
Research

Comparative Determination of Ascorbic Acid Content in Two Cashew Apple Varieties Using Iodometric and Dye Titration Methods

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Abstract: This study comparatively evaluated the ascorbic acid (vitamin C) content of two varieties of cashew apples: red (RCA) and yellow (YCA), as well as their 1:1 blend (RYCAB). The analysis was carried out using iodometric and dye titration methods. Results from iodometric titration showed that YCA contained the highest vitamin C content (203 mg/100 g), followed by RYCAB (194 mg/100 g) and RCA (185 mg/100 g). Similarly, the dye titration results indicated higher ascorbic acid levels in YCA (225 mg/100 g), followed by the RCA (206 mg/100 g), while the blend exhibited the lowest value (196 mg/100 g). Only slight variations were observed between the two analytical methods. Overall, the findings demonstrate that both cashew apple varieties are rich sources of vitamin C, with the yellow variety exhibiting comparatively higher concentrations, making them valuable for nutritional and health benefits.

Keywords: Ascorbic Acid, Cashew Apple, Comparison, Dye, Iodine, Titration.

1.0 Introduction

Vitamin C, which is also known as ascorbic acid, is a water-soluble antioxidant compound that is generally obtained from fruits and vegetables, such as blueberries, oranges, lemons, strawberries, peppers and tomatoes (Afuwape et al., 2022; Elhefian et al., 2019). It has a molecular formula of C₆H₈O₆, a molecular weight of 176.13 g/mol, and a

melting point of about 190°C (Elhefian et al., 2019). Ascorbic acid helps to boost the immune system, fight against infectious diseases, aid iron absorption, and facilitate the formation of collagen in humans and other animals (Andargie et al., 2023). It also contributes to the prevention of scurvy, provides relief from the common cold, plays a significant role in the wound-healing process, and helps to inhibit free radicals from being oxidised, thereby preventing cell damage; it is commonly used as a food additive (Azrina et al., 2017; Mulu et al., 2022).

The cashew tree (*Anacardium occidentale* L.) is a medium-sized tropical tree cultivated for its fruit (cashew nut) and pseudo fruit (cashew apple). It is also a multipurpose species that provides a broad range of services (Salim et al., 2023). The cashew tree is a fast-growing, hardy, and drought-resistant multipurpose tree that can be cultivated in many tropical climatic conditions (Kumar et al., 2019). The four major cashew-producing regions are India, Brazil, Nigeria, and Tanzania (Salim et al., 2023). Varieties of cashew are often distinguished by their colour (red or yellow) and the shape (pear-shaped, round, or elongated) of the swollen pedicel (cashew apple) to which the nut is attached. Locally recognised varieties are given names unique to their growing areas (Kumar et al., 2019).

Cashew apples are soft, juicy, slightly fibrous, and astringent in taste, covered with a thin, waxy skin that can easily bruise (Angela et al., 2023). They are mostly heart-shaped, 3-6 times or more larger than the nut, and when fully ripe, they are bright red, yellow, or a mixture of the two colours. Several edible products can be made from cashew apples, including cashew apple juice, cashew apple jam, cashew apple chutney, cashew apple wine, and cashew apple vinegar (Eliane et al., 2021). The photographs of red and yellow cashew apples are shown in Figures A and B, respectively.



Figure A: Red cashew apples



Figure B: Yellow cashew apples

Several analytical techniques have been reported in the literature for the evaluation of vitamin C, such as the redox titrimetric method (Timothy et al., 2021), spectrophotometric method (Eliane et al., 2021; Mohammed), fluorometric method (Dereje et al., 2015), and titrimetric method (Isam et al., 2017; Jalloul et al., 2024; Nagendra et al., 2023). However, the titrimetric methods, which are the focus of this study, are considered simple, rapid, and applicable methods for the quantification of vitamin C in food items.

It is well known among ordinary people today that the best sources of vitamin C are citrus fruits and juices. To make better use of fruits and vegetables as food, a clear understanding of their nutritional value, such as estimating the content of vitamin C, is necessary. The aim of this work has been to estimate the amount of ascorbic acid quantitatively in two varieties of cashew apples using iodometric titration and dye titration methods. This work helped to establish which of the cashew varieties will best meet the daily recommended dosage of ascorbic acid for healthy living.

