ISSN: 2347-467X, Vol. 12, No. (3) 2024, Pg. 1199-1209

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Detannying in Cashew Apple Juice (*Anacardium Occidentale* L.) By Chemical Agents

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Abstract

Vietnam is the leading exporter of cashew nuts globally; however, the cashew apple, constituting 75% of the total harvest from cashew trees, remains underexploited despite its rich nutrient content. The main reason is that cashew apple contains high amount of tannin which causes astringency. Consequently, this undesired compound poses a significant challenge for food product development. Thus, tannin removal is critical in cashew apple juice processing to improve sensorial characteristic and produce a nutritious beverage from cashew apple. Gelatin, modified cassava starch (MCS), and polyvinyl polypyrolidone (PVPP) are the most recommended agents for tannin reduction. This study evaluated the efficacy of these clarifying agents based on agent concentration, processing time, and juice pH. Results indicated that gelatin at 2 g/L for 30 mins at pH 3 reduced tannin by 34.31%; MCS removed 41.34% of tannins at 3 g/L for 30 mins at pH 3; and PVPP achieved tannin removal of 32.45% at 4 g/L for 30 mins at pH 4. All chemical treatments decreased nutritional values, including vitamins C, sugars, polyphenols, and antioxidant activity. While MCS demonstrated the highest tannin removal efficiency, the sensory quality of the treated juice requires further enhancement due to low pH.

Article History

Received: 28 December 2023

Accepted: 01 September 2024

Keywords

Cashew Apple;
Gelatin;
Modified Cassava Starch;
Pvpptannin Removal.

Introduction

Cashew (*Anacardium occidetale* L.) is a perennial plant belonging to the mango family (Anacardiaceae). The cashew tree, a tree native to Brazil, is a good product worldwide. Vietnam was the first country to export cashew nuts worldwide. It is estimated

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Doi: https://dx.doi.org/10.12944/CRNFSJ.12.3.17

that with the production output of cashew nuts (400 thousand tons per year), the main by-product which is cashew apple (2 million tons per year) is mostly discarded.

Cashew apples are rich in nutrients and antioxidants. Vitamin C contained in cashew apples is six times higher than in citrus fruits.[1] Cashew apple juice contains healthy ingredients, including Vitamin B1, B2 and B3; calcium; iron; and beta-carotene.[2] Cashew apples are rich in tannins (191 mgTAE/100mL),[3] which are astringent compounds. Besides, protein is obstructed the assimilation in the body by the tannin compound, ensuing nutritional deficiency.[1] Therefore, the removal of tannins is essential for producing beverage products from this fruit.

Tannins are phenolic compounds and classified into two main groups: condensed tannins and hydrolyzable tannins. Flavonoid polymers are the main tannins present in plants.[4] There are three subclasses of hydrolyzable tannins: simple gallic acid derivatives, gallotannins, and ellagitannins. Ellagitannins are found in high concentrations in some plants of high economic value as cashew nuts, pistachios, mangos, hazelnuts, persimmons, chestnuts, walnuts, and guavas. The biological activity of hydrolyzable compounds exhibits anti-cancer, anti-angiogenic, antioxidant, anti-inflammatory, and anti-ulcerative activities. Hydrolyzable tannins are natural antioxidants.[5] Condensed tannins are flavonoid phenols which are polymers of catechins. Condensed tannins are formed from flavan-3-ol or 3,4-diol units. Under the action of acids or enzymes, it is not hydrolyzed but forms red tannins or phlobaphene.[4]

Polyphenols, tannins (0.35%), and unknown slime (3%) present in the waxy layer of the skin cause astringency.[6] In cashew apple juice, the major polyphenolic compound and other phenolic acids (gallic acid, protocatechuic acid, conjugate cinnamic acid, and free cinnamic acid), known as leucodelphinidin.[7] The tannin content of fruit juice can vary between studies due to factors such as cultivar, extraction from different parts of the fruit, stage of maturity (harvest period), processing conditions, the composition of the juice, viscosity, and presence of other flavors affect the compounds in the juice.[8] Astringency is mainly caused by tannin, a water-soluble polyphenolic compound found in cashew apple juice.[9] When consumed, tannins react with salivary proteins, such as histatins and proline-rich proteins, to form protein-tannin complexes. These complexes interact with glycoproteins to develop a sensation of friction and astringency in the mouth. Tannins act as antinutritional factors by forming complex reactions with other digestive enzymes, starches, and proteins. Furthermore, these tannin-protein complexes cause turbidity in the juice.[8]

Tannins are considered nutritionally undesirable. Therefore, the removal of tannins from cashew apple juice to produce a beverage from this fruit is indispensable. Currently, there are three methods to reduce tannin in cashew apple juice: physical, chemical, and enzymatic method.

