

## Effects of tannin, ascorbic acid, and total phenolic contents of cashew (*Anacardium occidentale* L.) apples blanched with saline solution

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### Abstract

Cashew apple (*Anacardium occidentale*) is considered as a by-product of the cashew processing industry. Efficient utilization of this material source contributes to valorization of cashew and reduces the burden of agricultural waste placed on the environment. This study investigated the effects of various blanching conditions on the total tannins, the ascorbic content and the phenolic contents of cashew apples. The three parameters including blanching temperature, blanching duration and salt concentration of the blanching solution were considered. It was found that optimal blanching conditions (heating at 70°C, NaCl concentration at 1% within 2 mins) resulted in cashew apples with vitamin C content and tannin retention rate of about 78.125% and 45% compared to those of the fresh samples, respectively. The cashew apple texture seemed to be insensitive to the heating process, however, the colors (mainly red gamuts of carotenoids) were lost during blanching.

## 1. Introduction

Cashew (*Anacardium occidentale*) is a common cash crop that is widely propagated in countries with high temperature and humidity in the equatorial regions. According to the General Statistics Office of Vietnam (2020), Vietnam is among countries that are known and ranked high for cashew nut export, with a total production area amounting to more than 297,200 hectares and annual export of approximately 3.3 USD billion.

Processing of cashew fruits into nuts often leads to accumulation of a large amount of cashew apple, a by-product that finds limited applications despite its benefits and nutritional values. Previous studies have indicated that cashew apples are rich in vitamin C content, which is 5–6 times higher than that of lemons and 7–8 times higher than that of mandarin and pomelo (Assunção and Mercadante, 2003; Ahmed and Birnin-Yauri, 2008; Talasila *et al.*, 2012). In addition, cashew apples provide a wide range of essential minerals such as Fe, Ca and P. However, there are two major obstacles that limit

applications of cashew apples into food industries, which are the presence of tannins and the degradation of Vitamin C during prolonged storage. In particular, cashew apple contains up to 0.2 to 0.4% of tannins, a group of compounds that is responsible for the acid, bitter taste, causing difficulties in processing the apple (Assis *et al.*, 2007). As a result, cashew pulp has not been given adequate attention in food applications and is mostly discarded or used as fertilizer, causing huge waste and possibly generating pollution to the environment. On the other hand, it was suggested that prolonged storage time and inappropriate handling method significantly contributed to the gradual degradation of vitamin C of cashew apple over time (de Abreu *et al.*, 2013). Therefore, proper technological processes are essential to the preservation of bioactive ingredients in the material and to valorization of cashew apple as a food ingredient.

Blanching, which involves elevating the temperature of plant materials to a certain level and maintaining it for a specified period, is a common processing technique in

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the food industry to prolong storage time and to prevent spoilage of plant materials by inactivating enzymes and eliminating microorganisms that are harbored by fresh vegetables and fruits. Typically, the blanching temperature could range from 65 to 100°C and the duration for which blanching takes place varies from 15 s to 45 mins (Negi and Roy, 2000; Tsamo *et al.*, 2005). Among blanching methods such as hot water blanching, steam blanching, and microwave blanching, water blanching figures due to advantages in terms of ease of implementation and operation cost. However, improper thermal processing could reduce nutrient content, and adversely affect the visual and textural quality of the blanched materials (Song *et al.*, 2003; Van Linh Nguyen, 2019). The textural detriment was found to be more pronounced in some certain types of food, such as some varieties of potato, than in others due to higher time and temperature required to achieve enzyme inactivation. For example, Muftugil (1986) found that water blanching of green bean gave better color quality, higher acid ascorbic and chlorophyll contents compared to results obtained by other blanching techniques. In another study where blanching effects on sliced carrots were investigated with respect to varying time, temperature and different blanching solutions (water and sugar solution), it was found that blanching with sugar solution at 90°C gave product textures comparable to those obtained with water blanching and conferred protective effects on blanched materials (Neri *et al.*, 2011). Regarding nutrient preservation, Woyke and Szaniawska (1969) suggested the use of 2% saline solution as blanching medium to minimize acid ascorbic losses in cauliflower and broccoli occurring during extended frozen storage durations (3 or 6 months). In addition, saline blanching also contributed significantly to the preservation of organoleptic properties of the blanched product. Beneficial effects of saline solution were also confirmed in micro-wave blanched sliced potatoes to prevent material browning (Severini *et al.*, 2004).

