INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 22, No. 2, p. 92-101, 2023

RESEARCH PAPER OPEN ACCESS

Physico-chemical characterization of cashew apple (*Anacardium occidentale* L.) from Benin: cultivars and agro-ecological zone effects

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Key words: Cashew apple, Reducing sugars, Organic acids, Tannin, Vitamin C

http://dx.doi.org/10.12692/ijb/22.2.92-101 Article published on February 07, 2023

Abstract

The present study aimed to determine the physicochemical characteristics of cashew apple juice. The juice was produced using apples of two colors (red and yellow) harvested from two cultivars of cashew tree plantations located at two differents agro-ecological zones (AEZ) of Benin. Their physicochemical parameters were determined. Results showed that dry matter, pH, total soluble solids (TSS), and total titrable acidity (TTA) of juice samples varied from 7.4 to 11.4 %, 4.1 to 4.7,10.7 to 14.5 °Brix, 0.6 to 1.7%, respectively. Vitamin C content varied between 224 and 327 mg/100 ml; total phenols ranged from 1960 to 4785 mg/L; condensed tannins from 58 to 510 (mg Eq Catechin/100 ml); antioxidant capacity varied between 58.3 and 90.8 5 (% DPPH inhibition). Concentrations of glucose, fructose, raffinose, and saccharose varied between 17.3 to 34.6, 31.6 to 65.5, 1 to 2.4 and 0.0 to 1.6, respectively. Except for fructose, sugars were neither significantly ($P<0.05$) influenced by apple colors nor by cultivars or AEZ. Malic acid, acetic acid, citric acid, and oxalic acid contents ranged from 55.3 to 108.5, 33.2 to 6.9, 1.9 to 12.5, and 2.4 to 7.7, respectively. Citric, malic and acetic acids were significantly ($P<0.05$) influenced by apple colors, while oxalic acid of juice was significantly ($P<0.05$) influenced by AEZ. The main minerals, potassium, phosphorus, magnesium, and sodium levels varied from 930.5 to 1176.3,86.7 to 136.1,79.7 to 89.6,58.7 to 86.32, respectively. They were neither significantly ($P<0.05$) influenced by cultivar nor by AEZ. The ACP analysis clustered the juice into three homogenous groups.

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Introduction

Cashew tree (*Anacardium occidentale* L.) production is at high growth in some areas, such as West Africa (Oliveira *et al.,* 2020). Benin has favorable conditions for growing cashew trees; it is produced in 8 of the 12 departments, particularly in the northern and central regions. Benin is the seventh-leading global producer of cashew nuts (WB, 2020; FAOSTAT, 2021). The cashew peduncle represents 90% of the total weight of the fruit and can be bought at a very low price. For 1T of cashew nut, about 10-15 T of cashew apples are produced (Attri, 2009).

The vitamin C value of Cashew apple was 4-5 times higher than those of orange and pineapple (Akinwale, 2000). Thus, it is considered a good source of antioxidant compounds (Assunção, and Mercadante, 2003; Brito *et al.*, 2007), with a high level of reducing sugars (fructose and glucose), minerals salts and some amino acids (Lowor and Agyente-Badu, 2009; Adou *et al.*, 2011; Adou *et al.,* 2012). Cashew apple also presents functional properties such as cancer prevention and the prevention of severe gastritis caused by bacteria (*Helicobacter pillory*) (Carvalho *et al.*, 2007; Kubo *et al.*, 1999). Moreover, cashew apples are astringent due to polyphenols, tannins (0.35%), and unknown oily substances (3%) present in the waxy layer of the skin (Cormier, 2008; Michodjehoun-Mestres *et al.*, 2009). Physicochemical contents of cashew apples were significantly affected by the genetic, climatic variations and ecological zones (Agostini-Costa *et al.*, 2002; Lowor and Agyente-Badu, 2009; Adou *et al.*, 2011). Various processes of valorization of cashew apple in juice, alcohol, wine, jam and other products have been documented (Dèdéhou *et al.,* 2015).

Recently, some cultivars of cashew trees found in Benin were identified based on morphological characteristics (Chabi Sika *et al.,* 2015). But there has been no report on the physicochemical composition of these cultivars' fruits (nut and apple). The present study aimed to determine the physicochemical characteristics of apple juice from this cashew tree cultivars.