The physical methods of microfiltration (MF) and ultrafiltration (UF) can also reduce tannins from apple juice by 40.34% and 47.90%, respectively. Apple juice pretreated with 0.2% PVPP and filtered with MF and UF reduced tannin by 58-59%, more efficiently than using bentonite, activated carbon, pectinase, and amylase in combination with membrane filtration.[10] Treating cashew apple juice with hot water for 20 min reduced tannins by up to 92.6%.[11]

Adding tannase to myrobalan plum juice reduced the tannin concentration by 45.2% to 73.6%, thereby reducing the astringency of the juice.[12] The use of tannases from *A. foetidus* and *Rhizopus oryzae* reduced the tannin content in pomegranate juice by 25% after 120 min. The application of tannase with gelatin solution reduced the tannin content by 49% after 90 min.[13]

In terms of chemical method, the reduction of tannins using proteins (gelatin and casein), polysaccharides (starch, chitosan, sago, rice gruel, cassava starch), or synthetic polymers (polyvinyl polypyrrolidone) starch has been studied. Sago, which is a cheaper substitute than PVPP, was reported that it had a higher percentage of the recovery.[14] Using 0.375g/L gelatin removed 69.35% of hydrolyzable tannins in pomegranate juice, which was not significantly different from using casein at the same concentration (70.24% removal).[14] However, albumin at 0.125 g/L provided a lower efficacy in reducing tannin (58.99%), similar to that clarified by 0.375 g/ L xanthan gum (57.38% removal) and 0.5 g/L chitosan (60.54% removal).[15] The removal of tannins by protein-based substances depends on the pH of fruit juice. Proteins have a positive charge at a pH below the isoelectric point (pI) when they can bind to the negative charge of phenol compounds. Gelatin, albumin, and casein had pI of 7.0, 4.7, and 4.7, respectively, while pomegranate juice had a pH level of 3.15.[8]

Polyvinyl polypyrolidone (PVPP) is a cross-linked polyvinylpyrrolidone (PVP). Tannins and low-molecular-weight phenolic compounds are highly absorbed by PVP. Tannins are removed by PVPP by chelation mechanism. PVPP is widely used in the beverage industry because it is insoluble in water. Using PVPP with a concentration of 2 - 4 g/L can remove 31.86% tannins in cashew apple juice.[16]

By using polysaccharides, tannins can be removed from fruit juice. Before being added to the juice, starch is gelatinized or completely dissolved in warm water. Using tapioca starch (21% amylose) reduced the tannin content by 34.2%. Rice starch (24% amylose) reduced tannin content by 42.14%. The amylose-to-amylopectin ratio determines the efficiency of starch. Amylose created a more hydrophobic core for monomeric phenols, while amylopectin captured polymeric tannins without undergoing a chemical reaction.[17]

The objective of this study is to evaluate the efficacy of gelatin, modified cassava starch (MCS), and polyvinyl polypyrolidone (PVPP) as clarifying agents for tannin removal in cashew apple juice, based on varying concentrations, processing times, and pH. Additionally, the study aims to assess the impact of these treatments on the nutritional values and to identify potential improvements in the sensory quality of the treated juice. For that purpose, this study was carried out by using three clarifying agents, gelatin, MCS, and PVPP, at concentrations of 2, 3, and 4 g/L. The sample processing time were 15 min, 30 min, 60 min, 90 min, and 24 h. The juice pH was changed to 3, 4, and 5.

Materials and Methods

Extraction of Cashew Apple Juice

Fresh cashew apples, from Binh Phuoc province, Vietnam, were refrigerated and transported to the laboratory of the Department of Food Technology, Hanoi University of Science and Technology, within 24–36 h.

