American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 4 (4): 154-161, 2009
ISSN 1557-4571

Mineral and Proximate Composition of Cashew Apple (*Anarcadium occidentale* L.) Juice from Northern Savannah, Forest and Coastal Savannah Regions in Ghana

S.T. Lowor and C.K. Agyente-Badu
Cocoa Research Institute of Ghana, P.O. Box 8, Akim-Tafo, Ghana

Abstract: In this study, variations in the mineral, phenol, tannin, vitamin C and sugar composition of cashew apple juice from three agro-ecological zones (Northern Savannah, Forest and Coastal Savannah) of Ghana were investigated. The mean proximate composition (mg/100 mL) was as follows: phenolics (269.5), condensed tannins (266.0), Vitamin C (231.4) and sugars (12.05 mg mL^{-1}). The mineral composition (mg/100 mL) showed potassium (76.0) to be the highest, followed by calcium (43.0), magnesium (10.92), phosphorous (0.79) and sodium (0.41). Zinc, copper and iron concentrations were much lower and ranged from 0.05-0.08 mg/100 mL. The physicochemical properties of the juice were as follows: pH (4.31), colour (light yellow for juice from yellow apples and yellow with traces of red pigments for juice from red apples). Phenol and tannin contents in the juice showed significant ($p<0.05$) variation among the ecological zones. Thus, apples from the Forest transitional zone appear to be better for juice extraction because of their relatively low tannin, higher pH, higher sugar and less phenolic content. No significant differences in the quantitative composition of calcium, iron, zinc and phosphorus could be attributed to the ecological zone or colour from which the juice was extracted.

Key words: Macro-nutrients, agro ecological zone, sugars, phenolics, condensed tannins

INTRODUCTION

The cashew tree *Anacardium occidentale,* generally considered to be native to the Northern part of South America, is now found in many tropical areas. It thrives in three agro-ecological zones, known in Ghana as the Northern Savanna (Guinea and Sudan), Forest Savanna (Transitional zone) and Coastal Savannah, which have annual rainfall ranging between 900 and 1400 mm (Anonymous, 2006). In Ghana, interest in cashew development has emanated partly from the general recognition of the need to diversify the country's agricultural production and expand the export base. Cashew is gaining steady development and importance as one of the crops being promoted under the country's medium and long-term non-traditional export program. It is a seasonal crop harvested between January and March in Ghana. Until recently, its cultivation was restricted to small-scale rural farmers and making of the dried whole nuts for export was the only commercial activity. Currently, over 50 large farms (8-40 ha) and 15 plantations (≥40 ha) have been established across the country's cashew growing zone (Addaquay and Nyamekye-Boamah, 1998).

The cashew apple, which varies in colour from yellow to intense red, is generally discarded in Ghana but it is known to have many uses. For instance, processes exist that convert the apple juice into alcohol and non-alcoholic beverages, candied fruit, fresh juice, jam, jelly, syrup and pectin (Winterhalter, 1991). It is also a valuable source of minerals and vitamins. Indeed, cashew apple juice is reported to contain five times as much vitamin C as citrus juice (Akinwale, 2000; Azam-Ali and

Corresponding Author: Samuel Lowor, Physiology/Biochemistry Division, Cocoa Research Institute of Ghana, Box 8, New Tafo, Ghana Tel: +233243889880

Judge, 2001) and 10 times as pineapple juice (Ohler, 1988). The cashew apple juice also has medicinal uses. For instance, its high tannin content makes it a suitable remedy for sore throat and chronic dysentery in Cuba and Brazil (Morton, 1987). It is also reported to have anti-bacterial, anti-fungal and anti-tumor properties (Kubo *et al*., 1993a, b; Kozubek *et al*., 2001) as well as being anti-oxidant (Melo-Cavalcante *et al*., 2003) and anti-mutagenic (Cavalcante *et al*., 2005). The effect of the environment on the chemical composition of fruits has been studied for a number of crops (Shear, 1980), including tomatoes (Davies and Hobson, 1991) and grapes (Reynolds *et al*., 2007). Seed oils from sunflower grown in three different parts of Zanzibar from two sorts of seeds (black and cream) were found to be widely different in composition, with the oils from black seeds being more unsaturated than those from the cream seeds, but the main difference attributed to the place of growth (Barker and Hilditch, 2006).

