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Phenolic profile of cashew apple juice (*Anacardium occidentale* L.) from Yamoussoukro and Korhogo (Côte d'Ivoire)

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ABSTRACT

Objective: To evaluate the phenolic profile of the two varieties of cashew (*anacardium occidentale* L.) cultivated in Côte d'Ivoire, given the specific soil and climate in the various production areas. This was in order to determine the most suitable variety in terms of the astringency for production of juice and over product such as wine, syrup, alcohol.

Methodology and results: Cashew apple harvested from areas of Yamoussoukro and Korhogo were transported to the laboratory, crushed and the juice collected was analyzed. Total phenolic, flavonoids and anthocyanins were assayed by UV spectrometry, while the concentration of individual phenolic was done by HPLC. The composition (mg/L) of yellow and red apples juices in total phenolic determined in gallic acid equivalents using the Folin Ciocalteu assay ranged from 1653.8±2.3 to 2374.2±5.4, that of total flavonoids ranged from 298.6±1.5 to 479.8±2.6, while the anthocyanins 5.9 ± 0.8 to 7.4 ± 0.8. The concentration of individual phenolics gave values (mg/L) between 1.2±0.3 to 7.9±1.5 for quercetin, 4.3±0.7 to 10.3±3.6 for naringenin, 20.2±5.3 to 25.9±4.6 for caffeic acid, 2.5±0.9 to 7.9±1.1 for coumaric acid, 4.6±0.3 to 13.4±1.7 for ferulic acid and 1.9±1.6 to 15.4±2.2 for gallic acid. Catechin and syringic acid were not detectable. The levels of total phenolic, flavonoids and anthocyanins, gallic acid and cafeic acid in the juice showed a significant variation ($p<0.05$) according to the color of the cashew apples. Similarly, the ecological zone has an influence on the concentrations of total phenolic and flavonoids.

Conclusion and application: Thus, at the end of the study, the apples of Korhogo area seem best suited for juice production because of their relatively low content of total phenolic. Also, cashew apple juice appears is a good source of antioxidants for the population in Côte d'Ivoire.

Keys words: Juice, cashew, phenolic, ecological zone, HPLC

INTRODUCTION

The cashew tree (*Anacardium occidentale* L.) is native to the coast of Brazil. This is an extremely hardy tree that can grow on poor soils and under various climatic conditions. It produced at season as fruits and nuts which are very popular. The latter kidney shaped stalks are pressed in enlarged, fleshy and juicy commonly called cashew apple (Michodjehoun *et al.*, 2009). Cashew discovered by the Portuguese, was

introduced in their various colonies in Africa to fight against soil erosion (Rieger, 2006).

Figure 1: Photographs of yellow and red cashew apples

In Cote d'Ivoire, the cashew has quickly generated interest so that it became one of the main exports of the country shown by the 330 000 tons of cashew nuts exported in 2008 (Anonymous 1, 2008). Which is the equivalent of 2.84 million tons of cashew apples in the ratio of weight apple/nut that is 8/1 (Cornier, 2008). However, the cashew apple is often considered as a by-product because undervalued (Michodjehoun *et al.,* 2009). In Nigeria the use of cashew apple does not exceed 10 % of annual production (Oduwole *et al.*, 2006). Indeed, the nuts are valued at the expense of rotten apples or eaten by pests (Loeillet, 2001). The cashew apple is five times richer in vitamin C than orange juice and 10 times more than the pineapple juice (Akinwale, 2000). The juice is considered as an excellent remedy for sore throat and chronic dysentery (Morton, 1987). Phenolic compounds provide effective protection against cardiovascular disease and some cancers. Total phenolic content involved in these beneficial effects are mainly composed of flavonoids and phenolic acids (Hayatsu *et al.*, 1988; Sharma *et al.*, 1994; Stavric, 1994). These are powerful antioxidants *in vitro* (Van Acker *et al.*, 1996), and can inhibit lipid peroxidation and protect low-density lipoprotein or LDL against oxidation (Fuhrman *et al.,* 1995; Vinson and Dabbagh, 1998). They can also reduce platelet aggregation and lead to vasodilatation (Fitzpatrick *et al.*, 1993). Yet it is generally shown that fruits, nuts and vegetables play an important role in human nutrition, especially as sources of vitamins, polyphenols, minerals and dietary fiber (Quebedeaux and Bliss, 1988; Quebedeaux and Elsa, 1990; Craig and Beck, 1999; Wargovich, 2000). It was shown that the consumption of fruit and vegetable juices reduced risk of chronic diseases (Ruxton *et al.*, 2006). However, the physico-chemical composition of fruits could be influenced by the area of growing, environment, climate and variety (Karadeniz and Aziz, 2002; Lavinas, 2006; Pinheiro *et al*., 2006). The objective of this study is to determine the content of total polyphenols and phenolic composition of cashew apple juice in both cities of Côte d'Ivoire.