The anti-cancer and cardiovascular disease prevention capabilities of compounds such as phenolics and vitamin C, predominantly found in vegetables and fruits have been long known (Brat *et al.*, 2006). Of which, vitamin C is an essential compound that could not be synthesized by the human body and plays an important role in cellular biochemical reactions, reduction of free radical formations, slowing down of the aging process, generation of damaged blood vessels and prevention of cancers. In particular, it acts as a co-factor for the enzyme-N-trimethyl-L-lysine hydroxylase and butyrobetaine involved in the oxidation of fat in bones (Padayatty *et al.*, 2003). Since vitamin C is highly susceptible to pH changes, thermal treatment, light

irradiation, oxygen, enzyme, and a metal catalyst, its retention could serve as an indicator for food quality (Cieřlik *et al.*, 2006; Santos and Silva, 2008). On the other hand, retention of polyphenols, which consist of flavonoids, tocopherols and coumarins, is also an important measure that was taken into account in previous studies involving the evaluation of nutrient loss during the processing of vegetables and fruits such as soybeans (Song *et al.*, 2003), potatoes (Kozempel *et al.*, 1982), *Plectranthus amboinicus* (Nguyen *et al.*, 2020), *Gomphrena celosioides* Mart. (Thuy *et al.*, 2020) and green leafy vegetables (Gupta *et al.*, 2008).

The above-mentioned problems have called for further investigations regarding the effects of the salt blanching process on the quality of cashew apple, which have been relatively scarce recently. To our knowledge, only one study has attempted to investigate the effect of blanching and subsequent ultrasound treatment on some characteristics of dried cashew apple (Camel *et al.*, 2019). However, the examined blanching process was carried out under fixed conditions and measurement of tannin content, an important indicator for cashew apple, was lacking in that particular study. In this study, we evaluated the visual characteristics, the tannin content and the retention rate of ascorbic acid and total polyphenols of whole cashew apples blanched with saline solution. Different parameters including blanching temperature, time and salt concentration would be studied and appropriate parameters during the pretreatment of cashew material were determined. The results are expected to justify the blanching technique in reducing tannins in cashew apples, possibly contributing to valorization of this by-product in food applications.

## 2. Materials and methods

### 2.1 Samples

Whole cashew apples were collected in Binh Phuoc province (Coordinates 11°45'N 106°55'E), Vietnam, in the harvest season in March 2020 (Figure 1). Both red and yellow-colored apples were selected. The material was washed and manually picked. They were stored at 4°C until processing.



Figure 1. Cashew apples used in this study

## 2.2 Materials

Chemicals used in this study were supplied from Sigma-Aldrich (Folin-Ciocalteu Reagent, Gallic acid and  $\text{KMnO}_4$ ), India (dichlorophenolindophenol, abbreviated as DCPIP) and China (distilled water (with pH between 6.5 and 8), metaphosphoric,  $\text{Na}_2\text{CO}_3$  (99.5% purity) and ascorbic acid (99.7% purity)).

## 2.3 Process of blanching

The blanching was carried out at different temperatures, durations and salt concentrations. After being blanched, the apples were cooled in water at  $5^\circ\text{C}$ . The samples were then determined for retention of vitamin C, total phenolics and tannin contents. The examined temperatures ranged from  $65\text{--}80^\circ\text{C}$ . Blanching duration was either 1, 2, 3 or 4 mins and the salt concentration of the blanching solution was either 1, 2, 3, or 4%. Design the experiments with the relatively uniform size of cashew fruits.