Material and methods

Study area

The study was carried out in two zones of production of cashew nut in Benin: Bantè (Adjantè village) and Djougou (Founga village). These two localities were selected because they belong to the zones of high production of the cashew tree in Benin and are located in two different agro-ecological zones. Bantè (8° 25' 0" N and 1° 52' 60" E) is located in the agro-ecological zone 5 and Djougou (9°42′30″ N and 1°39′57″) is located in the agro-ecological zone 4 (Fig. 1).The agro-ecological zone 4 covers the western zone of Atacora's department, with a surface of 16 936 km². It is characterized by a Sudanian climate with an annual pluviometry of 800 to 1 300 mm/year. The agro-ecological zone 5 coincides with the production zone of central Benin with a surface of 32 163 km². It is characterized by a Sudanese-Guinean climate with two rainy seasons in the south and one in the north with an annual pluviometry 1 100 to 1 400 mm/ year.

Cashew apple sampling

The trees on which the apples were taken belong to two cultivars 1 and 2 identified in a previous study on the agro-morphological characterization of cashew trees in Benin (Chabi Sika *et al.,* 2015). Inside each cultivar, trees were selected according to the colors of the apples (three red cashew trees and three yellow cashew trees) and were considered replications. In total, 12 trees in each locality were taken into account.

Juice extraction

The collected cashew fruits were transported to the laboratory, where the nuts were detached. The apples were washed with distilled water. Then they were cut and ground with a Mixer. The juice obtained by pressing the mash through a muslin cloth was filtered through a 180 µm mesh sieve and then stored frozen at –10 °C for various physico-chemical analyses.

Physico-chemical analysis

Determination of dry matter, total soluble solid, pH, titrable acidity

The dry matter content was determined by oven drying 15 mL of cashew apple juice at 105°C until a

constant weight was reached (AACC, 44-15 A, 1984). The dry extract refractometric (°Brix) was given at 20 °C by a portable refractometer (Refractometer Fg-113 Brix/ATC 0-32%). Titratable acidity was determined by titration with NaOH (0,1N) in the presence of phenolphthalein. The pH was measured using a digital pH-meter (JENWAY, Model 3505, UK) calibrated with buffers at pH 4.0 and 7.0 (WTW, Weilheim, Germany).