2.0 Materials and Methods

2.1 Sample Collection

Fresh samples of two varieties of ripe cashew fruits (red and yellow) were randomly collected from two different gardens in Yankuzo village of Tsafe Local Government, Zamfara State, north-western Nigeria, and were botanically identified in the herbarium at the Department of Biological Sciences, Usman Danfodiyo University, Sokoto, Nigeria. All the samples were thoroughly cleaned with distilled water to remove adhering contaminants and then weighed using a weighing balance.

2.2 Sample Preparation

This method for sample preparation was adopted from Islam et al. (2017). The aqueous extracts for each of the two samples were prepared separately by accurately weighing 100 g of the freshly prepared fruit sample in a 500 mL beaker and blending vigorously with the aid of an electric blender, adding 30 mL of metaphosphoric acid (0.5% w/v) to obtain the fruit extract. Each of the mixtures was filtered using a clean muslin cloth, with the filtrate collected in a 250 mL conical flask. An aliquot of each sample was transferred to a 100 mL volumetric flask and then brought to the mark with metaphosphoric acid (0.5%).

2.3 Preparation of Blend of Red and Yellow Cashew Apples

A 1:1 ratio of the blend of red and yellow cashew apple samples was prepared as follows: 50 mL each of yellow and red cashew apple extract from section 2.2 was measured

and transferred to a 250 mL beaker. The resulting mixture was homogenised by mixing with the aid of a mechanical shaker for 15 minutes.

2.4 Preparation of Reagents and Standards

2.4.1 Preparation of 0.05 M Iodine Solution

This was carried out as reported by Manasa et al. (2019), by dissolving 1.75 g of iodine in a solution of 4.5 g of potassium iodide in 50 ml of water, followed by three drops of hydrochloric acid (HCl). The resulting solution was then made up to 250 mL of water in a volumetric flask.

2.4.2 Preparation of Starch Indicator

The 0.5% starch solution was prepared using the method reported by Eugena and Aba (2024), as follows: 0.25 g of starch was solubilised in a 100 mL beaker, and 50 mL of distilled water was added. The solution was heated with stirring at 79°C for 5 minutes. The resultant solution was allowed to cool to room temperature.

2.4.3 Preparation of Ascorbic Acid Standard (60 mg/L)

A 60 mg/L ascorbic acid standard solution was prepared by dissolving 0.0060 g of L-ascorbic acid in 25 mL of distilled water and then diluting to 100 mL with distilled water in a 100 mL volumetric flask, as reported by Eugena and Aba (2024).

2.5 Experimental Procedure

2.5.1 Titration of the reduced ascorbic acid (blank sample)

A 10 mL aliquot of standard ascorbic acid was pipetted into a conical flask and titrated immediately with the standard solution of 2,6-dichlorophenolindophenol to a pink endpoint that persists for 15 seconds, as reported by Manasa et al. (2019).

2.5.2 The Iodometric Titration Process

This method for the determination of ascorbic acid was adopted from Mulu et al. (2024). It is based on the iodometric titration of the sample with a standardized iodine solution.

To determine the amount of ascorbic acid in the samples, 20 mL of the clear solutions of the fruits were taken, and 1.5 mL of 0.5% starch solution was added to each extract. These solutions were titrated against the prepared iodine solution (0.05 M) with continuous shaking, and the endpoint was recorded for all the samples. The endpoint of the titration was taken at the first sign of the appearance of a blue-black colour that persists on swirling. Triplicate titrations were conducted, and the mean was taken. The ascorbic acid content was calculated using Equation 1 below.

$$\text{Conc. of ascorbic acid} = \frac{MI \times VI \times 176.12 \times 100}{W} \dots \dots \dots (1)$$

Where:

MI = Moles of Iodine (mol/L)

VI = Volume of iodine (ml)

176.12 g/mol = molar mass of ascorbic acid

W = sample weight

2.5.3 The Dye Titration Process

This method for the determination of ascorbic acid was adopted from Dao et al. (2021).

To determine the ascorbic acid content, exactly 10 mL aliquot of the extracted sample was pipetted into a conical flask and titrated against a solution of 2,6-dichlorophenolindophenol. The dye was added rapidly, dropwise, with constant shaking of the solution until the pink colour resulting from the neutralised dye persisted for 15 seconds. The same procedure was carried out for all the samples, and then triplicate titrations were conducted, with the mean taken. The mg of ascorbic acid per 100 g of the samples was calculated using Equation 2 below:

$$\frac{V \times T \times 100}{W} \dots \dots \dots (2)$$

Where V = volume of dye solution used for titrating a 10 ml aliquot of diluted samples (mL)