## 2.4 Determination of ascorbic acid (TAA)

To determine ascorbic acid content, method of AOAC 967.21 was adopted. The principle of the method bases on the oxidation of ascorbic acid into dehydroascorbic acid and colourless lenco derivatives using 2,6 dichlorophenolindophenol (DCPIP) (Puwastien *et al.*, 2011). The color of the solution will turn pink when excess DCPIP reacts with lenco derivatives. In this environment, a drop of excess blue DCPIP will make the solution turn pink. First, one gram of the sample was ground and extracted using distilled water. The extracted solution was added with distilled water to 100 mL. Then, 10 mL of the solution was added with 1 mL HCl 0.04 N in an Erlen flask. The flask was then titrated with 2, 6-dichlorophenolindophenol (DCPIP) until the solution changes into a pink color that lasts for 30 s. The standard solution was prepared by titrating 1 mL HCl in 10 mL of standard solution (pure L-ascorbic acid and distilled water 1:10 w/v) using in DCPIP. The ascorbic acid content was expressed in mg per gram of dry matter (mg/g dry matter).

## 2.5 Determination of total phenolic content (TPC)

To determine total phenolic content, a modified Folin-Ciocalteu colorimetric method was adopted [27]. First, one gram of apples was ground and extracted with distilled water. The afforded extract was added to 100 mL using distilled water, allowed to stand at room temperature for 30 mins and then filtered through Whatman No.1. The collected filtrate (0.5 mL) was transferred into a dark tube, followed by addition of 2 mL Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 2.5 mL sodium carbonate solution

(20% w/v). The mixture was then incubated in the dark for 1 hour before being measured photometrically at an absorption wavelength of 765 nm. The total phenolic content was expressed in mg of gallic acid equivalent per gram of dry matter (mg GAE/g dry matter).

## 2.6 Determination of tannin content (TTC)

The tannin content was determined by the Lowenthal method as follows (Helvich, 1990). First, cashew apples were pressed at room temperature ( $30^\circ\text{C}$ ) to afford the extract. Then, 10 mL of the extract was transferred into a 250 mL Erlen flask, followed by the addition of 1 mL of indigo-carmin indicator and 100 mL of distilled water. The flask was titrated using  $\text{KMnO}_4$  0.1N until the colour of the flask changes to yellow.

The tannin content is calculated as follows:

$$\text{Tannin (mg/100 mL)} = \frac{(V_1 - V) \times 0.25 \times 100}{m}$$

Where  $V_1$  (mL) and  $V$  is the  $\text{KMnO}_4$  quantity used for titration of the cashew apple extract sample and control sample (distilled water), respectively.  $m$  is the amount of titrated sample (10 mL).

## 2.7 Statistical analysis

Each data point is a result of triplicate experiments and is expressed mean  $\pm$  standard deviation. MS Excel was used to process the experiment data and SPSS was used to perform one-way analysis of variance (ANOVA) test with the level of significance at 5%.






## 3. Results and discussion

### 3.1 Effects of temperature of the water blanching process on TTC, TAA, and TPC of blanched cashew apple

Table 1 summarizes total tannin, vitamin C and total phenolic content and shows images of cashew apples blanched at different temperatures (other conditions include: 1% salt concentration in 1 min). Visually, the apples blanched at  $65$  and  $70^\circ\text{C}$  showed no clear distinguishable changes in their hardness and colour compared to the fresh, unblanched samples. This is possibly due to the inability of the heating to cause noticeable damage to the cell walls as well as to the color of the material. When the samples were blanched at  $75^\circ\text{C}$ , the cashew pulp became softer and at  $80^\circ\text{C}$ , wrinkles were observed in the epidermis of the fruit due to promoted cell wall disruption and cell deaths. This result is also presented in the study of Andersson *et al.* (1994) where higher the temperature was found to associate with a more unstable texture of potato due to hardened cell walls. Similar texture loss was also reported by Abu-Ghannam and Crowley (2006) when blanching potatoes at the temperature of  $80^\circ\text{C}$ . In addition, the darker yellow



Table 1. Picture, TTC, TAA, and TPC of cashew apple in blanching process at different temperature