The cashews were seeded and washed. Cashew apples were pretreated with 0.01% enzyme preparation Pectinex Ultra SP-L in 2 h to increase juice yield. Then, the juice was collected by juicer model Sharp KS-888, followed by centrifuge and filtering to obtain a clear juice and stored at -20°C for further analysis. The nutritional and bioactive composition of collected juice were determined in the our previous study.[18]

Detanninfication Treatments

A quantity of PVPP was added directly to the juice19, whereas MCS was dissolved in water, followed by gelatinization at 90°C before being mixed with the juice sample.[20] Regarding gelatin, it was allowed to react with juice after being added into water and incubated in a water bath (50°C) for 2 h.[21] Three variables were studied, including agent's concentration, reaction time and pH of juice, to select the most effective condition in removal tannin of cashew apple juice. The variable levels described in table 1.

After reaction with clarifying agents, the precipitation was eliminated by centrifuge and filtered through filter paper for further analysis.

Table 1. Variable levels of chemical agents

No	Agent	Concentration	Exposure time	pH
1	MCS	2, 3, 4 (g/L)	15, 30, 60, 90 minutes, 24 hours	3, 4, 5
2	PVPP	2, 3, 4 (g/L)	15, 30, 60, 90 minutes, 24 hours	3, 4, 5
3	Gelatin	2, 3, 4 (g/L)	15, 30, 60, 90 minutes, 24 hours	3, 4, 5

Determination of Detannying Efficiency

The tannin-removing capacity was determined by quantifying the tannin content of fresh and treated cashew apple juice.[1]

Detannying efficiency (%): [(T0-T)/T0]×100

Where T0 is the tannin content of sample before tannin removal, and T is that of treated sample with clarifying agents.

Physiochemical Analysis

pH – meter (Lab 845, SI Analytics) was used in pH determination and total soluble solids was quantified by refractometer.

The AOAC 942.15 method[22] was adopted in order to measure the total titratable acitity.

The concentration of reducing sugar in the sample was determined by employing Miller's method.[23] Diluted sample (0.5mL) was reacted with DNS reagent (3mL) in 100^{o}C for 5 minutes. The cooled mixture then was measured the absorbance at 540nm and using a calibration curve created by glucose (0-0.5g/L). The same method was used for determination of total sugar after hydrolyzing it in 2% HCl, 1 hour.

The vitamin C was determined following the iodine titration method.[24] By using 1% starch indicator to determine the endpoint of titration, the iodine solution and sample was titrated against 1% ascorbic acid solution. Vitamin C = (V1/V2)×1000 (mg/100mL). (V2 is titre from the titration of the standard ascorbic acid solution and V1 is that of the sample solution). Total polyphenols and tannins was estimated using the Folin-Ciocalteu method.[3] For polyphenols, the gallic acid was used in the calibration curve and the absorbance was measured at 765nm, while tannic acid and absorbance at 700nm was employed in determination of tannin.

The scavenging activity or trapping percentage P was evaluated by the DPPH method.[25] Briefly, the sample was mixed with DPPH and reacted in darkness in 1 hour before measuring the absorbance at 517 nm. P = (Ab0-Ab1)/Ab0×100. (Ab0 is absorbance of the white, Ab1 is absorbance of the sample). From P value obtained and vitamin C calibration curve, the antioxidant activity (IC50) value was estimated at at y=50%.

The method of estimating pectin as calcium pectate[26] was employed to determined pectin content in the sample.

Statistical Analysis

The results of triplicate determinations were expressed as mean values and standard deviations. The mean differences were determined using analysis of variance (ANOVA) and t-test at a significance level of $P \leq 0.05$. The results of triplicate determinations were expressed as mean values and standard deviation.