MATERIAL AND METHODS

Material: The plant material consists of red and yellow cashew apple from two producing areas of Côte d'Ivoire: Yamoussoukro (center) and Korhogo (north). The ripe and intact Cashew apples harvested in these areas were transported in a refrigerated chamber to the laboratory for juice extraction and analysis. Both cities collection inside the country are: The city of Yamoussoukro (Lake District), located in the center of the country 380 km from Abidjan. This area is considered unfavorable for the cultivation of cashew. The city of Korhogo (Savannah Region) in the north of country 600 km from Abidjan. This area is considered favorable for the cultivation of cashew. The yellow

apples (YAY and YAK) and red apples (RAY and RAK) were harvested from the same period.

Table 1: Source of samples of cashew apples

N°	Source	Samples	Abréviation
1	Yamoussoukro	Yellow apple	YAY
2	Yamoussoukro	Red apple	RAY
3	Korhogo	Yellow apple	YAK
4	Korhogo	Red apple	RAK

Reagents and standards: All reagents used in this work were of analytical grade. Sodium carbonate, aluminum chloride and the phenolic standards (gallic acid, naringenin, catechin, quercetin, p-coumaric acid, ferulic acid, syringic acid, cafeic acid) are all from Merck Co. (Darmstadt, United Kingdom). The Folin Ciocalteu was supplied by Panreac. Ethanol (EtOH) and pure methanol (MeOH) obtained from Prolabo.

Methods

Extraction of juice: Cashew apples transported to the laboratory, were detached from the nuts. Apples were washed thoroughly with clean water. Then the apples were cut and ground by a Mixer (Blender LB20E, Torrington, United States, 2002). The juice obtained by pressing the ground material was filtered through a 0.5 mm mesh sieve and then stored frozen (Fiocchetti, Mazzara, Italy) at -80°C for different analysis.

Determination of total phenolic: The total phenolic content in juice was determined in terms of gallic acid equivalents (GAE) using Folin Ciocalteu method (Singleton and Rossi, 1965). An aliquot (0.1 ml) of cashew apple juice was mixed with 1 ml of distilled water and 0.5 ml of Folin Ciocalteu to 2 N. The mixture was left in contact for 3 min. Then 3 ml of 2% Na_2CO_3 are added. The solution is centrifuged for 15 min. Finally, the absorbance is measured at 750 nm using a UV/VIS (JASCO-V-530). The total phenolic content is calculated in terms of Gallic acid equivalents

Determination of total flavonoids: The flavonoids content of various samples was determined in terms of rutin equivalents by the method of Jia et al. (1999). An aliquot (0.5 ml) of apple juice was mixed with 1.25 ml of distilled water and 75 µL of solution of 5% $NaNO_2$. The mixture is left in contact for 6 min. Then 150 µL of solution of aluminum chloride 10 % were added. The resulting solution is centrifuged for 5 min. Then 0.5 ml of sodium hydroxide at 1 M and 2.5 ml of distilled water are added. The absorbance was finally measured at 510 nm.

Determination of total anthoyanins: The assessment of the amount of anthocyanins in the juice of red and yellow cashew apples was performed using the method of Ribereau and Stonestreet (1965). In an aliquot (1) ml of sample were added successively 1 ml of ethanol and 20 ml of Hcl solution. Then 10 ml of the mixture are evenly distributed in two tanks. Each tank respectively contents: tank A: 5 ml of the mixture + 2 ml of distilled water, tank B: 5 ml of the mixture + 2 ml of bisulfate. These tanks are read in a spectrophotometer at 520 nm.

