

Full Length Research Paper

## Assessment of some aspects of phytonutrients of cashew apple juice of domestic origin in Nigeria

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**Phytochemical screening, assessment of some biochemical characteristics and antioxidants indices of juice obtained from red and yellow varieties of cashew apple of domestic origin in Western Nigeria were done with view to gain insight to its phytonutrients and health maintenance potentials. Assessment revealed the presence of phytochemical of the therapeutic importance principally: total phenolics, flavonoids, tannins and anthraquinones. Quantitative assessment showed that ascorbic acid (mg/mL of juice) ranged from 0.24 to 4.00. Total sugar (mg glucose/mL of juice) ranged from 3.50 to 111.88; titratable acidity (mg citric acid equivalent/mL of juice) ranged from 5.89 to 15.71 and total phenolic content (mg ferulic acid equivalent/mL of juice) ranged from 1.20 to 1.68. Results of reducing power and hydroxyl scavenging power suggest antioxidative endowment.**

**Key words:** Cashew apple juice, domestic origin, phytochemical screening, antioxidant potentials.

### INTRODUCTION

Fruits and vegetables are important for good health and certainly good for all age categories because the class of foods forms an important portion of a healthy diet (Zhang et al., 2010). Regarding phytonutrients, both fruits and vegetables contain many hundreds of compounds with potential antioxidant activity, including the antioxidant vitamins C and E. There are also carotenoids, chlorophyll and a wide variety of phytochemicals such as a simple phenolics compounds, flavonoids glycosides and in some foods, complex polymeric tannins (eprocyanidins and gallotannins) (Pellegrini et al., 2007). As a corollary to this, there exist a plethora of literature expressing epidemiological evidence suggesting positive association between consumption of a diet high in fruits and vegetables and decreased of lifestyle diseases (cancer, cardio vascular hypertension, etc) (Agte and Tarwadi, 2005).

Of all the tropical fruits trees in Western Nigeria, cashew tree is the most widely domesticated food plant, ahead of mango and pawpaw trees. The cashew

(*Anacardium occidentale* L) is native to tropical America. Originating in Brazil, the plant has become naturalised in many Tropical countries such as Vietnam, India and the African countries such as Nigeria, Tanzania, Ivory Coast, Mozambique and Benin. Like any other fruit juices, cashew apple juice is notably characterised by sugar, organic acids and vitamin C (Akinwale, 2000). Further studies have indicated antitumor, antimicrobial (Cavalcante et al., 2005) and antioxidant activity (Alves et al., 2010) among others. However, the source of the samples is either agricultural research station or commercial farm. Treatments offered in agricultural experimental stations or commercial farms for the sake of yield improvement or disease resistance modifies the naturalness of the juice nutrients obtained from such fruits and therefore, its biological value. Like any other biological system, other factors that could cause variation in nutritional and biochemical profile of cashew apple juice are: genetic, edaphic, application of nutrients, climatic, processing method, geographical and pest

control. Therefore, it is incorrect to assume the nutrient profile or biochemical compositions of cashew apple juice from structural sources such as experimental stations or commercial farms are same with juice from domestic origin. Added to this, it is inappropriate to rely on imported data regarding cashew apple juice for local applications. Besides, only miniscule amount of cashew apple of domestic origin is consumed as relish. Leaving a larger portion to waste in form of ground drop apple due to lack of processing technology. The quantity of the wasted cashew apple could be adequate for cottage juice production or more. Therefore, there is need for quality data on the juice of apple of domestic origin. Such information could be useful for juice quality standardization and nutrient-health promotion campaign.

Hence, the objective of this study was the evaluation of the nutritional and biochemical characteristics of cashew apple juice of domestic origin in Western Nigeria.

## MATERIALS AND METHODS

Fully matured yellow and red varieties of cashew apples obtained from cashew tree on the premises of Federal Polytechnic, Ado-Ekiti, Nigeria in April 2011 were used for the expression of juice while the two apples varieties were ripe, and unripe sample was selected for diagnostic study. Juice was extracted by two methods tagged: 'squeezed' and 'blended'. Juice obtained by squeezed method was by manual pressing of apple to yield juice while the other method involve extraction of juice using juice extractor which blended the apple to yield higher obtained using muslin cloth for filtration prior to preservation and clarification treatments.

Each group was pasteurized at a temperature of 65°C for 30 min after the addition of gelatine at 50 mg/100 mL (Nagaraja et al., 2011). Sodium benzoate (0.7 g/L) was added for preservation, cooled at room temperature (28 ± 2°C) and used for subbasement analysis. The juice samples were allowed to stand for overnight in a refrigerator. The clarified juice was filtered using muslin cloth. The quantity of the juice recovered was expressed as percentage of the cashew apple used. Some physical characteristics of the juice was subjectively assessed and described in terms of clarity, cloudiness and colour as adjudged by the eye.