No work, however, has been done on the juice from cashew originating from the three agro-ecological zones in Ghana with respect to the effect of environment on the proximate composition and mineral concentration of the apple juice and how they vary between the red and yellow apples among the unknown source of the population stands of Ghana's cashew.

Therefore, this study seeks to assess the differences that may exist in the contents of apple juice from two different coloured apples from three agro-ecological zones and interaction between environment and apple colour.

MATERIALS AND METHODS

Preparation of Juice

During the 2008 and 2009 cashew seasons (January to March), fresh cashew fruits were harvested from the Cocoa Research Institute of Ghana (CRIG) Bole substation in the Northern Savanna zone, Wenchi Ministry of Food and Agriculture (MOFA) cashew farm in the Forest Savanna (Transitional zone) and in Farmers' farms at Dodowa in the Coastal Savannah zone. The number of trees sampled per each region/location was 100. The fruits were sorted into yellow and red, washed with clean water, macerated and the juice sieved (cheese cloth) using sterile equipment and thereafter frozen at -20°C until analyzed.

Determination of Sugar Contents

Juices were clarified by centrifuging at 7,500 rpm (Sorvall RC 5C Plus, Kendro Laboratory Product, Asheville, USA) for 5 min. The supernatants were decanted and deionized using Amberlite resin. Total sugar was estimated directly on resulting juice using the method of Dubois *et al*. (1956).

Analysis of Minerals in the Juice

Juices were digested for mineral analysis following the procedure of Beltrán-Gonzále *et al*. (2008). Thus, 15 mL of juice were treated with 5 mL of 65% (w/v) Nitric acid in Pyrex tubes and the tubes heated in digestion blocks for 180 min at 130°C. The solutions were cooled by allowing them to sit at room temperature, transferred to 25 mL volumetric flask and topped up to the mark with deionized water.

Levels of iron, copper, zinc, magnesium, sodium, potassium and calcium were determined using an atomic absorption spectrometer (SpectraAA 200FS, Varian Australia Pty Ltd). Phosphorous was determined as PO_4^{3-} by the vanadium phosphomolybdate method in which the phosphorous present as orthophosphate reacts with a vanadate molybdate reagent to produce a yellow-orange complex. The absorbance was measured at 420 nm using a Cecil CE 7400 spectrophotometer (Super Aquarius, Cecil Instruments, Cambridge, England). Minerals were analyzed in four replicates. All the chemicals/reagents used were of the analytical grade and products of sigma, Germany.

pH of the juice was determined at 25°C using a pH meter (Mettler-Toledo GmbH, 8603 Schwerzenbach, Switzerland) equipped with a standard single-junction glass-Ag/AgCl combination electrode, which was calibrated at pH 4, 7 and 9.

Total Titratable Acidity (TTA)

Total titratable acidity (% citric acid) was determined by placing 10 mL of juice into a beaker and titrating with 0.1 mol L^{-1} NaOH to pH 8.2±0.1 (AOAC, 2000). All samples were analyzed in 4 replicates.

Total Polyphenolic Content

The total phenolic content was analyzed using Folin-Ciocalteu reagent, following a modified procedure of Singleton and Rossi (1965). Samples were appropriately diluted with methanol to give 80% aqueous methanol. One mL of the appropriately diluted sample was mixed with 5 mL of Folin-Ciocalteu reagent (1:10, v/v, diluted with distilled water). The reaction was neutralized by adding 4 mL of 75 g L^{-1} sodium carbonate. Samples were held for 2 h at 25±2°C and the absorbance of the resulting blue color was measured at 760 nm against a reagent blank on a Cecil UV-visible spectrophotometer (CE 7400, Cecil Instruments, Cambridge, England).