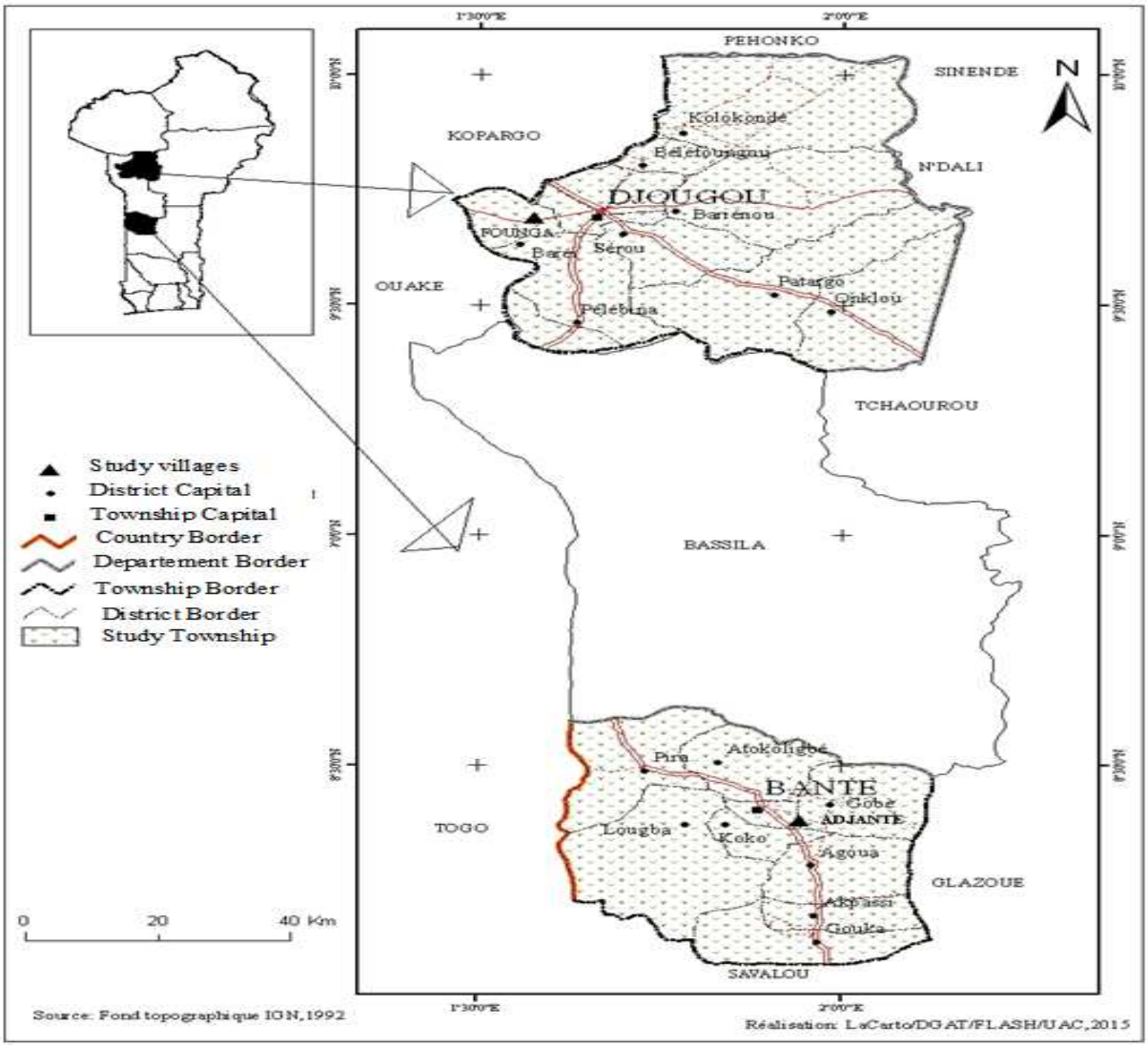

Fig. 1. Study area.

Determination of total phenols, condensed tannins, vitamin C and antioxidant capacity of juice

Total phenolic content in the cashew apple juice was determined using the protocol of Folin-Ciocalteu (FC) reagent in the colorimetric analysis described by Singleton and Rossi (1965) modified by Kayodé *et al.* (2006). The content of condensed tannins was determined by the method of vanillin and was expressed as equivalent to catechin, according to Broadhurst and Jones (1978). Vitamin C was determined by titration with iodine (AOAC, 2000) and the antioxidant capacity was determined by 2,2-diphenyl-2-picrylhydrazyl Radical (DPPH) method.

Determination of sugars and the organics acids

Sugars (fructose, glucose, raffinose and saccharose) and organics acids (citric, acetic, malic, propionic and oxalic) were determined by HPLC. 15 mL of juice are added to 10 mL bidistilled water. The mixture was homogenized. Approximately 0.5 mL of juice was taken with a syringe and filtered using the filter syringes (0.45 µm).The filtrate is collected in a vial of

1 mL for analysis. Soluble sugars and the acids contained in the juice were separated HPLC using an Aminex HPX-87H⁺cation-exchange column (BioRad Hercules, USA) at a column temperature of 37 °C with UV-(210 nm) and IR-detectors (Mestres and Rouau, 1997). Twenty microliters (20µL) of juice were injected and diluted with sulphuric acid 5mM with a flow of 0.6 ml/min. The eluent was 5 mM sulphuric acid at a flow rate of 0.6 mL min^{-1}.

The injection volume of the sample was 20 µL. Sugars and acids were identified by their time of retention and were quantified using the coefficient of response in refractometry determined from the solutions of standards.

Statistical analysis

Procedure General Linear Model (GLM) of the software Statistical Analysis System version 9.2 (SAS v. 9.2) was used for the statistical analyses. These analyses primarily consisted of variance analyses of three factors (agro-ecological zones, cultivars and color of apples). The average values were then compared between them using the test of Student Newman-Keuls at a significance level of $p<0.05$. Principal Component Analyses (PCA) were performed for physico-chemical parameters of cashew apple juice data analysis.

Results

The physico-chemical composition of cashew apple juice produced is indicated in Tables 1 to 4. The dry matter, the pH, the total soluble solids (TSS), and total titrable acidity (TTA) varied from 7.4 to 11.4 %, 4.1 to 4.7, 10.7 to 14.5 °Brix, 0.6 to 1.7% respectively (Table 2). The color of apples (yellow and red) significantly ($p<0.05$) influenced dry matter, TSS and TTA of cashew apple juices of cultivars 2 collected from Djougou. These parameters are neither influenced by cultivars nor by agro-ecological zone.