### Phytochemical screening

Phytochemical investigations on cashew apple juice/liquor were accomplished as described by Trease and Evans (2002). Briefly, Meyers reagent was used for alkaloids, Molish Test for glycosides, Iron (III) chloride Test, for phenolic, reducing compounds and tannins, Mg-HCl reagent for flavonoids, Born Trager test for anthraquinones, Fehling solution for reducing sugars, Sulphuric acid test for carotenoids and frothing method was for detection of saponins.

### Refractive index determination

Refractive index of samples was measured using Abbey refractometer (ABBE 325, ZUZI) and the value recorded without modification.

### pH determination

The pH measurement was accomplished by dispensing 50 mL of sample into 100-mL beaker and read directly on a pH meter (omega

H.HPx digital pH meter) which has been previously standardized with buffer 4 and 9 solutions. Value was recorded when equilibrium pH was attained.

### Titrateable acidity determination

Titrateable acid was determined by titrating 100 mL of the sample against 0.1 M NaOH using phenolphthalein as indicator of end point, and results were expressed in citric acid equivalent/100 mL of sample.

### Total sugar determination

Total sugar in samples was determined colourimetrically using phenol-sulphuric acid method as described by Dubois et al. (1956). Briefly, sample was treated with phenol sulphuric acid and absorbance measured using spectrophotometer at wavelength of 490 nm. Total sugar was calculated using glucose standard curve.

### Ascorbic acid determination

Ascorbic acid content was determined by the indophenols method (Ruck, 1969). Ascorbic acid in juice samples was extracted with 0.4 % oxalic acid and the extract titrated with standardized sodium 2,6 dichlorophenol indophenols.

### Evaluation of total phenolic content

Total phenolic content was evaluated according to the method described by Taga et al. (1984). Briefly, A 100 µL of Folin-Ciocalteu reagent (2N wrt acid Fluka Chemic AG-Ch-9470 BUCHS) was added to each sample (20 µL) and well mixed after addition of 1.58 mL of water. After 30 s, 300 µL of 2% sodium carbonate solution was added and the sample tubes were left at room temperature for 2 h. The absorbance (A) of the developed blue colour was measured at 750 nm using Unicam Helios and UV/VIS/Spectrophotometer. A plot of  $A_{750nm}$  against corresponding concentration was used to calculate phenolic content (mg/g ferulic acid equivalent).

### Determination of reducing power

Reducing power of each sample was determined in accordance with the method of Oyaizu (1986). Simply, each sample (1 mg/ mL) in methanol (2.5 mL) was mixed with sodium phosphate buffer (pH 6.6). The buffered sample was mixed with conditioning reagents (1%  $K_3FeCN_6$ , 10% TCA, 0.1 %  $FeCl_3$ ) centrifuged, diluted using distilled water and absorbance was measured at 700 nm.

### Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined according to the method of Klein et al. (1991) with little modification. 1 ml of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA (0.018 %), and 1 mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to 2 mL of juice sample, and the reaction was initiated by adding 0.5 mL of 0.22% ascorbic acid. The test tubes were capped tightly and heated on a water bath at 80°C for 15 min. The reaction was terminated by the addition of 1 mL ice cold TCA (17.5% w/v). 3 mL of Nash reagent (75.0 g of ammonium acetate, 3 mL of glacial acetic acid and 2 mL of acetyl acetone were mixed and raised to 1 L with distilled water)

**Table 1.** Phytochemical characteristics of cashew apple juice .

Sample	PC	RC	Glycosides	Flavonoid	Alkaloid	Saponin	Carotenoid	Anthraquinone	Tannin	RS
CJ-YAB	+	+	-	-	+	-	-	-	+	++
CJ-YAS	++++	+++	+	+	+	-	++	++	++++	++
CJ-RAB	+++	+++	++++	+++	+	-	+	+	++++	++
CJ-RAS	++	++	+	++	+	-	-	+++	++	++
CJ-UYAS	+++	+++	+++	+	+	-	-	++++	++	+
CJ-URAS	++	+++	++	++	+	-	-	++	++	+

was added to all of the test tubes and left at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured spectrophotometrically at 412 nm against reagent blank. The percentage hydroxyl radical scavenging was calculated as follows: % hydroxyl radical scavenging activity = 1 - (difference in absorbance of sample/difference in absorbance of blank) x 100.

#### Statistical analysis

Statistical comparison of some biochemical characteristics of cashew apple juice samples was accomplished using student's t-test. Difference at  $p=0.10$  was considered to be significant.

## RESULTS AND DISCUSSION

### Phytochemical screening of cashew apple juice

The result of the phytochemical screening of the cashew apple juice as accomplished in this study is presented in Table 1. All the samples showed positive for phenolics and its sub-class principally, flavonoids, and tannins. This is in agreement with the results of Sivagurunathan et al. (2010). In addition, NNMDA (2006) reported presence of tannins as well as traces of alkaloids in cashew apple fruit. However, no saponins were detected in this study.