Characterization of phenolic compounds

Extraction of phenolic compounds: The phenolic compounds are extracted from samples of apple juice, adding 25 ml of juice; 75 ml of methanol diluted to 80% saturated NaCl (sodium chloride). After a period of stirring for 30 min, the resulting solution is centrifuged at 2000 g for 5 min at 5°C with a refrigerated centrifuge (Sigma Aldrich 2-PK). The supernatant was recovered and the pellet was extracted twice under the same conditions. The supernatants were combined and evaporated at 35°C to 50 ml using a rotary evaporator (HEILDOLPH Control Laborat 4003, Schwabach, Germany). The remaining solution is diluted to 100 ml with distilled water.

Analysis of phenolic compounds: The test samples were filtered through Whatman paper No. 4, then through a 0.45 microns Millipore membrane (Roth, Karlsruhe, Germany). The phenolic composition of cashew apple juice was analyzed by the method of Donovan and al. (1998) on an HPLC system (Shimadzu, France) equipped with a binary pump (LC-20A) coupled to a UV-VIS detector (SPD-20A). The column used for this analysis is the hypersyl ODS C_{18}, 250 x 4.6 mm, 5µm (Thero, Runcom, England). The mobile phase consists of 50 mM, pH 2.6 $NaH_4H_2PO_4$ (eluent A), a solution of acetonitrile/$NaH_4H_2PO_4$ (80:20, v/v) (eluent B) and 200 mM o-acid phosphoric acid at pH 1.5 (eluent C). The flow rate was 0.5 mL/min. The elution program was carried out as follows: 100% A (0-5 min), 92% A, 8% B (5-8 min), 14% B 86% C (8-20 min), 16.5% B 83.5% C (20-25 min), 21.5% B 78.5% C (25-35 min), 50% B 50% C (35-70 min), 100% A (70-75

min), 100% A (75-80 min). The spears are then identified by comparison of retention times and spectra with authentic reference substances.

Statistical Analysis: The statistical analysis was made by the software STATISTICA (99th ed). The significance of the variation in phenolic content of different cashew apple juice was calculated with Duncan's test at significance level P<0.05.

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RESULTS AND DISCUSSION

Changes in total phenolic, flavonoids and anthocyanins of four samples of cashew apple juice (YAY, RAY, YAK, RAK) from the two cities are given in Table 2. Levels (mg/L) observed range from 1653.8±2.3 to 2374.2±5.4 for total phenolics, total flavonoids those range from 298.6±1.5 to 479.8±2.6 while anthocyanins ranged from 5.9±0.8 to 7.4±0.8 and are present only in samples of red juice. Figure 1 shows an example of HPLC chromatogram of purified phenolic extracts obtained from red cashew apple juice from Korhogo. The composition of phenolic acids is summarized in Table 3

Table 2: Contents (mg/L) in total phenolics, flavonoids and anthocyanins of cashew apples juices.

localisation	Color of apple	Total phenolic	Total flavonoids	Total anthocyanins
Yamoussoukro	Red	2374,2 ± 3,4[a]	479,8 ± 2,6[a]	7,4 ± 0,8[a]
	Yellow	2237,6 ± 5,5[b]	410,2 ± 2,2[b]	nd
Korhogo	Red	1752,7 ± 4,2[c]	327,6 ± 2,5[c]	5,9 ± 0,8[a]
	Yellow	1653,8 ± 2,3[d]	298,6 ± 1,5[d]	nd

Value with similar superscripts arranged vertically is not significantly different from each other (p<0,05) in the same variety of mineral, value are expressed as mean **± SEM (**n = 3 determinations), nd = no detected.