Table 1. Physico-chemical characteristics of cashew apple juice.

Zones	Cultivars	Color	Dry matter (%)	pH	SST (°BRIX)	TTA (%)
Bantè	1	Red	7.6±0.2a	4.4±0.2a	10.7±0.3a	0.6±0.1a
		Yellow	8.2±0.6a	4.3±0.1a	11.3±0.9a	0.9±0.1a
	2	Red	9.4±0.4a	4.3±0.0a	11.5±1.5a	1.3±0.1b
		Yellow	7.4±0.1a	4.7±0.1a	10.7±0.9a	0.6±0.1a
Djougou	1	Red	9.2±0.5a	4.3±0.1a	11.6±1.1a	0.9±0.0a
		Yellow	9.5±0.6a	4.4±0.3a	12.6±1.5a	0.9±0.1a
	2	Red	11.4±0.3b	4.1±0.1a	14.5±1.1b	1.7±1.2b
		Yellow	8.9±0.1a	4.5±0.2a	11.5±1.2a	0.6±0.0a

In the same column the means followed by the same alphabetical letters are significantly ($p<0.05$) similar ic following the test of Student-Newman-Keuls.

Table 2 shows the level of vitamin C, total phenols, condensed tannins, and antioxidant capacity of juices. The vitamin C content varied between 224 and 327 mg/100 mL; total phenols ranged from 1960 to 4785 mg/L; condensed tannins from 58 to 510(mg Eq Catechin/100 mL); antioxidant capacity varied between 58.3 and 90.8 5(% DPPH inhibition). For these four parameters, there was no significant ($p<0.05$) difference between the samples of juice. The three factors which are color, cultivars and agro-ecological zone, did not significantly ($p<0.05$) influence the content of vitamin C, total phenols and the antioxidant capacity of cashew apples.

Table 3 presents the levels of sugars in cashew apple juices. The concentrations (mg/mL) of glucose, fructose, raffinose, and saccharose varied respectively between 17.3 to 34.6, 31.6 to 65.5, 1 to 2.4 and 0.0 to 1.6. There was no significant ($p<0.05$) difference between glucose, raffinose, and saccharose contents of the samples. Except for fructose level, which was influenced by agro-ecological zone, sugars of cashew apple juice are either influenced by apple colors, nor by cultivars, or agro-ecological zone.

Table 2. Levels in Vitamin C (mg/100g), total phenols, condensed tannins and antioxidant capacity of cashew apples juices.

Zones	Cultivars	Color	Vitamin C (mg/100 mL)	Total phenols (mg Ac gali/L)	Condensed tannin (mgEqCatechin/100 mL)	Antioxidant capacity (% inhibition DPPH)
Bantè	1	Red	306.8 ±21.81a	2360.0±976.5a	96±17a	73.9±11a
		Yellow	319.4±60.1a	2731.7±1034.17a	92±29a	90.7±2.6a
	2	Red	319.0±83.03a	3417.5±1297.5a	286±199a	85.6±10a
		Yellow	327.9±84.3a	3688.3±1495.4a	314±240a	58.3±16.4a
Djougou	1	Red	326.2±28.7a	2350.0±1235.1a	118±30a	90.3±12.1a
		Yellow	259.6±32.0a	2880.0±1115a	301±262a	81.3±11.0a
	2	Red	224.4±39.7a	4785.0±1695.3a	510±398a	90.8±8.2a
		Yellow	260.4±31.8a	1960.0±520.6a	58±33a	78.4±9.5a

In the same column the means followed by the same alphabetical letters are significantly ($p<0.05$) similar ic following the test of Student-Newman-Keuls.

Table 3. Levels of sugars (mg/mL) of cashew apple juice.

