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Effect of Pretreatment Methods on Juice Extraction Yield and Nutritional Composition of Cashew Apple (*Anacardium Occidentale* L.) Harvested from Binh Phuoc (Vietnam)

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Abstract

Nowadays, cashew trees are mainly cultivated to collect cashew nuts, leading to the large amounts of cashew apples (known as the by-product of the cashew industry) being eliminated due to astringent taste caused by tannin. This is the reason why although cashew apples contain many nutritious compounds as vitamin C, polyphenols, sugars, antioxidants are still underutilized in manufacturing beverages. Hence, aiming to evaluate the potential of cashew in terms of effect of pretreatment methods on extraction yield and nutritional values of extracted cashew apple juice, the organic cashew apples collected in Binh Phuoc, Vietnam were examined. The impact of 11 pretreatment methods including blanching cashew apple in different media and incubating in pectinase on extraction yield, tannin and other nutritional values were evaluated. Using enzyme Pectinex Ultra SP-L 0.01% in 2 hours gave extraction yield of 83.89±0.4% which was highest compared to other examined pretreatment methods in the study. Enzymatic pretreatment resulted in 36.5% reduction in tannin content and other nutritious values changed slightly. The obtained results emphasis the potential of cashew apples as a raw material for nutritional beverage production in hope of reducing the quantity of discarded cashew apples. Consequently, this study developed methods in order to add economic value to cashew apple and decrease the negative impacts of the cashew industry to the environment.

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Introduction

In Vietnam, a country in Asia, the cashew industry has seen significant growth. Vietnam has become largest cashew nut producer[1] and the main exporter worldwide.[2] Due to the growth of the cashew nut industry, the amount of a by-product called cashew apples has been increasing. Cashew apples (*Anacardium occidentale* L.) are attached to the nuts, accounting for 90% weight of the whole fruit.[3] It is estimated that 1 ton of cashew nuts produced leads to 10-15 tons of cashew apples formed.[4]

Cashew apples have been proven to contain many nutrient compounds which are beneficial for human health, such as sugars, amino acids, vitamins, fibers, and minerals.[3,5,6] The content of vitamin C being 4-6 times higher than that in oranges and mangoes[7–9] and the antioxidant activity in different varieties of cashew apples[3,10] suggest that they are a good source of nutrients. The previous study of authors[11] on cashew apples harvested in Binh Phuoc (Vietnam) has also shown the same results.

Although cashew apples are abundant and nutritious, it is not utilized effectively and wisely in industrial scale in the country. This fruit is mostly considered as an agricultural waste[12,13] and a large quantity is left to be spoiled.[14] The main reason is that cashew apples contain tannin, a substance causes astringency[15,16] and turbidity in juice,[17] thus making the fruits unfavorable for consumption. Tannins range between 0.01 to 197 mg/100 mL in cashew apples depending on the processing method and other variants.[18,19] Besides, the inadequate knowledge about potential uses[6] accompanying with the lack of available processing and post-harvest techniques[13] contribute to said underutilization.

There are several studies on processing technology that explore and increase the economic value of cashew apple. This by-product has been used as a snack or fermented into alcoholic drinks[20,21] and bioethanol.[22] Additionally, cashew apples have been involved in the production of not only ice cream, juice, and other delights, but also enzymes such as tannase or pectin esterase.[23]

There have also been certain studies on post-harvest processing technology aiming to at the shelf life extension of cashew apples, such as using chemical preservatives,[24–26] thermal treatment,[25,27] or combination of heat and additives.[28] A saline solution at 1% was introduced as a blanching agent to help reduce tannin content and preserve vitamin C in cashew apples.[29] The study suggested that the pretreatment method of cashew apples with saline solution resulted in a high tannin reduction rate (45%).

Different studies on juice extraction methods was conducted. Introducing fruit into hot water before extracting was studied on banana and showed the ability to increase juice yield.[30] Besides, hot water extraction had been conducted on roselle and the significant rise in juice content was obtained comparing hot water and cold water blending or screw press.[31] Adding enzyme in juice recovery process is another promising method. Study on palm showed the efficiency of enzyme in juice obtained regarding both quality and content.[32] Using a combination of enzyme, resulted in higher juice quantity in kiwifruits.[33] However, there is a limitation in the number of research on evaluating the effect of processing method on cashew apple juice extraction yield and nutrition.