The total phenolic content was determined from a catechin calibration curve prepared and analyzed concurrently with the juice samples. Samples were analyzed in four replicates.

Total Proanthocyanidin Contents (Condensed Tannins)

To 20 mL of juice was added 80 mL of acetone to yield 80% aqueous acetone solution and shaken for 10 min at room temperature (25°C). The proanthocyanidins content was measured according to the vanillin-HCl method (Tiitto-Julkunen, 1985). Briefly, to 0.1-0.5 mL of extract was added 3 mL of 4% vanillin (w/v) in methanol and shaken vigorously. 1.5 mL of concentrated HCl was immediately added and the tube shaken again. Absorbance was read at 500 nm after being allowed to stand for 20 min at room temperature. The results were expressed as catechin equivalents (mg/100 mL) of the fresh juice.

Vitamin C Determination

Vitamin C was determined by iodine titration (AOAC, 2000; Food Chemicals Codex, 1996). To 25 mL of juice in a 150 mL beaker was added 35 mL starch-sulphuric acid solution. The resulting solution was titrated with standardised 0.1 M iodine solution (covered from light), while stirring until the first stable blue colour appeared. For the blank, juice was replaced with distilled water. Ascorbic acid (mg/100 mL) was calculated from the formula:

$$\text{Ascorbic acid (mg/100mL)} = \frac{\text{Net mL titrant}}{\text{mL sample}} \times 880.6$$

where:

$$\text{Net mL titrant} = \text{mL titrant for sample} - \text{mL titrant for blank}$$

Experimental Design and Statistical Analysis

The factors considered were effects of agro-ecological zone (location) and colour of apple from which juice was extracted. All data were tested by analysis of variance (two-way ANOVA procedure) with the Genstat 5 release (3.2) program package. Means were separated by the LSD test at ($p \leq 0.05$).

RESULTS AND DISCUSSION

The cashew apple juice from the three ecological zones was found to contain between 206.2 and 268.6 mg/100 mL vitamin C (Table 1). These values compare favourably with the value of 203.5 mg/100 mL reported for Nigerian cashew apple juice (Akinwale, 2000). The differences were not statistically significant and therefore location and colour of apple did not significantly influence the amount of vitamin C in the juice. Assunção and Mercadante (2003) however, reported non statistically higher levels of vitamin C in both yellow and red apple varieties from Northeast of Brazil compared to those from the southeast. The yellow varieties also had higher levels of vitamin C compared to the red. The levels of vitamin C in the cashew apples as shown in this study were much higher than those found in many other fruits. The vitamin C could supplement the nutritional requirement for vitamin C (30 mg day^{-1}) for growers and African people in the study (Akinwale, 2000).

The macro-elements, Ca, Fe and P contents of the juice are given in Table 1. Values ranged from 0.60-0.98 mg/100 mL for P, 30.5-60.9 mg/100 mL for Ca and 0.04-0.07 mg/100 mL for Fe. There were no significant differences that could be attributed to the ecological zone or colour of the apples from which the juice was extracted.

The concentrations of copper in the red and yellow coloured apples from the three ecological zones were low and ranged from 0.07-0.12 mg/100 mL of juice (Table 2). This is probably because the soils on which the cashew is being cultivation in these agro ecological zones do not contain high deposits of copper since, according to Vesk and Allaway (1997), high sources of copper in plants are usually attributed to environment (air/water) and soils on which the plants grow or to copper based fungicides applied. Juice from the coastal savannah zone appeared to have significantly higher levels of copper than those from the Northern savannah and forest transitional zone (Table 2).