Results and Discussion

The reduction of tannins in cashew apple juice is influenced by various factors, including pH, reacting time, and the concentration of tannin removal agents. Tannins are polyphenolic compounds that are sensitive to pH changes. At different pH levels, the solubility and reactivity of tannins can vary significantly. Besides, the duration of contact between the tannin removal agent and the juice affects the extent of interaction and binding. Sufficient reacting time ensures that the agents have enough opportunity to interact with and bind to the tannins, forming complexes that can be removed. Moreover, for effective tannin removal, different agents have optimal concentration beyond which additional amounts do not significantly increase tannin removal and may lead to diminishing returns or unwanted side effects. Thus, these parameters have been researched to investigate their impacts on tannin removal efficiency.[1,17,27,28]

Effect of Gelatin on Tannin Reduction of Cashew Apple Juice

Figure 1A shows that the tannin content decreased inversely with gelatin concentration. In this experiment, the treatment time was fixed at 30 min, the solution pH = 4 at a gelatin concentration of 2 g/l, and the greatest ability to reduce tannin content was 21.7%. According to the research conducted by Dagadkhair and others,[1] when using a gelatin concentration of 1-4 %, the tannin content can be reduced to 33.54%. The research of Lam Quoc and co-authors concluded that using gelatin at 2.7-3.0 g/L removed 24.00% tannins.[29] Meanwhile, 2 g/L gelatin helped to reduce 26.37% tannins in the work of Talasila and colleagues.[16] In another study, gelatin was mixed with cashew apple juice at a rate of 0.67 g/100 mL for 15 minutes to create the ideal conditions for tannin precipitation, which eliminated 79% of the juice's tannins.[27] Over-optimal dosages of removal agents can lead to self-interaction, where agents start binding to each other instead of to the tannins. This self-binding can reduce the number of active sites available for tannin binding. Besides, an excess of gelatin molecules can lead to the formation of larger gelatin-tannin complexes. These large complexes can encapsulate other juice components, including desirable flavors and nutrients, leading to a decrease in overall juice quality. Meanwhile, a low gelatin concentration of gelatin provides fewer binding sites for tannins. This might allows for a more selective removal of tannins while minimizing the capture of other juice components.

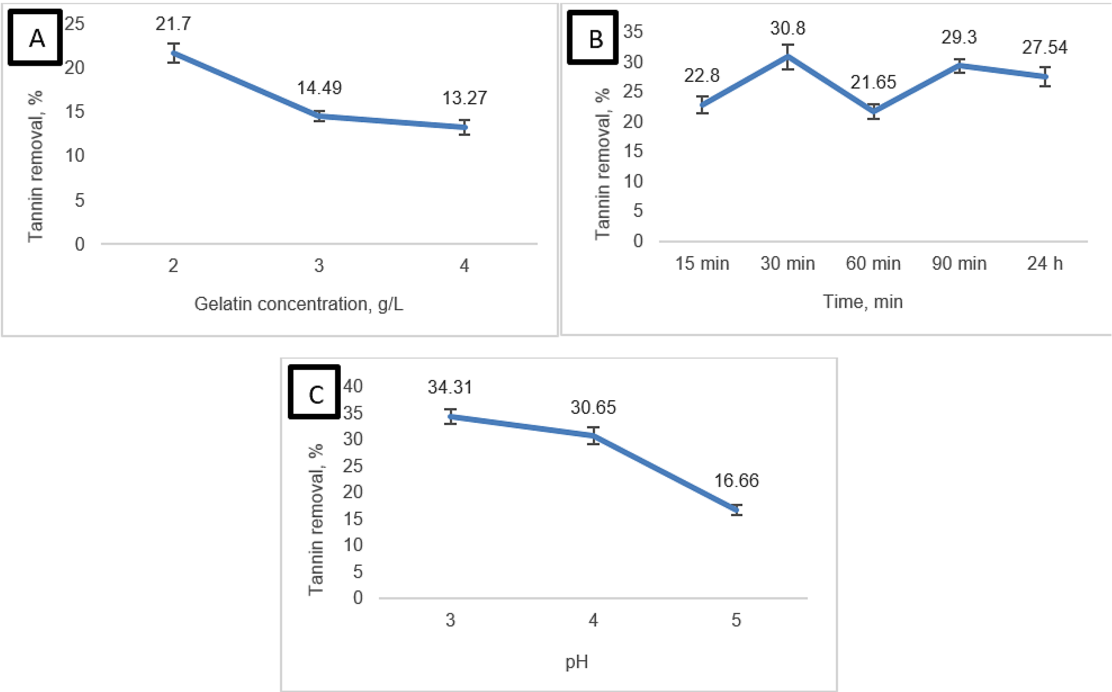

Fig.1. Effect of gelatin on tannin reduction of cashew apple juice when change variable A = gelatin concentration, B = gelatin reaction time, C = pH of juice