This study performed on organic cashew apples collected in Binh Phuoc (Vietnam) and aimed to develop cashew apples into a nutritious beverage in order to eliminate food waste and add value to a promising byproduct of the cashew industry.[11] pretreatment methods were evaluated based on juice extraction yield, tannin reduction and changes in nutritional values.

Materials and Methods

Raw Materials

Cashew trees (*Anacardium occidentale*) - BP18 were grown on infertile basalt red soil with tropical wet and savanna climate in Binh Phuoc, Vietnam. 200 kg (equivalent of approximately 2500 apples) ripe organic cashew apples (10.2±0.4 ^{o}Bx) were selected in the harvest season in March 2023, transported to the laboratory in 24-36 hours, and preserved by dried ice during transportation.

All chemicals used were of analytical grade. Pretreatment agents include citric acid, chemical preparation (50% Na2S2O5, 45% NaHSO3, 2% NaCl, 3% acid citric), and enzyme preparation Pectinex Ultra SP-L.

Processing of Cashew Apple

Cashew apples were sorted and cleaned before undergoing 4 methods of pretreatment.

Group 1, consisting 3 coded samples (M11, M12, M13), were blanched by water in either 1, 3, 5 minutes, respectively. In group 2, the three samples M21, M22, M23 were blanched by water with chemical preparation being added at 0.1% in 1, 3, and 5 minutes. The third group consists of three samples M31, M32, M33 that were blanched by water added citric acid 0.3% in 1, 3, and 5 minutes. Group 4 has two samples M41, M42 that were added with enzyme preparation Pectinex Ultra SP-L 0.01% in 1 and 2 hours. All samples then were pressed using a screw presser Sharp KS-888 to separate the juice and bagasse. The experiment was performed in triplicate.

Table 1: Pretreatment methods

Group	Sample	Pretreatment agent	Time
	M0	Untreated	
1	M11	Blanching by water (90-95 °C)	1 minute
	M12		3 minutes
	M13		5 minutes
2	M21	Blanching by chemical preparation 0.1% (90-95 °C)	1 minute
	M22		3 minutes
	M23		5 minutes
3	M31	Blanching by citric acid 0.3% (90-95 °C)	1 minute
	M32		3 minutes
	M33		5 minutes
4	M41	Adding pectinex ultra SP-L 0.01%	1 hour
	M42		2 hours

After pressing, cashew apple juice and cashew apple bagasse were separated, and the juice extraction yield was determined. All samples were preserved at -20 °C for further analysis.

Determination of juice extraction yield

Extraction yield (%) = m1/m2×100%

Where m1 is the weight of obtained juice and m2 is weight of cashew apple.

Determination Nutritional Values

Titratable acidity, pH and °Brix - total soluble solids

The AOAC 942.15 method[34] was employed in quantifying the titratable acidity. 20 mL distilled water was added to 5 mL of sample. NaOH solution at 0.1N was used with 3-4 drops of phenolphthalein as the indicator for titration of each 25 mL of sample. The end-point was when pink color appeared and persisted for 30 seconds. The pH value was measured by pH meter (Model SI Analytics Lab Meter 845). Determine the total soluble solids by the portable refractometer. These tests were performed in triplicate.

Reducing Sugars and Total Sugars

The reducing sugars were quantified by Miller's method.[35] This colorimetric assay involves the oxidation of the aldehyde functional group in reducing sugars to the corresponding acid while DNS is simultaneously reduced. After completion of the reaction, an intense red-brown color appeared, which was measured by spectrophotometry at 540 nm. The reaction mixture was 0.5 mL sample and 3 mL of DNS. It was then boiled (100 °C - 5 minutes) before stopping the reaction in an icy water bath (10 minutes). The absorbance was measured by a UV–vis spectrophotometer (Model Apel PD-3000UV) at 540 nm. Glucose (0.1–1 g/L) was used in the standard curve.

Regarding the total sugars, the sample is hydrolyzed by HCl 2% at 100 °C - 45 minutes to yield reducing sugars, followed by neutralization by NaOH 10%

before conducting Miller's method. Experiment was conducted in triplicate.

