day 12; J16: Day 16.

The number of mesophilic aerobic bacteria (MAG) varies statistically depending on the level of chitosan used in

the juices and the shelf life (p<0.05) (Fig. 1). The chitosan-free juice contains 250.104 CFU/mL of G MAG on Do. But this number increases significantly (p <0.001) to reach a figure of 348.104 CFU/mL after 16 days of juice storage. On the other hand, with the introduction of chitosan (0.30%. 0.50% and 1%) the MAG numbers are established between 92.104 and 96.104 CFU/mL on Do. Then decrease statistically during storage (p <0.001) to result in a total absence of colonies on D16 (Fig. 1).

In the control juices (0%), the numbers of yeasts and molds increased significantly (p <0.001) from 141.104 to 393.104 CFU/mL (from Do to D16). Whereas with the addition of chitosan in the juice, the number of yeasts and molds are 126.104 to 134.104 CFU/mL on day 0. Then drops significantly during storage (p <0.001) until there is a total absence of colonies on day 16. The number of yeasts and molds is significantly higher in juices without chitosan than in juices with chitosan (Fig. 2).

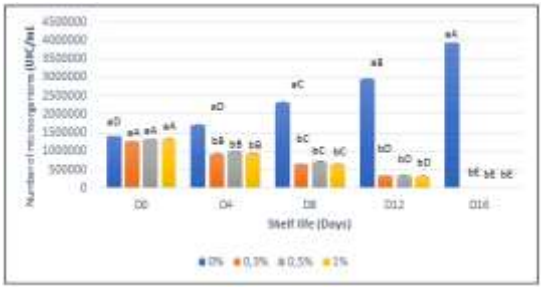

Fig. 2. Yeasts and molds in cashew apple juice during storage and as a function of chitosan concentration (CFU/mL)

Means within the same row or column. Each containing the same lowercase letter. do not show a significant difference (p = 0.05). Do: Day 0; D4: Day 4; D8: Day 8; D12: Day 12; D16: Day 16.

The number of coliforms present in cashew apple juice varies statistically according to the chitosan concentration and storage time (p<0.05) (Fig. 3).

In juices without chitosan (0%), there is a significant increase (p<0.001) in coliforms from 48 x 10^4 to 86 x 10^4 CFU/mL (from day 0 to day 16). However, in juices with chitosan (0.30%. 0.50%. and 1%), there is a progressive decrease in coliforms. The numbers range from 7 x 10^4 to 9 x 10^4 CFU/mL on day 0 and decrease significantly during storage (p<0.001) until a complete absence of coliforms was observed on day 16. At each storage time, the number of coliforms is significantly higher (p<0.001) in the control juice (0%) than in the juices with chitosan.

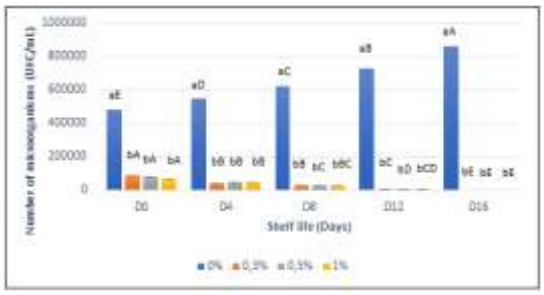

Fig. 3. Coliforms in cashew apple juice during storage as a function of chitosan concentration (CFU/mL)

Means within the same row or column, each containing the same lowercase letter. Do not show a significant difference (p = 0.05). Jo: Day 0; J4: Day 4; J8: Day 8; J12: Day 12; J16: Day 16.

DISCUSSION

This study aimed to evaluate the effect of chitosan on selected physicochemical, phytochemical, and microbiological parameters of cashew apple juice. During storage, a significant increase in pH (4.65–4.92) was observed in the control juices, whereas a significant decrease in pH (4.66–4.56) was observed in the juices containing chitosan. The juices remained acidic both in the absence and presence of chitosan. A chitosan concentration of 0.50% had the optimal effect on pH, regardless of shelf life. This difference in pH between the control juices and those containing chitosan could be due to the acidic nature of chitosan, the pH of which varies between 4 and 4.6 (Cissé, 2012). This pH reduction phenomenon has been observed by other authors in studies on the clarification of cashew apple juice using gelatin, cashew gum, and gum arabic. Indeed, Kouamé work (2021) revealed that adding gum arabic and gelatin to cashew apple juice led to decreases in pH from 4.64 to 4.57 and from 4.64 to 4.55, respectively. Similarly, Kouassi *et al.* (2017) and Touré (2015) recorded decreases in pH from 4.31 to 4.27 and from 4.74 to 4.00, respectively.