Figure 1B shows that when fixed at a gelatin concentration of 2 g/L selected from the above experiment, a pH of 4 at 30 min gave the highest ability to reduce tannin at 30.8%. When the time was increased to more than 30 mins, the effect of reducing the tannin content was decreased. While sufficient reacting time is crucial, excessively long reacting times can lead to re-dissolution of gelatin-tannin complexes, reducing the overall efficacy of tannin removal and potentially leading to loss of other desirable components in the juice.[29] Therefore, in this experiment, 30 min was the appropriate time to treat the gelatin solution.

Figure 1C shows that when the gelatin concentration is fixed at 2 g/L and the solution treatment time is 30 minutes, the ability to reduce the tannin content tends to decrease when increasing the solution pH at points.[3, 4, 5]

The use of protein-based substances to remove tannins from juice depends on the pH of the juice because gelatin carries a positive charge (+) when the pH is less than the isoelectric point of gelatin (pI = 7), while tannin compounds carry a negative charge.[8] Therefore, the ability to precipitate gelatin tannins at pH 3 was optimal.

Effect of Modified Cassava Starch (MCS) on Tannin Reduction of Cashew Apple Juice

There is an optimal concentration of modified cassava starch where tannin binding is maximized. At this concentration, the starch molecules have sufficient active sites to interact with tannins, forming complexes that can be precipitated out of the juice. If the starch concentration is too low, there are not enough binding sites available, resulting in incomplete tannin removal. Meanwhile, if the concentration is too high, it can lead to aggregation of starch molecules, reducing their surface area and effective interaction with tannins. Additionally, excess starch may not contribute to further tannin removal and can affect the juice's texture and clarity.

Figure 2A shows that the MCS concentration had a significant effect on tannin removal. When the MCS concentration increased from 2 g/L to 3 g/L, the percentage of tannin removal increased from 26.78% to 28.57%. When the concentration was increased to 4 g/L, the tannin removal percentage decreased to 27.52%. By adding sago or starch at a rate of 2 g/L higher than that of the same substance at a rate of 4 g/L, tannins in cashew apple juice may be further eliminated.[17]

In the MCS treatment time range of 15 min to 24 h, the tannin removal percentage varied greatly. At 30 min, the highest tannin removal percentage was 36.86%. Given enough time, the reaction can reach equilibrium, maximizing the interaction between starch and tannins, leading to optimal removal. After 30 min, the processing time gradually increased, and the ability to remove tannins decreased. At 24 h, the tannin removal ability decreased to 13.54%. Thus, adequate reacting time allows starch molecules to interact thoroughly with tannins, forming complexes that precipitate out of the solution.