Total Polyphenols and Tannin

Total polyphenols was quantified according to the modified version of the Folin-Ciocalteu method.[18] 0.2 mL of diluted cashew apple juice was accurately transferred to a test tube and 1 mL of Folin-Ciocalteu's phenol reagent was added into the solution. 0.8 mL of 7.5% (w/v) Na2CO3 was added after 3 minutes of incubation, following by 1 hour reaction in the dark. The standard curve was gallic acid (25 mg/L - 125 mg/L) at 765 nm.

Using the same method in determination tannin, however the wavelength used for tannins was 700 nm, and tannic acid as standard (20 mg/L -100 mg/L). Experiment was conducted in triplicate.

Vitamin C

The iodine titration method was applied.[36] Standardize the iodine solution (dissolving 5.00 g KI, 0.268 g KIO_3 in 200 mL of distilled water before adding 30 mL of 3M H_2SO_4, then making up the volume to 500 mL) by titrating it against 5 mL of 1% ascorbic acid solution with 1% starch indicator. The blue starch-iodine color appeared indicates the equivalent point. The experiment conducted in the same way with 5 mL of the samples.

Ascorbic acid = V1/V2 ×1000 (mg/100 mL), where V1 is titre (mL) from the titration of the sample solution and V2 is that of standard ascorbic acid solution. Experiment was conducted in triplicate.

Pectin Content

Pectin content was determined by estimating of pectin as calcium pectate.[37] 20 mL of sample was mixed with 100 mL NaOH 0.1N and then allowing this mixture to react in 7 h. Then, add 50 mL CH_3COOH 0.1N. After 5 minutes, 50 mL of $CaCl_2$ 1N was added and reaction took place in 1 hour, followed by boiling the mixture in 5 minutes and then filtrating by dried filter paper. Rinsing the precipitate on filter paper by hot distilled water and stopping when no chloride ions remained (using AgNO3 1%). Drying the filter paper at 105 °C until its weight unchanged.

Pectin = (m×0.92)/V×1000, g/L, where m is the weight of dried precipitate and V is the sample's volume. Experiment was conducted in triplicate.

Scavenging and Antioxidant Activity

The DPPH method[38] was employed to determine the scavenging activity. The mixture of DPPH solution and the sample was reacted 1h in the darkness and then at 517 nm, the absorbance was recorded. The trapping percentage was determined: P = (AbW-AbS)/AbW ×100. AbW is absorbance of the blank, AbS is that of the sample. Calculate the antioxidant activity (IC_{50}) from P and vitamin C standard curve at y=50%. Experiment was conducted in triplicate.

Statistical Analysis

The experiment results from triplicate assay were expressed mean±SD. One-way ANOVA and t-test at p-value < 5% was performed by MS Excel was used in statistical analysis.

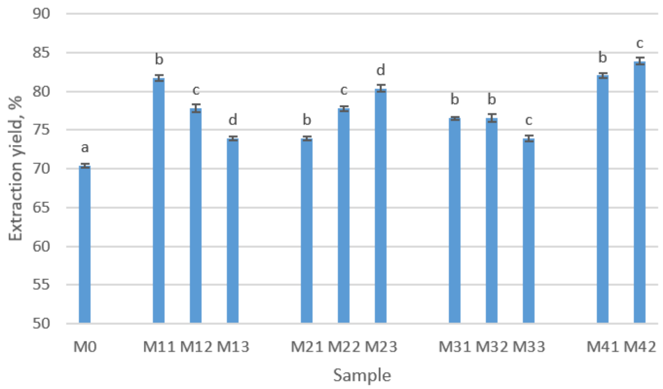

Fig.1: Effect of pretreatment method on juice extraction yield of cashew apple juice Values are mean ± SD of 3 replicate (n=3), followed by different lowercase superscripts in a group are significantly different at p<0.05