Other phytochemicals detected were anthraquinones, glycosides and similar compounds (sugars) at varied quantities. One of the modes of therapeutic activity of phytochemicals is by reduction therefore screened. All the samples showed vivid reducing property. The result of phytochemical screening revealed that cashew apple juice whether obtained from squeezed or blended was endowed with some bioactive components that could exhibit antioxidative activities. Results of reducing power, vitamin C and phenolic content of the juice suggest it can be a source of antioxidant food drink. This is because natural antioxidants can be phenolic compounds (tocopherols, flavonoids, phenolic acids), and nitrogen compounds (alkaloids, chlorophylls, amino acids, amines, peptides). Others are carotenoids and ascorbic acids (Velioğlu et al., 1998) with concomitant biological antioxidant functions in the nature of antimutagenicity, anti-aging, anticarcinogenicity (Cook and Samman, 1996). Pellegrini et al. (2007) stated that besides

minerals and vitamins, also fruits contain other phytochemicals some of which include simple phenolic compounds, organic acids, flavonoids glycosides and complex polymeric tannins. These phytochemicals are characterized with antioxidant potentials and associated health benefits. This assertion informed the phytochemicals screening of juice obtained from cashew apple of domestic origin with view to ascertain its phytochemical endowment.

### Selected physical and physicochemical characteristics of the cashew apple juice

Two varieties of cashew apple fruit namely the red and yellow were used in this study. Besides, an additional sample of unripe yellow variety was used. The juice was obtained from the fruit samples by squeeze and blend processes. Selected physical and physicochemical characteristics of the cashew apple juice obtained from the expression processes are presented in Table 2.

All the juice samples obtained by squeeze were of higher clarity in comparison to samples obtained from blends preparations. This is reasonable because blends will facilitate comminution of fibres into the mash and eventually in the juice resulting to cloudy juice. The colour of the obtained juice is a reflection of the colour of the skin of the variety of cashew fruit used. However, colour intensity was influenced by the method (squeezed or blended) of juice expression. The juice yield (%) (Table 2) is high for expression obtained using blending in comparison to manual squeeze. This observation is logical as more liquor is liberated by blending from structural and non-structural cells of the fruit apple. However, such yield requires further processing (centrifugation or sieving) to obtain juice of comparable clarity with that obtained using squeeze. The refractive index of all the samples ranged from 1.351 to 1.354. This value could be useful for quality control assessment during preparation of cashew apple juice.

The pH of the juice samples ranged from 3.61 to 3.82, this is similar with an earlier report by Talasila et al. (2011). This classifies cashew apple juice as acid food same class as that of orange (Ihekoronye and Ngoddy, 1985).

**Table 2.** Physical and Physicochemical characteristics of cashew apple juice.

Sample	Parameters				
	pH	R.I.	Juice yield (%)	Colour	Clarity
CJ-YAB	3.72	1.354	62.58	Light yellow	-
CJ-YAS	3.75	1.354	40.48	Clear	+
CJ-RAB	3.82	1.352	59.79	Pink	-
CJ-RAS	3.70	1.352	43.23	Light pink	+
CJ-UYAS	3.65	1.351	35.23	Milk white	+
CJ-URAS	3.61	1.351	32.40	Milk white	+

CJ-YAB, Cashew juice-yellow apple blended; CJ-YAS, cashew juice-yellow apple squeezed; CJ-RAB, cashew juice-red apple blended; CJ-RAS, cashew juice- red apple squeezed; CJ-UYAS, cashew juice-unripe yellow apple squeezed; CJ-URAS, cashew juice-unripe red apple squeezed.

**Table 3.** Selected biochemical characteristics of cashew apple juice.

Sample	Biochemical characteristic					
	Ascorbic acid	Total sugar	Titratable acidity	Total phenolic content	Reducing power	Hydroxyl radical scavenging index
CJ-YAB	0.255	7.500	5.891	1.200	100	36.58
CJ-YAS	4.000	95.000	6.480	1.350	80	55.57
CJ-RAB	2.580	96.887	7.534	1.350	90	44.18
CJ-RAS	3.000	111.880	10.799	1.650	100	39.44
CJ-UYAS	0.240	4.000	15.714	1.680	110	78.60
CJ-URAS	0.242	3.500	15.000	1.640	110	76.70

CJ-YAB, Cashew juice-yellow apple blended; CJ-YAS, cashew juice-yellow apple squeezed; CJ-RAB, cashew juice-red apple blended; CJ-RAS, cashew juice- red apple squeezed; CJ-UYAS, cashew juice-unripe yellow apple squeezed; CJ-URAS, cashew juice-unripe red apple squeezed. Ascorbic acid = mg ascorbic acid equivalent/ml of juice; Total sugar = mg glucose equivalent/mL of juice; Titratable acidity = mg citric acid equivalent/mL of juice; Total phenolic content = mg ferulic acid equivalent/mL of juice; Reducing power = mg ferulic acid reducing power equivalent/100mL; hydroxyl radical scavenging = %.