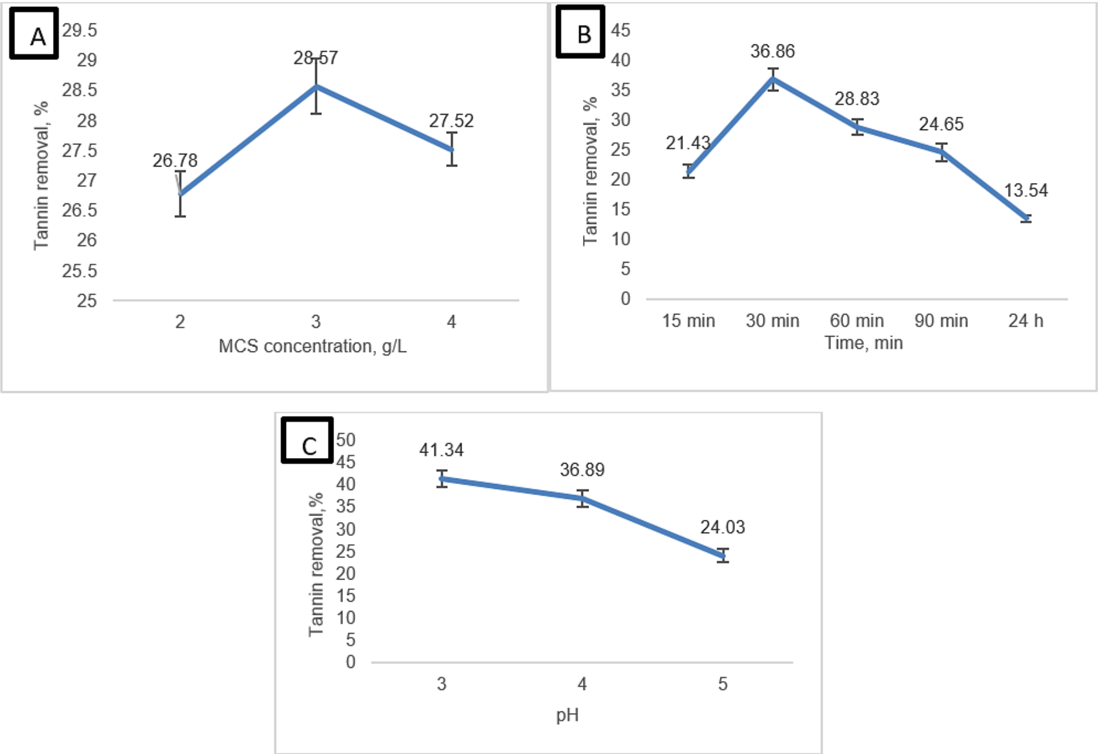

Fig.2. Effect of MCS on tannin reduction of cashew apple juice when change variable A = MCS concentration, B = MCS reaction time, C = pH of juice

Figure 2C shows that pH has a significant impact on tannin content, with tannin removal decreasing with increasing pH. The binding affinity between tannins and starch can vary with pH. At optimal pH levels, starch molecules can more effectively interact with tannins, facilitating their precipitation and removal from the juice. At pH 3, the tannin removal percentage was approximately 44.34%. Tannins are polyphenolic compounds whose solubility and reactivity are influenced by pH. In more acidic conditions (lower pH), tannins tend to be more soluble, which can enhance their interaction with starch. When the pH was increased to 4, the tannin removal percentage decreased by approximately 36.89%. When the pH reached 5, the percentage of tannin removal decreased by approximately 24.03%. Thus, the results showed that pH adjustment can significantly affect the tannin content of cashew fruit juice after treatment with MCS. Specifically, the ability to remove tannins decreased as pH increased from 3 to 5.