Results and Discussion

Effect of Pretreatment Methods on Juice Extraction Yield

The juice extraction yield of cashew apple after pretreatment is presented in Figure 1. The result for group 1 (M11 to M13) shows that the apples blanched at 1 minute show the highest yield at 81.68% and the yield decreases when the blanching duration increases. Boiling water helps remove microorganisms on the surface of the fruit and softens the flesh of the fruit, thus creating favorable conditions for the juice collection.[39,40] The blanching time increased but the yield decreased because the over hydrolyzation can reduce the porosity of cashew apple, thus making the juice extraction process more challenging when increase blanching time. Hot water extraction is a method that has been applied in juice production due to its ability to increase juice yield. Banana juice recovery increased 9% by using hot water.[41]

In terms of using chemical preparation 0.1% as a blanching agent (group 2, M21 to M23), the yield of extraction cashew apples increased with time and at 5 minutes it reached the highest value at 80.38%. By adding the chemical mixture containing sulfites used for the purpose of inactivation polyphenol oxidase, color change is prevented.[42] Under heat treatment in acidic medium, protopectin is hydrolyzed into soluble pectin.[43] Chemical mixture contains 45% $NaHSO_3$ which then is dissociated into Na^+ and HSO_3^- ions in water, thus creating acidic blanching medium. As a result, this medium fostered hydrolyzation and after 5 minutes of heat treatment with chemical preparation, the extraction yield reached its highest value in this group. In addition, the added mixture has an anti-mold effect since it contains sulfites, which inhibiting the activity of microorganisms.[44]

For group 3 (M31 to M33 samples) when using citric acid at 0.3% as blanching agent, the yield is highest at 3 minutes of blanching at 76.49%. Acid can hydrolyze starch[45] and pectin, other high-molecular substances presented in the fruit.[46,47] Therefore, by adding citric acid at 0.3% into the blanching medium, the juice extraction process from apples could be easier. However, because of the aforementioned reason, over hydrolyzation can reduce the porosity of cashew apple, resulting in the juice extraction yield being reduced after 3 minutes.

The fourth pretreatment method is enzyme preparation mixed into cut cashew apples at 0.01% (M41 and M42). Under enzymatic treatment, the pectin is degraded and as a result, its water holding ability was decreased. Consequently, free water in the cashew apple is released, thus there was an increase in the juice extraction yield.[48,49] The pectin content in raw cashew apple was 1.26 g/100 mL in the author's previous work,[11] and it reduced to 0.71 g/100 mL after enzymatic treatment in 2 hours. From the graph, there is a slight increase in the yield to 83.89% from 1 hour to 2 hours in incubation time. Therefore, adding 0.01% pectinase in 2 hours helped to increase the juice extraction yield of cashew apples. The efficiency of enzymatic extraction by Pectinex Ultra-SPL has been reported in the research on banana juice.[50] This study showed that notably higher juice content (60.4%) was extracted after incubating 2 hours with enzyme compared to mechanical method (54.1%). When combine these two methods: pretreatment by enzyme and then performing mechanical extraction, the significant increase in juice yield was obtain.[51] In kiwifruits, the juice yield increased by 23.02% when using a mixture of enzymes including pectinase.[33] Optimum palm juice recovery was obtained at 87.9±0.66% when using pectinase and cellulase.[32] The data obtained once confirms that enzymatic extraction is a superior method over thermal and mechanical methods.[51]

Nutritive Composition of Cashew Apples after Pretreatment

The effectiveness of the pretreatment method was evaluated based on the efficiency of the juice yield (>80%) and the decrease in total tannin content after pretreatment. Table 2 summarizes the results of fruit yield and the nutritional values of cashew apples after the three pretreatment methods that were considered the most effective.

From the result obtained, pretreating cashew apples with pectinase 2 hours (M42) before pressing gave the highest amounts of fruit yield and tannin reduction. Therefore, the most effective pretreatment method is adding enzyme in 2 hours (M42). The juice extraction yield reached 83.89% and total tannin decreased by 36.5% compared to raw cashew apples (193.29±7.65 mgTA/100 mL) in the authors' previous publication.[11] The result is obtained by enzyme action on pectin, thus enhancing juice purity and decreasing juice viscosity, increasing juice yield.[52,53] The second most effective pretreatment method is blanching cashew apples in a chemical preparation for 5 minutes (M23). This pretreatment method gave 80.38% of juice yield, along with the total tannin content reduced by 19.23%. The third pretreatment method chosen was blanching cashew apples in boiling water for 1 minute (M11). The recovery efficiency of this method reached 81.68%, and tannin content decreased by 11.04%. High tannin content in juice negatively affects sensorial quality,[54] thus removal tannin should be take into consideration.