T = dye factor

W = grams of the sample in the aliquot titrated

3.0 Results and Discussion

Table 1: Titrations of dye solution against the standard ascorbic acid

Burette readings (mL)	1 st titration	2 nd titration	3 rd titration
Final burette reading (mL)	4.90	9.90	14.70
Initial burette reading (mL)	0.00	4.90	9.90
Volume of dye solution used (mL)	4.90	5.00	4.80

$$\text{Average volume of dye solution used} = \frac{4.9+5.00+4.80}{3} = 4.90 \text{ ml.}$$

Table 2: Amount of Vitamin C in the samples determined by iodometric titration

S/N	Samples	Titer values obtained after triplicate titrations (mL)			Average Titer(mL)	Amount of ascorbic acid (mg/100 g)
		1 st Titration	2 nd Titration	3 rd Titration		
1	YCA	4.70	4.70	4.50	4.60	203
2	RCA	4.20	4.20	4.20	4.20	185
3	RYCAB	4.50	4.30	4.40	4.40	194

Key: RCA (Red cashew apple); YCA (Yellow cashew apple); RYCAB (Blend of Yellow and Red cashew apples)

Table 3: Amount of Vitamin C in the samples determined by dye titration

S/N	Samples	Titer values obtained after triplicate titrations (mL)			Average Titer(mL)	Amount of ascorbic acid C (mg/100 g)
		1 st Titration	2 nd Titration	3 rd Titration		
1	YCA	4.50	4.70	4.50	4.60	225
2	RCA	4.10	4.10	4.30	4.20	206
3	RYCAB	3.80	4.10	4.10	4.00	196

Key: RCA (Red cashew apple), YCA (Yellow cashew apple), RYCAB (Blend of yellow and red cashew apples)

The results of the determination of the ascorbic acid content of two varieties of cashew apples and their blend via iodometric and dye titrimetric methods are shown in Tables 2 and 3, respectively. The ascorbic acid contents obtained by the iodometric titration method were: yellow cashew apple (203 mg/100 g), red cashew apple (185 mg/100 g), and the blend of red and yellow cashew apples (194 mg/100 g). Meanwhile, the dye titration method yielded 225 mg/100 g for the yellow cashew apple, 206 mg/100 g for the red cashew apple, and 196 mg/100 g for their blend.

From the column of their average titer values in both Tables 2 and 3, it can be inferred that the higher the titers, the greater the quantity of ascorbic acid. This means that the quantity of ascorbic acid present in a sample of fruit is directly proportional to the titer values.

The overall result indicated that yellow cashew apple has a higher vitamin C content than red cashew apple, and the blend of the two cashew apple varieties (yellow and red) provides a moderately improved ascorbic acid content in favour of red cashew apple. However, with respect to the methods used, the dye titration method demonstrated slightly better ascorbic acid content than the iodometric method.

Generally, the results obtained from the present study are in agreement with results from other studies and also in disagreement with some reported results from the literature. For example, Quttera et al. (2016), in their study on cashew apples, showed a vitamin C content of 211.79 mg/100 mL. The results obtained in the present study are also comparable to those of Egbekun and Otiri (1999), who found the vitamin C content of a cashew apple sample to be 224.7 mg/100 g, and Carvalho et al. (2007), who reported 219 mg/100 g. However, our findings are in disagreement with the work of Landing et al. (2022), who found the vitamin C content of raw cashew juice to be 290 ± 0.02 mg/100 mL and 270 ± 0.02 mg/100 mL for red and yellow cashew apples, respectively.

The deviation of this result from those reported by some other researchers for the same fruit samples analysed may be due to other factors that affect the ascorbic acid level in fruits. These factors include climate, degree of ripeness, and the amount of fertiliser used in cultivation. Climatic conditions such as light and temperature have been reported to affect the chemical composition of horticultural crops, as suggested by Dioha et al. (2011).

Conclusion

This study quantitatively determined the ascorbic acid content of red and yellow cashew apple varieties, including their 1:1 blend, using iodometric and dye titration methods. The results revealed noticeable differences in vitamin C levels between the two analytical techniques, although the variations were minimal. In both methods, the yellow cashew apple consistently exhibited higher ascorbic acid content compared to the red variety and its blended sample. These differences may be attributed to factors such as environmental conditions, maturity stage, and post-harvest handling. Despite these variations, both cashew apple varieties were confirmed to be rich sources of vitamin C and can significantly contribute to meeting daily nutritional requirements. Increased consumption, particularly of the yellow variety, is therefore recommended for improved dietary health.

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