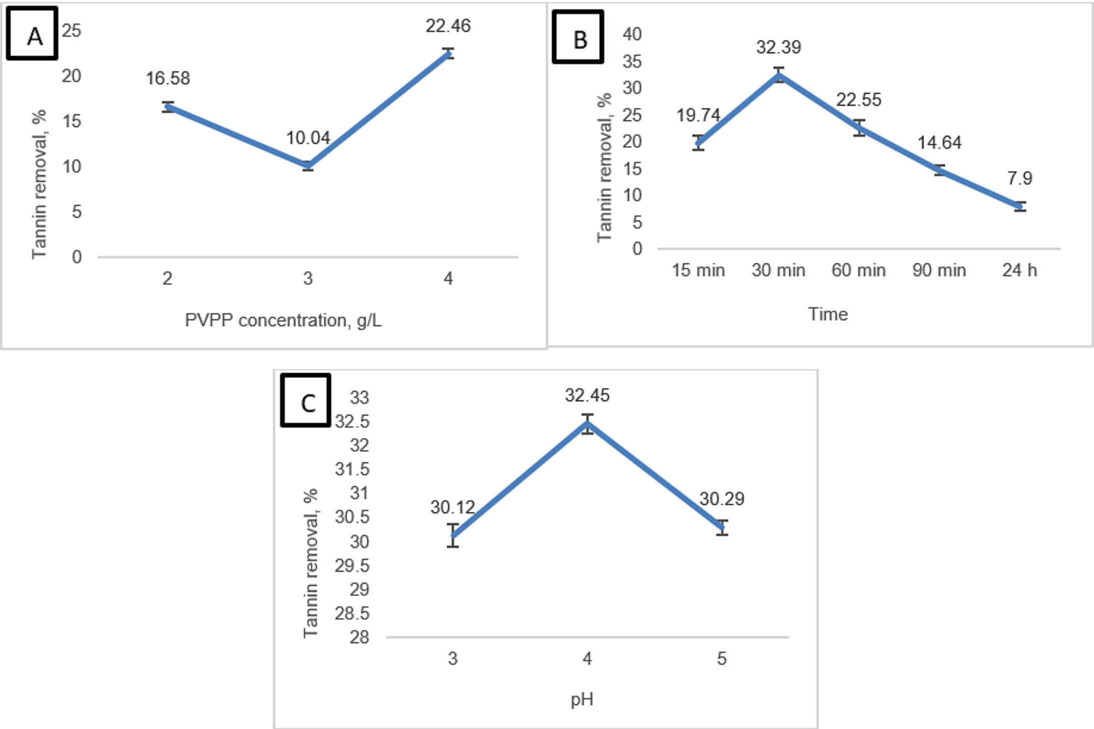

Fig.3. Effect of PVPP on tannin reduction of cashew apple juice when change variable A = PVPP concentration, B = MCS reaction time, C = pH of juice

PVPP is a synthetic polymer known for its high affinity for polyphenolic compounds like tannins. Figure 3A shows that PVPP precipitates tannin better at a higher concentration of 4 g/L, whereas at 3 g/L, the ability to remove tannin was only 10.04%. This pattern of change was similar to that reported by Dagadkhair and others[1]: at concentrations of 3 and 4 g/L, the reductions in tannins reached 30.17% and 34.3%, respectively. Whereas, 4g/L PVP reduced 31.86% tannins in the study of Talasila and team.[16]

Figure 3B shows that the best tannin removal was achieved when treated with PVPP for 30 min (32.39%). The optimal mixing time where the PVPP has enough contact with the tannins to effectively adsorb them. During this period, the PVPP will effectively bind with the tannins, leading to a more efficient removal process. The longer the time, the lower the efficiency decreases, this has also been demonstrated in the study of Vardi and others[30] when pomegranate juice was treated with PVPP at a concentration of 2 g/L, stored for 5ºC, for about 16 h, the total phenolic compounds reduction efficiency is only 18.09%. Extending the mixing time further may not provide additional benefits and could even lead to other issues. Over-mixing might result in the formation of a gel-like precipitate that can be difficult to remove.

Figure 3C shows that the pH range from 3 to 5 slightly influence the tannin removal capacity of PVPP. In acidic conditions (low pH), tannins tend to be less dissociated, which can enhance their interaction with PVPP. The reduced ionization can lead to more effective adsorption of tannins onto PVPP. As the pH moves towards neutral or alkaline conditions, tannins can become more dissociated and may interact differently with PVPP. This can sometimes lead to reduced effectiveness in tannin removal because the PVPP might have a lower affinity for the more dissociated forms of tannins.

Nutritional Composition and Biological Value of Cashew Apple Juice After Removal of Tannin

Table 2 summarizes the nutritional and biological values of cashew apples after tannin removal. Using MCS at 3g/L, 30 min and pH 3 was considered to be most effective in removing tannin, as after treating cashew apples with MCS, the remaining tannin content was 72.00±2.77 mg TAE/100mL of tannin. Whereas treating cashew apples with gelatin and PVPP kept 80.63±2.82 and 82.91±2.40 mg TAE/100mL of tannin, respectively.

In general, most of other nutritional values in three samples decreased after detannying compared to the values of enzymatic treated cashew apple juice as reported in one under-review manuscript of the authors. However there is a minor difference between the nutritional values of sample treated by MCS compared by gelatin and PVPP. Regarding total and reducing sugars, there were no significant differences between these juice samples. Meanwhile, polyphenols remained highest in MCS. In terms of vitamin C, antioxidant acitivity, the remaining quantities recorded in MCS were slightly lower than PVPP. However, the tannin-removal efficiency by MCS was significantly higher than PVPP, thus it was considered as the most effective method. Besides, the total titratable acidity recorded in MCS was lowest in comparison with gelatin and PVPP, but organic acids are easily added in later production process if needed.