Table 1: Ca, P, Fe and vitamin C concentration (mg/100 mL) in juice extracted from yellow and red coloured cashew apple from three Agro ecological zones

	Forest Savannah		Northern Savannah		Coastal Savannah		
Minerals	Yellow	Red	Yellow	Red	Yellow	Red	LSD (p = 0.05)
Ca	40.30	46.10	48.20	60.90	31.80	30.50	ns
P	0.60	0.63	0.69	0.94	0.91	0.98	ns
Fe	0.05	0.05	0.05	0.07	0.06	0.04	ns
Vitamin C	268.60	206.20	210.70	252.40	244.10	206.20	ns

ns = Not significant at ($p<0.05$)

Table 2: Mineral concentration (mg/100 mL), pH, polyphenol and sugar (mg mL^{-1}) in cashew apple juice from different locations and apple colours

Locations	Apple color	Copper	Zinc	Sodium	Potassium	Magnesium	pH	Polyphenol (mg/100 mL)	Sugar (mg mL^{-1})
Forest savannah	Yellow	0.07	0.05	0.45	32.2	6.45	4.46	221.9	13.46
Forest savannah	Red	0.07	0.05	0.45	30.8	5.57	4.59	184.7	14.45
Northern Savannah	Yellow	0.07	0.07	0.33	69.8	9.71	4.34	215.1	11.20
Northern Savannah	Red	0.07	0.03	0.33	86.4	12.75	4.21	412.8	14.43
Coastal Savannah	Yellow	0.12	0.15	0.45	156.2	16.70	4.19	366.9	10.32
Coastal Savannah	Red	0.08	0.13	0.45	80.6	14.32	4.08	215.6	8.44
Location LSD ($p<0.05$)		0.01	0.02	0.02	14.7	1.80	0.03	21.2	0.80
Colour LSD		0.01	0.02	ns	11.9	ns	0.02	17.2	0.70
Interaction LSD		0.02	ns	ns	20.7	2.70	0.04	29.9	1.20

ns = Not significant at ($p<0.05$)

Copper is an essential and beneficial element in human metabolism and the average daily dietary requirement for copper in the adult human has been estimated at 2 mg and for infants and children at 0.05 mg kg^{-1} b.wt. (Browning, 1969; WHO, 2004). The NRC (1980) reported estimated safe and adequate daily dietary intakes of copper ranging from 0.5 to 0.7 mg day^{-1} for infants 6 months of age or less up to 2-3 mg day^{-1} for adults. The copper concentration in the cashew apple juice from Ghana is within the safe prescribed limits of infants also. Juice from both the red and yellow obtained from the coastal savannah zone appeared to have significantly higher levels of zinc than those from the Northern savannah and Forest transitional zone (Table 2). The juices were also low in sodium (0.33- 0.45 mg/100 mL) with colour not playing a significant role (Table 2). Location however, had a significant effect since juices from the coastal and transitional zones had higher sodium levels than that from the Savannah. The gradient of sodium present in the juice could be due to the availability of sodium in the cashew growing belts, with coastal plains worldwide having higher Na concentration in soils then the hinterlands (Misra *et al*., 1995).

Potassium was the most concentrated element among the macro-elements assayed, ranging from 30.8-156.2 mg/100 mL of juice (Table 2). The yellow apple juice from the Coastal Savannah had significantly high ($p<0.05$) levels of potassium than juices from the other zones. Magnesium was present in relatively moderate amounts, ranging from 5.52 -16.70 mg/100 mL (Table 2). Colour was found not to significantly influence the levels, however, location had a significant effect with the coastal savannah juice having higher levels and forest savannah the lowest.

pH of juice from the yellow apples was generally significantly higher ($p<0.05$) than those of the red apples from the forest savannah (Table 2). Location significantly influenced the acidity of the juice. Juice from the coastal savannah tended to be more acidic compared to those from the forest Savannah, with the Northern savannah juice being intermediate. Red apples from coastal and Northern savannah were more acidic ($p<0.05$) than their corresponding yellow apples. The pH range was 4.19-4.59. Osho (1995) reported a pH value of 3.8 in Nigeria. pH of juice reported from two different regions in India were not affected by region of origin or colour of the apple from which they were extracted (Gunjate and Patwardhan, 1995).