	Fresh	65°C	70°C	75°C	80°C
Image					
Tannin (mg/g)	2.00±0.35	1.58±0.00	1.13±0.03	1.56±0.05	1.31±0.01
Vitamin C (mg/g)	0.64±0.02	0.58±0.03	0.45±0.01	0.41±0.01	0.37±0.03
Total Phenolic (mg/g)	7.60±0.3	4.35±0.18	3.21±0.03	3.18±0.17	0.91±0.13






colour of the blanched apples is also attributable to the decomposition of colour pigments at high temperatures and in turn, activation energy changes, which is also mentioned in studies regarding colour decomposition kinetics (Weemaes *et al.*, 1999; Ahmed *et al.*, 2002). Regarding TAA content, generally, as the temperatures rise, the TAA content tends to decrease and reaches the lowest value at 80°C (0.37±0.03 mg/g). However, ANOVA results indicated that differences between TTA obtained at 70°C and that obtained at 75°C were insignificant. Vitamin C is a strong antioxidant that could be contributory to reduced incidence of some cancers (Frei *et al.*, 1989) but is highly susceptible to environmental factors and catalyzed oxidations (Oboh, 2005). During blanching, the depletion of vitamin C occurs mainly due to temperature and water exposure. At the blanching temperature of 80°C, even though accelerated decomposition of vitamin C was observed, resulting fruit texture was softer and thus the subsequent grinding process could lead to higher vitamin C extraction. This proposition was consistent with previous studies where the loss of ascorbic acid was found to be affected by the structure of the green asparagus (Zheng and Lu, 2011) and Brussels sprouts (Olivera *et al.*, 2008) during blanching. Regarding TPC, the polyphenol content experienced a drastic drop when being heated from 65 to 80°C. There was no significant difference between TPC at 70°C (3.21±0.03) and at 75°C (3.18±0.17). The decomposition of phenolics could be primarily attributable to high temperature (Gonçalves *et al.*, 2010). On the other hand, since polyphenol oxidase enzyme, which catalyzes the oxidation of monophenolic compounds, is inactivated in the temperature range of 70-90°C, raising the temperature from 75 to 80°C caused a sharp drop in TPC due to inhibition of the enzyme

(Bravo, 1998; Queiroz *et al.*, 2008; Cheng *et al.*, 2013). For TTC, its relationship with increasing temperature was similar to those of TPC and TAA (Andreou *et al.*, 2018). Because tannins are and thermally sensitive antioxidants that are highly soluble in water, heating the blanching mixture at 65-70°C caused a moderate loss of tannins. At 75°C, the TTC value (1.56±0.05 mg/g dry matter) was almost identical to that at 65°C (0.58±0.01). Other contributing factor leading to tannin loss after blanching may include the presence of oxidizing components (Ahmed *et al.*, 2002). Another study also indicated that nearly 36.04% of the tannin content in cashew apple was lost after heating and processing and the loss was more than 74.07% after storage (David and Prasad, 2015).

### 3.2 Effect of blanching time on TTC, TAA, and TPC of blanched cashew apple

Changes in texture, color of vitamin C, TPC, and TTC of cashew apple at 70°C at different times were recorded and shown in Table 2. Compared with fresh cashew apples, samples treated for more than 4 mins showed slight color and textural changes, which can be explained by polyphenol oxidation and chlorophyll degradation caused by elevated blanching temperature. The oxidation may cause browning due to production of o-quinones, expressed by the darker color of the apples. The vitamin C content decreases gradually after increasing the time and reaches the lowest value within 4 mins (0.75±0.03). In addition, the vitamin C content of apples obtained at 2 and 3 mins was statistically similar. Due to the high thermal sensitivity and solubility of Vitamin C in water, prolonged heat treatment may strongly oxidize vitamin C. Our current result is in line with results of Gupta *et al.* (2008) where an inversely

Table 2. Picture, TTC, TAA, and TPC of cashew apple at different time periods

	Fresh	1 min	2 min	3 min	4 min
Image					
Tannin (mg/g)	2.00±0.35	1.58±0.07	1.13±0.01	1.56±0.06	1.31±0.06
Vitamin C (mg/g)	0.64±0.02	0.91±0.01	0.76±0.01	0.74±0.01	0.75±0.03
Total Phenolic (mg GAE/g)	7.60±0.3	5.14±0.68	2.32±0.20	4.25±0.49	2.28±0.32

proportional relationship between blanching time and vitamin C was observed in blanching of some tropical leafy vegetables.