Besides, when compare the amount of some remaining nutrients by using MCS as agent with other chemical approach in detannying in cashew apple juice, total sugars were reduced to 4.55g/100mL which were similar as the amount recorded when dried okra pod powder (DOP) was employed (4.8g/100mL).[31] In terms of vitamin C, MCS resulted in reduction to 35.2mg/100mL which was higher than when DOP was used (8.1mg/100mL).[31]

Table 2. Nutritional composition and biological value of cashew apple juice after tannin removal

Component	Unit	Removal tannin by		
		Gelatin	MCS	PVPP
Total of titratable acidity	gMAE/L	3.21±0.12^{a}	1.11±0.05^{b}	2.39±0.1^{c}
Total soluble solids (Bx)	^{o}Brix	1.8±0.01^{a}	1.0±0.03^{b}	1.8±0.01^{a}
Total sugar content	g/100mL	5.11±0.21^{a}	5.03±0.18^{a}	4.96±0.18^{a}
Reducing sugar content	g/100mL	4.68±0.19^{a}	4.55±0.17^{a}	4.78±0.14^{a}
Total polyphenol content	mgGAE/100mL	26.56±0.92^{a}	27.59±1.14^{a}	20.8±0.74^{b}
Vitamin C content	mg/100mL	21.12±0.8^{a}	35.2±1.65^{b}	38.72±1.12^{c}
Total tannin content	mgTAE/100mL	80.63±2.82^{a}	72.00±2.77^{b}	82.91±2.40^{a}
Scavenging activity	%	5.87±0.21^{a}	9.67±0.45^{b}	10.68±0.32^{c}
Antioxidant activity (IC_{50})	mg AAE/100mL	0.050±0.002^{a}	0.092+0.030^{b}	0.103±0.005^{c}

Values are demonstrated in means ± standard deviation (n=3). Mean followed by difference lowercase superscripts in a row are significantly different at $p < 0.05$.

GAE: Gallic Acid Equivalents, TA: Tannic acid, AAE: Ascorbic Acid Equivalents.

On top of that, the MCS sample was at pH 3 which created unfavorable taste when consumes the sample. By this reason, further research need to be conducted to overcome this disadvantage in order to successfully utilise cashew apple in food production. Regarding cost when using chemcial agents in tannin migration, low cost food grade materials have been studied but resulting in lower efficiency (defatted soymeal was 34.3%, dried potato powder 28.6% and bajara flour 24.0%)[1]. For DOP, the energy requirement needed to take into consideration since it is proportional to the particle size.[31]

Conclusion

This study investigated tannin reduction in cashew apples harvested from Binh Phuoc, Vietnam, with the goal of mitigating their astringency and enhancing their use in fruit juice processing. Three clarifying agents (MCS, gelatin, and PVPP) were evaluated for their effectiveness in tannin removal and their impact on nutritional and antioxidant properties. The results showed that MCS was the most effective, reducing tannins by 41.34% while maintaining nutritional and biological values with minimal changes when used at 3 g/L for 30 minutes at pH 3. The astringency of the cashew apple juice has been reduced due to the low remaining tannin content, and thus it can be applied in beverage production.

Acknowledgement

The authors gratefully acknowledge Hanoi University of Science and Technology (HUST) and Viet Nam National University of Agriculture (VNUA) for facilitating this study and the research team for their support.

Funding Sources

This research is funded by Hanoi University of Science and Technology (HUST) under project number T2022-PC-098 and ĐTĐL.CN - 57/22.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Author Contributions

- **Nguyen Thi Hanh:** Data Collection, Data Curation, Validation, Writing (Original Draft, Review, Editing)
- **Nguyen Thi Trang:** Data Collection, Data Curation
- **Nguyen Thi Minh Anh:** Data Collection
- **Nguyen Thi Huong:** Data Collection
- **Nguyen Van Hung:** Supervision
- **Vu Thu Trang:** Project Administration
- **Nguyen Thi Thuy:** Funding Acquisition
- **Nguyen Viet Long:** Funding Acquisition

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