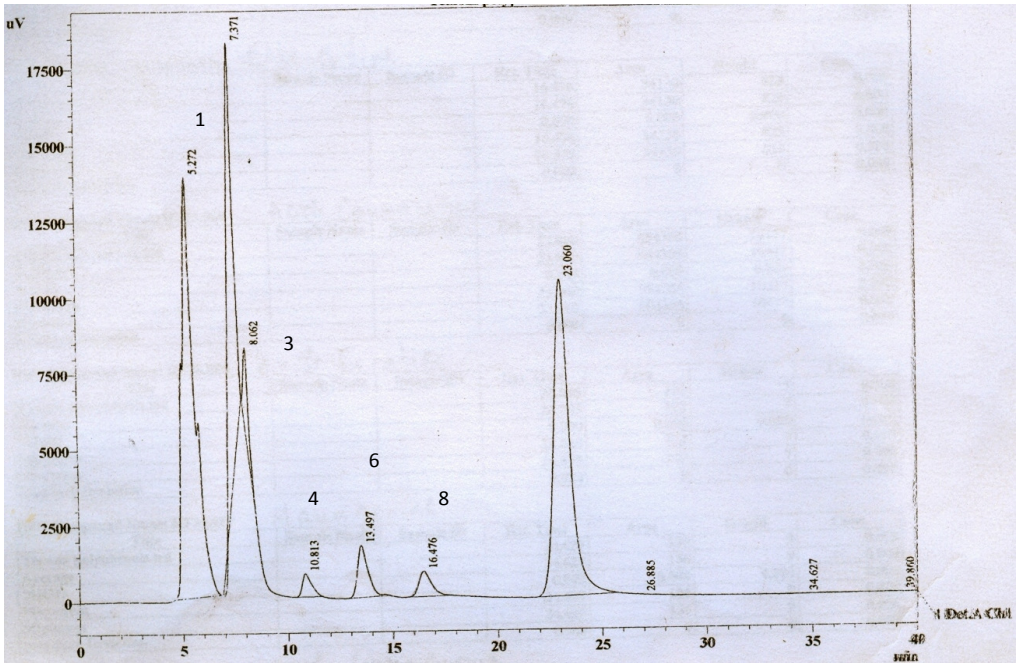

Figure 2: Chromatogram of red cashew apple juice of Korhogo **1** : naringenin, **3**: cafeic acid, **4** : gallic acid, **6** : p-coumaric acid, **8** : syringic acid.

Table 3: Contents (mg/L) in acid phenolic of cashew apples juices

	Yamoussoukro		Korhogo	
Compounds	RA	YA	RA	YA
Cafeic acid	23,6 ± 1,3[a]	22,5 ± 2,7[a]	25,9 ± 4,6[a]	20,2 ± 5,3[a]

p-coumaric acid	2,5 ± 0,9[a]	7,9 ± 1,1[b]	nd	nd
Ferulic acid	nd	nd	13,44 ± 1,7[a]	4,6 ± 0,3[b]
Gallic acid	1,9 ± 1,6[a]	13,3 ± 3,40[b]	4,4 ± 0,7[c]	15,4 ± 2,2[b]
Syringic acid	nd	nd	nd	nd
Naringenin	10,3 ± 2,9[a]	5,3 ± 1,67[b]	4,3 ± 0,7[b]	10,3 ± 3,6[a]
Catechin	nd	nd	nd	nd
Quercetin	7,9 ± 1,5[a]	4,2 ± 0,34[b]	3,7 ± 0,3[c]	1,2 ± 0,3[d]

Value with similar superscripts arranged vertically is not significantly different from each other (p<0,05) in the same variety of phenolic, value are expressed as mean **± SEM (**n = 3 determinations), nd = no detected. RA: Red Apple, YA: Yellow Apple/

Levels (mg/L) range from 1.2±0.3 to 7.9±1.5 for quercetin, 4.3±0.7 to 10.3± 3.6 for naringenin, 20.2±5.3 to 25.9±4.6 for caffeic acid, 2.5±0.9 to 7.9±1.1 for coumaric acid, 4.6±0.3 to 13.4±1.7 for acid ferulic, 1.9±1.6 to 15.4±2.2 for gallic acid. On total phenolics, there is a significant difference (P<0.05) between the apples of different colors from the same area, but also between the ecological zone. Moreover, the levels of total phenolics in apple juice of Yamoussoukro are higher than those of Korhogo. However, the sample values of YAY, RAY and RAK are compared favorably with those found (172-4128 mg/mL) in apple juice in some areas of Ghana (Lowor and Agyente, 2009), but against that of the sample YAK is much lower. These observations are explained by the fact that the phenolic content of fruit is generally influenced by genetic factors, variety, maturity and also by environmental conditions (Bravo, 1998). In this study, the variety and ecological zone have influenced the content of total phenolic of juice. Thus, the city of Yamoussoukro recorded the highest levels of total phenolic and red apples juices seem to be richer in this compound. The presence of this high amount of total phenolic in the juice of cashew apples could be advantageous for cashew producing countries. Many studies have indeed shown that phenolic are effective in reducing risk of cardiovascular disease (Johnson and Fenwick, 2000; Pfannhauser *et al.*, 2001; Margetts and Buttriss, 2003). Yet 80% of deaths from cardiovascular disease occur in countries with low or middle income where people who have little access to effective health services (WHO, 2011). The cashew apple juice appears to be an alternative for these countries including Côte d'Ivoire, through better knowledge and extension. As for total flavonoids, the contents of the juice samples YAY and RAY are higher than those samples YAK and RAK. Similarly, the levels of red apple juice seem to be higher than those of yellow apple juice. It also confirms the influence of variety and ecological zone. However, these levels are higher than those of apple juice (20.3 to 92.11 mg/L) reported by Gliszczynska and Tyrockonoka (2003). Flavonoids are among the most studied phytochemicals components in plant foods. They contain within them, a variety of molecules which can result in diverse biological activities (Hidalgo *et al.*, 2010). Numerous studies have revealed their character and their antioxidant action on free radicals in the human body (Rice-Evans, 1996; Plumb, 1998).