Regarding changes in TPC and TTC, Both TPC and TTC tend to decrease during prolonged heat exposure. However, the content of TPC and TTC decreased in the first and second blanching mins. Then, this compound was held for more at 3 mins. The maintenance of high total polyphenols at 2 mins is due to short-term exposure to temperature and water environment and inhibition of the enzyme polyphenol oxidase. This result is similar to that reported by Nguyen *et al.* (2019) in which total polyphenol content decreased after increasing blanching time in asparagus butt segment. At the same time, the tannin decomposition also took place when heating the sample at 70°C for 1 min (0.42 mg/g lower compared to the original) and was lowest at 2 mins sample (1.13±0.01 mg, no significant difference compared to 4 min sample with  $p < 0.05$ ). This is in line with the result of another study where more than 19% of tannin content in tea leaves is lost after 10 mins of heating (Amanto *et al.*, 2019). The relatively high TTC at 3 and 4 mins of treatment could be due to the entrapment of lipids and other compounds in joint protein particles formed by heat (Peng *et al.*, 2017).

### 3.3 Effect of sodium chloride concentration

The results of Table 3 showed that the salt concentration induces moderate declining effect on the decomposition of biological compounds in cashews apple. Rising the salt concentration from 0 to 1% reduces

the TPC value from 7.60±0.30 mg of the original sample to 4.69±0.55 mg. The TPC was further reduced to 3.98±0.27 mg, which is 47.63% relative to the original TPC, at 2% NaCl. However, TPC differences between at salt concentration of 3 and 4% are not significant. The non-existent reduction of TPC occurring when blanching at high salt concentrations is likely due to the blocking of intermediate products of chloride ions in polyphenol oxidation reaction (Anipsitakis *et al.*, 2006) and inhibition of enzyme polyphenol peroxidase (Pizzocaro *et al.*, 1993). On the other hand, increasing salt supplementation seemed to induce a declining trend in TAA levels. To be specific, at 1% salt concentration, TAA was reduced by 21.88% compared to the TAA of the fresh sample. At 3%, the TAA was reduced by 46.88% compared to that of the fresh sample. Increasing the concentration from 3 to 4% does not seem to induce a further decline in TAA. The decline could be attributable to the combination of both salt and heat that induce degradation of vitamin C located in the outer layer and the inner flesh of the apples. Regarding TTC, the minimum TTC value stayed at 0.65±0.04 mg at the highest salt concentration of 4%, which is not statistically different from TTC of the 3% sample. At 2% salt concentration, the TTC was 0.83±0.01 mg/g, which is only slightly lower than TTC obtained at 1% salt treatment. Considering the major loss of TPC and TAA suffered when rising the salt concentration from 1 to 2%, 1% NaCl was selected as the suitable blanching solution.

## 4. Conclusion

The present study investigated the impact of three

Table 3. TTC, TAA, and TPC of cashew apple at different concentration

	Fresh	1%	2%	3%	4%
Tannin (mg/g dry matter)	2.00±0.35	0.90±0.07	0.83±0.01	0.79±0.06	0.65±0.06
Vitamin C (mg/g)	0.64±0.02	0.50±0.02	0.42±0.01	0.34±0.01	0.32±0.02
Total Phenolic (mg GAE/g)	7.60±0.30	4.69±0.55	3.98±0.27	3.59±0.05	3.52±0.13

blanching parameters including the temperature, time and salt concentration on texture, TPC, TAA and TTC of cashew apples. The blanching conditions that could both maintain reasonable textural and nutrient quality of the cashew apples and minimize the tannin content consist of blanching temperature of 70°C, blanching solution of 1% NaCl salt and duration of 2 mins. Under these conditions, the vitamin C and polyphenol content reached 0.50±0.02 mg/g (to reduce 22%), 4.69±0.55 mg/g (to reduce 38%), respectively and the tannin content was reduced by 55% compared to that of the fresh samples. The study results justify the use of cashew apples in the manufacture of jam, juice, dried jelly, possibly contributing to the reduction of agricultural wastes. Further studies should contemplate applicability of other pretreatment methods and the effect of peroxide enzyme inactivation.

### Conflict of interest

The authors declare no conflict of interest.

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