Concerning titratable acidity, the control juices had a titratable acidity (54.53–71.66 meqH+/100 mL) approximately twice as high as that of the juices containing chitosan (27.48–30.66 meqH+/100 mL), regardless of chitosan concentration and shelf life. The greatest reduction in the titratable acidity of cashew apple juice was observed with 0.30% chitosan, in contrast to the greatest reduction in pH, which was observed with 0.50% chitosan. This difference between titratable acidity and pH could be related to the fact that these parameters do not express the same quantities. Indeed, according to Konan *et al.* (2013), pH reflects the presence of free H+ or H3O+ in the medium, whereas titratable acidity takes into account not only H3O+ but also the presence of CO2 and free organic acids (amino acids, fatty acids, and other acids).

These results are consistent with those obtained by Kouassi *et al.* (2017) and Soro (2012) in their studies on the clarification of cashew apple juice using gelatin and membrane processes, respectively. These authors observed decreases in titratable acidity from 31 meqH+/100 mL to 25 meqH+/100 mL (Kouassi *et al.*, 2017) and from 2.61 g of acid malate/kg to 2.57 g of acid malate/kg (Soro, 2012).

The soluble solids content of the control juice decreased significantly from 11 to 6 °Brix, whereas that of the juices containing chitosan remained high and stable (11.90–12 °Brix), regardless of storage time or chitosan concentration.

However, the soluble solids content of the juice did not vary as a function of the amount of chitosan added. This could be due to the existence of a chitosan solubilization threshold (0.30%) in cashew apple juice. Some authors found higher soluble matter levels of more than 14% in their studies on the clarification of cashew apple juice (Touré, 2015; Soro *et al.*, 2017). However, the levels of soluble matter in juices containing chitosan comply with the standard (greater than or equal to 10 °B) required by the General Standard for Fruit Juices and Nectars (CODEX STAN 247. 2005).

The addition of chitosan had no effect on the dry matter content (9.33–13.66%) of cashew apple juice, regardless of chitosan concentration or storage time. These results differ from those of Kouassi *et al.* (2017), who showed that gelatin influenced the dry matter content of clarified juice, with levels decreasing from 8.03 g/100 g to 7.33 g/100 g.

The vitamin C contents of chitosan-free juices varied little during storage (45.54–53.46 mg/mL). However, a significant decrease in vitamin C content was observed in juices containing chitosan. The values decreased from 27.27 to 19.80 mg/mL (0.30%), from 29.46 to 23.54 mg/mL (0.50%), and from 41.36 to 27.28 mg/mL (1%) during storage. Furthermore, the vitamin C contents of the control juices were higher than those of the treated juices, regardless of shelf life. The values were approximately twice those of juices containing 0.30% and 0.50% chitosan. Thus, chitosan may have a degrading effect on vitamin C in the juice. This degradation of vitamin C during the clarification of cashew apple juice has been observed by several authors, regardless of the clarifying agent used. Indeed, Kouassi *et al.* (2017), Kouamé (2021) and Kouassi (2022) showed significant decreases in vitamin C content, respectively, from 175.255 to 147.551 mg/100 mL (gelatin), from 526.31 to 315.78 mg/100 mL and from 526.31 to 398.47 mg/100 mL (gelatin and chitosan), and from 350.70 to 210.75 mg/mL (Citrus limon essential oil).

The polyphenol content ranged from 127.83 to 331.68 mg Eq Ac Gal/100 mL, and a significant increase was observed in the juices regardless of chitosan concentration and shelf life. The highest levels were obtained in juices without chitosan, regardless of shelf life, except for juices containing 0.50% (D8, D12) and 1% (D4, D8, D12). However, the loss of polyphenols in the juice may be acceptable given the reduction in tannin content. Soro work (2012) also showed that the polyphenol content of cashew apple juice increased significantly from 2401 to 2926 mg/kg after juice clarification. The tannin content of juices containing chitosan (0.30%, 0.50%, 1%) significantly decreased from 46.81 to 26.34 mg EAT/100 mL,

whereas that of the control juice (0%) significantly increased from 72.44 to 81.53 mg EAT/100 mL.