In general, the values of nutritional values changed slightly than those in the raw material that was reported in our published study.[11] Most of the cashew apple's nutritional compounds after treatment reduced by a minor amount. Regarding M11, polyphenol and tannin were destroyed by heat and created precipitation with protein, so they were trapped inside the cashew apple bassage, reducing the quantity in juice. For M42, these values were decreased because they were at the bassage as their linkages with other substances in cashew apple were not affected by the enzyme.

Scavenging and antioxidant activity were measured by DPPH assay which has been applied in many studies of antioxidant activity of different plant matrices such as blood orange juice,[55] wild grape[56] and other fruits and vegetables.[57] In pretreated cashew apple juice, scavenging and antioxidant activity content increased slightly compared to the raw material. These compounds increased in quantity after pretreatment since their linkages with other substances in cashew apple were weaker under heat and blanching agents effect; therefore, the extraction efficiency increased.

Even though the three pretreatment methods evaluated turned out to have lower effectiveness in reducing tannin content (36.5%) compared to the previous study that used saline solution as a blanching agent (reducing to 45%),[29] the colors of cashew apples were preserved after the pretreatments by using non-heat treatment (M42), blanching agents having ability to preserve color (M23) or short heat-exposing time (M11).

Table 2: Fruit yield as well as the nutritional values of cashew apples after pretreatments

Criteria	Unit	M42	M23	M11
Recovery efficiency	%	82.90±0.40^{a}	80.38±0.42^{b}	81.68±0.35^{c}
Total acidity (MAE)	gMAE/L	3.24±0.16^{a}	1.92±0.08^{b}	1.83±0.07^{b}
Total soluble solids	^{o}Brix	6.60±0.31^{a}	8.80±0.41^{b}	9.4±0.45^{b}
Total sugars	g/100 mL	5.85±0.29^{a}	7.46±0.37^{b}	8.67±0.41^{c}
Reducing sugars	g/100 mL	5.48±0.25^{a}	6.09±0.27^{b}	6.12±0.29^{b}
Total polyphenols	mgGAE/100 mL	105.99±5.18^{a}	260.54±11.0^{b}	121.34±6.05^{c}
Total tannins	mgTAE/100 mL	122.74±6.12^{a}	156.12±6.81^{b}	171.95±7.81^{b}
Vitamin C	mg/100 mL	214.72±9.61^{a}	274.56±12.54^{b}	199.47±8.32^{a}
Scavenging activity	%	95.70±4.71^{a}	93.50±4.52^{a}	87.00±4.23^{a}
Antioxidant activity (IC50)	mgAAE/100 mL	1.04±0.05^{a}	0.94±0.036^{b}	1.02±0.043^{ab}

GAE: Gallic Acid Equivalent, TA: Tannic Acid Equivalent, AAE: Ascorbic Acid Equivalent, MAE: Malic Acid Equivalent. Values are mean ± SD of 3 replicate (n=3), followed by different lowercase superscripts in a row are significantly different at $p<0.05$.

Despite some slightly changes, nutrients such as sugars, vitamin C, organic acids, antioxidant activity still remain in the juice after pretreatment process. Therefore, pretreatment methods help to increase juice yield accompanying preserving nutrients which are beneficial for consumers' health.

Conclusion

The work evaluated pretreatment methods to increase juice yield and partially reduce tannin which is a substance responsible for causing astringency in cashew apple juice. All of the pretreatment methods shown the ability to enhance extraction yield and decrease tannin. However, the most effective pretreatment method is determined to be the addition of enzyme preparation Pectinex Ultra SP-L into the cashew apples for 2 hours before extraction since this method increased 12.52% juice volume and reduced 36.5% tannin compared to untreated sample. Further research should be conducted to reduce tannin in extracted juice by physical, chemical, and enzymatic methods, yet still maintain the nutritional values in juice. It can be concluded that cashew apple is a potential and promissory source for the food industry, and technologies should be integrated to add value to this material and diversify food products such as juice, cyder, or wine.

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Conflict of interest

The authors declare no conflict of interest.

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