### Selected biochemical characteristics of the cashew apple juice

The results of assessed selected biochemical characteristics of the cashew apple juice are shown in Table 3. The ascorbic acid content (mg/mL) ranged from 0.240 to 4.000. The result is similar to earlier report by Nagaraja et al. (2011). However, a cursory look at the values singly revealed highest value for the juice obtained from squeeze processes most especially CJ-YAS, while the least value was obtained from juice from unripe fruit. The trend of result can be explained on the basis that the other samples had lower values probably due to oxidative enzymic action resulting from destruction of cellular structure of fruits as a result of blending used for expelling juice from the fruit.

The total sugar content (mg glucose equivalent/mL) ranged from 3.500 to 111.880; were similar to values reported by Nagaraja et al. (2011). Examination of the values with respect to the method of juice expression showed that blend preparation gave liquor with lower total sugar. This suggests that blending offered the release of enzyme which probably caused glycosylation thereby

causing reduction in the number of carbonyl group for sugar reaction. Juice obtained from CJ-UYAS were characterized with lowest amount of sugar; this is reasonable since the fruit was unripe.

Titratable acidity (mg citric acid equivalent/mL) of the sample ranged from 5.891 to 15.714. Although, the literature at my disposal did not give report on acidity of cashew apple juice but the fact that the sample with the lowest pH has the highest value of titratable acidity proved the plausibility of the trend of results obtained in this study. In addition, the preparation from unripe fruit has the highest 15.000 to 15.714 titratable acidity values in comparison to low 5.891 to 10.799 values obtained for ripe samples irrespective of mode of juice expression. More importantly, the trend of result is justified by the fact that fruit acidity decrease with ripeness. Total phenolic content (mg ferulic acid equivalent/mL) of the samples ranged from 1.20 to 1.68 (Table 4). The trend of result shows that higher value of total phenolics for juice was obtained from unripe fruits and juice obtained by squeeze method from red variety. Generally, the juice samples were characterized by tangible amount of total phenolic content.

**Table 4.** Statistical comparison of some biochemical characteristics of cashew apple juice.

Parameters	Apple variety		Students T test value
	Yellow	Red	
Ascorbic acid	0.24 – 3.40	0.242 – 0.300	0.968
Total sugar	0.004 – 0.095	0.0035 – 0.11188	1.5693
Titrateable acidity	5.891 – 15.714	7.534 – 15.000	1.204
Total phenolic content	0.0012 – 0.00168	0.00135 – 0.00164	4.1675*
Reducing power	0.0008 – 0.001	0.0009 – 0.0011	0.794
Hydroxyl radical scavenging	36.58 – 78.60	39.44 – 76.70	0.5048

Value 0.01, 0.05, 0.10 = 31.598, 4.303, 2.92, n = 3.

Two antioxidant indices namely relative reducing power and hydroxyl radical scavenging index were employed to gain insight to health maintenance potentials of the juice. To my knowledge, this has not been reported in literature. The relative reducing power (mg ferulic acid reducing power equivalent/100 mL) of the samples ranged from 80 to 110. All the samples were characterized with good relative reducing power suggesting a high health maintaining potentials. The hydroxyl radical scavenging index (%) of the samples varied from 36.58 to 78.60. The high variance within the hydroxyl radical scavenging index could be explained in terms of many factors in nature of oxidation and reduction that influence hydroxyl radical scavenging which manifest through the biochemical changes in ascorbic acid, titrateable acidity and total phenolic compounds. The high value of hydroxyl radical scavenging index for the juice obtained from unripe fruit was probably due to synergistic activity of ascorbic acid, titrateable acidity and total phenolic content of the samples.

Statistical analysis of the assessed phytonutrients and antioxidant indices of the juice samples were accomplished using pair comparison students t-test with respect to yellow and red variety, regardless of method of juice expression. Analysis showed that no significant difference was detected in all the parameters evaluated except in total phenolic content in which significant difference exist at  $p=0.10$ . The consequence of this result is that either of the fruit variety could be used to prepare juice with no significant difference in the biochemical properties assessed in this study.

## Conclusion

Phytochemical and nutritional assessment showed that juice obtained from two varieties of cashew apple of domestic origin in Western Nigeria is endowed with phytochemical and nutritional constituents that could play a role in health maintenance. As suggested by assessed antioxidant tests. The findings may be useful for quality control and nutrient-health promotion campaign.

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