Zones	Cultivars	Color	Saccharose	Glucose	Fructose	Raffinose
Bantè	1	Red	1.4±0.1a	28.1±2.8a	51.8±3.8 b	1.0±0.5 a
		Yellow	1.0±0.4a	31.4±2.2a	56.7±3.2 b	1.7±0.0 a
	2	Red	0.0a	34.6±0.7a	65.5±0.7 b	1.5±1.5a
		Yellow	1.7±0.1a	29.1±2.3 a	53.5±3.9b	2.4±0.3 a
Djougou	1	Red	1.6±0.1a	20.8±8.3 a	36.8±0.5 a	1.9±0.3 a
		Yellow	0.7±0.3 a	20.7±8.2 a	36.8±0.3 a	1.3±0a
	2	Red	1.4±0.3 a	27.4±0.3 a	52.0±0.6b	1.6±0,1 a
		Yellow	0.6±0.7 a	20.3±9.1 a	31.6±4.3 a	1.5±0.3 a

In the same column the means followed by the same alphabetical letters are significantly ($p<0,05$) similar ic following the test of Student-Newman-Keuls.

Table 4 gives the organic acids contents of cashew apple juices. The malic acid leads with levels (mg/mL) ranging from 55.3 and 108.5, followed by acetic acid 33.2 to 6.9; citric acid (1.9 to 12.5), oxalic acid (2.4 to 7.7) and finally propionic acid.

The citric, malic and acetic acids of cashew apples juice were significantly ($p<0.05$) influenced by apple colors. While oxalic acid of juice was significantly ($p<0.05$) influenced by agro-ecological zone. Propionic acid was either influenced by apple colors, nor by cultivars, or agro-ecological zone.

Principal components analysis (PCA) on physico-chemical parameters of cashew apple juices

The principal components analysis showed that the first three axes explained 76.24% of the total variation (Table 5). First and second principal components explained respectively 35.73% and 27.48% of the initial variation, while the third accounted for 13.03%. The contribution of each quantitative variable to the formation of the PCs revealed that the phenolics compounds, reducing sugars and organics acids except propionic acid were negatively correlated to the first axis (Table 6).

Table 4. Levels of organics acids (mg/ml) of cashew apple juice.

Localities	Cultivars	Color	Oxalic acid	Citric acid	Malic acid	Acetic acid	Propionic acid
Bantè	1	Red	4.2±0.7b	5.0±3.4a	98.3±5.5 a	6.9±3.6 a	0.0a
		Yellow	5.8±0.1b	2.4±1.7a	83.1±41.8a	16.9±6.2 b	1.0±0.9 a
	2	Red	7.7±0.0b	12.5±8.1b	137.9±15.7 b	27.4±5.1 b	0.0a
		Yellow	6.1±0.4b	3.4±1.7a	85.6±5.4 a	33.2±2.7c	0.5±0.2 a
Djougou	1	Red	3.0±0.3 a	3.7±0.5a	62.5±6.3 a	10.1±2.3 a	0.0a
		Yellow	3.5±0.1a	3.0±0.3 a	53.5±4.6 a	12.9±0.3 a	0.2a
	2	Red	3.9±0.4a	3.8±0.3a	108.5±9.2 b	18.8b	1.1±0.9a
		Yellow	2.4±0.5a	1.9±0.7a	55.3±5.2a	8.5±3.8a	0.4±0.3 a

In the same column the means followed by the same alphabetical letters are significantly ($p<0,05$ similar ic following the test of Student-Newman-Keuls.

Cashew apple juices classification and groups' description

The ACP analysis clustered the juice into three homogenous groups (Fig. 2). Cluster 1 was composed of juices of cashew apples (red and yellow) of cultivar 2 from Bantè and juice of red cashew apples of cultivar 2 from Djougou, while the cluster 2 was formed by juices of cashew apple colors (red and yellow) of cultivar 1 from Bantè. Finally, cluster 3 was formed of juices of cashew apple colors (red and yellow) of cultivar 1 and juice of yellow cashew apples of cultivar 2 from Djougou. The results of the ACP were subjected to an analysis of variance (Table 7). The chemical characteristics of clusters are presented in Table 7. Cluster 1 was characterized by cashew apples with high phenolics, reducing sugars, and malic and acetic acid contents.

The second cluster was mostly characterized by cashew apples with low phenolics and acetic acid contents, high reducing sugars and middle malic acid levels. Cluster 3 contained cashew apples with low phenolics, reducing sugars, malic and acetic acid contents.

Table 5. Values of the five first principal components.