Total polyphenol in the juice ranged from 184.7-412.8 mg/100 mL (Table 2). Polyphenols in plant foods are greatly influenced by genetic factors, variety, degree of ripeness and environmental conditions (Bravo, 1998). In this study, location had a significant effect on the level of phenolics in the juice. Thus, the dryer Northern Savannah environment registered the highest mean values of phenolics, while the forest savannah zone, which usually experiences much higher rainfall, had the lowest phenolics. The values reported here compare favourably with 295.25 mg/100 g for cashew fruit reported by Mélo *et al*. (2006).

Cashew apple juice is rich in carbohydrates. The levels of sugars found in the juices from the three-agro ecological zones are shown in Table 2. The respective lowest and highest values were as follows (mg mL^{-1}): for red, 8.44 (Coastal Savannah) and 14.45 (Forest Savannah); for yellow, 10.32 (costal savannah) and 13.46 (Forest savannah). Sugar levels in apple juice from the Forest savannah zone were significantly ($p<0.05$) higher than those from the Northern and Coastal Savannah. Less sugar will therefore be needed to ferment the juice from the forest savannah zone when such juices are used in wine production where percentage alcohol levels (in the wine) are expected to exceed 11%. Fermentable sugars from the juice of red apples were generally significantly higher ($p<0.05$) than those of yellow apples from the same location. Again, the indication is that red cashew apples may be more suitable for wine production in relation to higher alcohol content.

Biologically active compounds with antioxidant properties include condensed tannins or proanthocyanidins (Hagerman, 1998; Havsteen, 2002; Melo, 2006; Kitao *et al*., 2006). They are widely distributed in fruits and have health implications (Hagerman *et al*., 1998). The proanthocyanidin contents of the cashew apple juices varied from 145.3 to 512 mg /100 mL of fresh juice (Table 3). Juice

Table 3: Total titratable acidity (% citric acid) and Condensed tannin concentration in *Anacardium occidentale* juice (n = 4)

Location	Colour of apple	Total titratable acidity (%)	Condensed tannins (mg/100 mL)
Forest Savannah	Yellow	0.20	170.6
Forest Savannah	Red	0.19	145.3
Northern Savannah	Yellow	0.26	228.6
Northern Savannah	Red	0.37	512.4
Coastal Savannah	Yellow	0.23	232.8
Coastal Savannah	Red	0.37	306.4
Location LSD ($p<0.05$)		0.05	10.5
Colour LSD ($p<0.05$)		0.02	5.5

from the Northern Savannah registered significantly highest proanthocyanidin content than those from the two other zones. Also, significant differences occurred between the yellow and red apples from same locations. Total Titratable Acidity (TTA) expressed as citric acid ranged from 0.19 to 0.37% with a mean value of 0.27% (Table 3). The TTA varied significantly for juices from the ecological zones. Similar results have been reported in India where both red and yellow apples from the Karnataka region had higher levels of acidity compared to those from the Kerala region (Gunjate and Patwardhan, 1995).

CONCLUSION

Juices from both the red and yellow cashew apples from the three agro ecological zones in Ghana were similar and rich in concentrations of Cu, P, Ca and Fe. Cashew apple juice from all the three agro ecological zones is rich in vitamin C and should be considered as a potential supply source of adequate daily nutritional requirement for children and adults. Juice from the forest transitional zone had higher pH and less phenol and therefore less astringent compared to juices from the other zones. The forest transitional zone apples also appeared to be better for apple juice extraction because of its relatively low tannin and relatively higher sugar content and could be exploited in breeding for varieties with higher sugar content. No varietal difference was noticed with respect to qualitative composition of sugars, organic acids and phenols. Low variation in the genetic base of the cashew plants resulted in no marked differences in the chemical composition of the juices despite the distinct rainfall and temperature conditions in the ecological zones from which the study was conducted.

ACKNOWLEDGMENTS

I thank Messers Peter Denkyi, David Kumah, Ebenezer Ofori, Divine Addo, Seth Avoka and Miss Nancy Ohene Darko for their technical assistance. This study is published by the kind permission of the Executive Director of the Cocoa Research Institute of Ghana.

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