The greatest reduction in the tannin content of cashew apple juice was observed with 0.30% chitosan, regardless of shelf life. This decrease could reflect the formation of tannin/chitosan complexes that were subsequently eliminated during filtration. Furthermore, this reduction in tannin content in juices containing chitosan could reduce their astringency.

This reduction in tannin content was also observed by Kouassi *et al.* (2017) and Soro (2012), who obtained complete removal of tannins from cashew apple juice after clarification using gelatin and tangential microfiltration, respectively. Kouamé (2021) also showed a significant decrease in the tannin content of cashew apple juices after clarification, from 6.44 to 6.05% (gelatin) and from 6.44 to 5.97% (chitosan).

During storage, a significant increase in the number of mesophilic aerobic bacteria was observed in the control juice (250 x 10^4 to 348 x 10^4 CFU/mL), whereas the number recorded in the juice containing chitosan decreased from 96 x 10^4 CFU/mL to zero. The lowest chitosan concentration with an optimal effect on mesophilic aerobic bacteria was 0.30%. Cashew apple juices were of satisfactory microbiological quality (GAM), regardless of chitosan concentration, according to the microbiological quality standard published by the Codex Alimentarius (3 x 10^5 CFU/mL) (Codex Alimentarius, 2001). A reduction in the number of GAMs was demonstrated by Kouassi (2022) after clarifying the juice with Citrus limon essential oil.

This author observed GAM counts ranging from 50 to 280 x 10^5 CFU/mL (juice without essential oil) and from 39 x 10^5 CFU/mL to 0 CFU/mL (juice with essential oil). Regarding yeasts and molds, complete elimination was also observed in juices containing chitosan (from 134 x 10^4 CFU/mL to 0 CFU/mL) during storage, compared with an increase in their numbers in juices without chitosan (from 141 x 10^4 to 393 x 10^4 CFU/mL). At 0.30%, chitosan had an optimal reducing effect on yeasts and molds in cashew apple juice. Based on the Codex Alimentarius (2000) microbiological quality standard for fungi (1 x 10^5 CFU/mL), cashew apple juices were of satisfactory microbiological quality after 12 days of storage. Kouassi (2022) work on the clarification of cashew apple juice also indicated the complete elimination of yeasts and molds in juices containing Citrus limon essential oil. For total coliforms, a significant increase in their number was observed in the control juice (0% chitosan), rising from 48 x 10^4 to 86 x 10^4 CFU/mL, whereas a decrease to complete elimination (9 x 10^4 to 0 CFU/mL) was observed in the juices containing chitosan.

The presence of coliforms in cashew apple juice could indicate ineffective aseptic treatment of the cashew apples with chlorinated water, a lack of pasteurization, or contamination during juice production. Kouassi work (2022) showed a complete absence of coliforms during juice storage, particularly in juices with or without essential oil. This difference could be attributed to the author's practice of pasteurizing the juices before storage. These results indicate the antimicrobial activity of chitosan against GAMs, coliforms, yeasts, and molds in cashew apple juice during storage. This inhibitory effect on the microorganisms responsible for juice spoilage could be explained by both environmental conditions and the intrinsic antimicrobial activity of chitosan. Indeed, the acidic pH of the juice does not promote bacterial growth, according to Dossou *et al.* (2019) and Talasila *et al.* (2012). These authors indicated that most bacteria do not grow at low pH levels. Furthermore, chitosan may possess antimicrobial properties (Cissé, 2012). Chitosan could therefore be used to preserve cashew apple juice at an optimal concentration of 0.30%.

CONCLUSION

The objective of this study was to evaluate the effect of chitosan on selected physicochemical, phytochemical, and microbiological parameters of

cashew apple juice stored at room temperature. The results showed that chitosan reduced acidity, vitamin C and polyphenol contents, as well as the numbers of GAM, yeasts, molds, and total coliforms, at incorporation rates ranging from 0.30% to 1%. In contrast, a preservative effect was observed on the soluble solids (°Brix) and dry matter contents of the juices at chitosan concentrations of 0.30% and above. These effects of chitosan improved juice quality in terms of Brix level and microbiological standards. Therefore, the use of chitosan for preserving cashew apple juice could represent an innovative and potentially useful alternative to chemical additives and intensive heat treatments, which may negatively affect the nutritional value of these products.

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