Axis	Values	Proportions	Cumulated proportions
1	6.80	35.73	35.73
2	5.22	27.48	63.21
3	2.48	13.03	76.24
4	2.17	11.40	87.64
5	1.53	8.05	95.69

Discussion

The color of apples (yellow and red) significantly ($p<0.05$) influenced dry matter, TSS and TTA of cashew apple juices of cultivars 2 collected from Djougou. These parameters are neither influenced by cultivars nor by agro-ecological zone. Apple juice pH values obtained in the present work (4.1 to 4.7) are similar to those (4.4 to 4.5) reported by Adou *et al.* (2012) in Côte d'Ivoire and by Lowore and Agyente (2009) (4.1 to 4.5) in Ghana. But, these values are lower than those reported (4.9 to 5.5) in India (Sivagurunathan *et al.*, 2010). Lowore and Agyente (2009) found that the cashew apple color and the ecological zone significantly ($p<0.05$) influence the pH and the acidity of the juice. However, Gunjate and Patwardhan (1995) in India reported that the color or the origin did not affect the pH.

The values of total soluble solids found (10.7 to 14, 5°Brix) were above the minimum value required by Brazilian law which is 10 °Brix (Brasil leis, 2000). The dry matter variation was similar to the results found by Adou *et al.* (2012) in samples of red and yellow cashew juices from Yamousokro (Côte d'Ivoire).

Table 6. Parameters associated with the first tree principal components.

Parameters	PC.1	PC. 2	PC. 3
Vitamin C	-0,14	-0,69*	-0,43
Phenols	-0,72*	0,38	0,25
Tannin	-0,57*	0,53	0,08
Saccharose	0,21	0,00	0,40
Glucose	-0,83*	-0,53	0,07
Fructose	-0,86*	-0,48	0,07
Raffinose	-0,31	0,27	-0,08
Oxalic acid	-0,84*	-0,44	-0,21
Citric acid	-0,65*	-0,23	-0,57
Malic acid	-0,86*	-0,25	-0,02
acetic acid	-0,85*	0,10	-0,18
Propionic acid	-0,26	0,24	0,81*

*= significant ($P<0.05$) computed using standard linearPearson correlation.

The factors, which are color, cultivars and agro-ecological zone, did not significantly ($p<0.05$) influence the content of vitamin C, total phenols and the antioxidant capacity of cashew apples. The levels of ascorbic acid (vitamin C) in cashew apple juices in this study are lower than those obtained by Adou *et al.* (2012). These levels are higher than those found by Lowore and Agyente (2009) which vary between 206.2 and 268.6 mg/100 g. As reported by some authors (Assuncao and Mercadante, 2003; Lowore and Agyente, 2009), the color and location did not influence the vitamin C content in the present study. However, the cashew apple color influences the vitamin C content of red and yellow cashew juices from Yamousokro (Côte d'Ivoire) (Adou *et al.*, 2012).

The total phenols and condensed tannins values obtained in this study are similar to those found by Lowore and Agyente (2009) who worked on the cashew apple juice from three areas of Ghana. These authors reported that the ecological zone significantly ($p<0.05$) influenced the level of phenols in juice. But in this study, the color, the cultivars and the agro-ecological zones do not significantly ($p<0.05$) influence this component.

Table 7. Physico-chemical characteristics of clusters.

Parameters	Cluster			%CV	p value	Significance
	1	2	3			
Phenols	3963,6b	2545,85a	2396,667a	30,2	0,036	*
Tannin	370,00	94,00	159,00	70,7	0,077	Ns
Glucose	30,37b	29,75b	19,6a	22,8	0,011	*
Fructose	57,00b	54,25b	35,06a	24,4	0,008	**
Saccharose	1,03	1,20	0,97	55,8	0,932	Ns
Raffinose	1,83	1,35	1,57	25,8	0,503	Ns
Malic acid	110,67c	90,70b	57,10a	33,9	0,034	*
Acetic acid	26,47b	11,90a	10,50a	55,5	0,038	*
Oxalic acid	5,90	5,00	2,97	39,2	0,105	Ns
Citric acid	6,57	3,70	2,87	75,8	0,446	Ns
Propionic acid	0,53	0,50	0,20	111,0	0,686	Ns
Vitamin C	290,43	313,10	282,07	13,4	0,747	Ns
Composition	B2R	B1R	D2Y			
	B2Y	B1Y	D1Y			
	D2R		D1R			

Values with different letters on the same line are significantly different at 5% according to SNK test.