Unlike total phenolics and total flavonoids present in all juice samples, total anthocyanins are present only in red apple juice of the localities studied. Concentrations are significantly lower than that of "blood" orange juice (68 mg/L) reported by Shaoqian et al. (2009). In fact, anthocyanins are pigments responsible for the color red, purple and blue of fruits and whose concentration depends on the intensity of the color (Paliyath *et al.*, 2008). The color of the apple has therefore a significant influence (P<0.05) on the total anthocyanin content of juice. Low concentrations of anthocyanins of red cashew apples limit their use in food industries especially in finding natural pigments. The five phenolic acids characterized in this study were also detected in cashew apples from Benin by Michodjehoun et al. (2009). Satyanarayana et al. (1978) observed gallic acid in a variety of Indian cashew apple. These phenolic compounds therefore appear to be evidence of authenticity of cashew apple juice. The caffeic acid is the compound most concentrated among the phenolic acids detected in cashew apple juice. Levels are influenced by the variety and ecological zone. Thus, the red apple juice was richest in caffeic acid and the samples YAY and RAY have a higher average. The second most important component in the samples is gallic acid, whose levels are significantly different (P<0.05) in the varieties and ecological zones. Thus, samples YAY and YAK are richer than the samples RAY and RAK in gallic acid. As for ferulic acid and p-coumaric, they are present respectively in samples of Yamoussoukro and samples of Korhogo. Biologically active compounds with antioxidant properties, belonging to flavonoids include quercetin, naringenin and catechin. The cashew apple juice while being rich

in flavonoids, does not contain catechin. The concentrations in quercetin of cashew juice ranged from 1.18 to 7.95 mg/L of pure juice. The red apples juices showed significantly higher levels of the quercetin in this study. Gökmen et al. (2001) have reported by against 59.5 mg/L of quercetin in the apple juice.As for the amounts of naringenin, they range from 4.34 to 10.31 mg/L with an average grade of 7.57 mg/L of juice. These low levels obtained could be explained by the fact that the individual phenolic compounds are more common in fruits in the esterified form as in the free form (Mattila *et al.*, 2006). The presence of these biologically active phenolic compounds can confirm the therapeutic properties attributed to the juice of cashew apples. These antioxidants also have multiple interests that are utilized in the cosmetic industry and dermopharmacy. Hence, the cashew apples could be a raw material in these different industries.

CONCLUSION

The red and yellow cashew apples juice from the two agro-ecological zones of Côte d'Ivoire, have high amounts of total phenolics and total flavonoids. These juices also contain bioactive phenolic compounds such as quercetin, naringenin and phenolic acids (caffeic acid, p-coumaric acid, ferulic acid, gallic acid). The variety and growing area of cashew apples significantly influence the concentrations of phenolics in juice. Phenolic compounds are partly responsible for the astringency of the cashew apple. Regardless of the negative organoleptic characteristics, the richness of these compounds and other nutrients such as vitamin C and minerals show the cashew apple as an alternative nutritionally and medicinally. In view of the different levels of phenolic compounds in juice, cashew apples of Korhogo would be best suited for juice production because of their relatively low phenolic content. Similarly, it will be interesting to investigate the antioxidant activities (*in vivo* and *in vitro)* of the juice of cashew apples harvested in Côte d'Ivoire to locate their sphere of action.

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