Legend : D1R :Djougou Cultivar 1 color Red ; D1Y :Djougou Cultivar 1 color Yelow ; D2R :Djougou Cultivar 2 color Red ; D2Y :Djougou Cultivar 2 color Yelow ; B1R :Bantè Cultivar color Red ; B1Y :Bantè Cultivar 1 color Yelow ; B2R :Bantè Cultivar 2 color Red ; B2Y :Bantè Cultivar 2 color Yelow.

There was no significant ($p<0.05$) difference between glucose, raffinose, and saccharose contents of the samples. Except for fructose level which was influenced by agro-ecological zone, sugars of cashew apples juice are either influenced by apple colors, nor by cultivars, or agro-ecological zone. Glucose, fructose and saccharose are the major sugars found in cashew apples, as also noted by other authors (Sivagurunathan *et al.*, 2010; Adou *et al.*, 2012). The levels of saccharose, glucose and fructose determined in the present study were lower than those found by Adou *et al.* (2012). Lowore and Agyente (2009) found that the cashew apple color and the ecological zone significantly ($p<0.05$) influence the level of sugars in cashew apple juices.

The citric, malic and acetic acids of cashew apples juice were significantly ($p<0.05$) influenced by apple colors. While oxalic acid of juice was significantly ($p<0.05$) influenced by agro-ecological zone. Propionic acid was either influenced by apple colors, nor by cultivars, or agro-ecological zone.

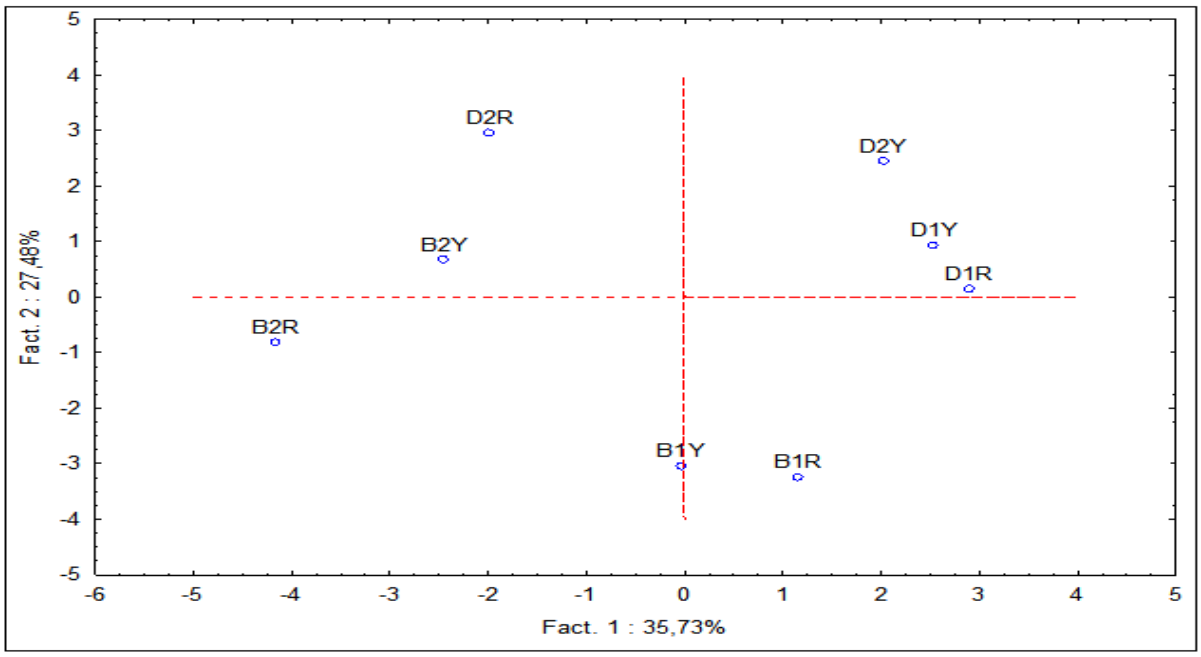

Fig. 2. Projection of the individuals on the factorial design 1*2.

Legend : D1R :Djougou Cultivar 1 color Red ; D1Y :Djougou Cultivar 1 color Yelow ; D2R :Djougou Cultivar 2 color Red ; D2Y :Djougou Cultivar 2 color Yelow ; B1R :Bantè Cultivar color Red ; B1Y :Bantè Cultivar 1 color Yelow ; B2R :Bantè Cultivar 2 color Red ; B2Y :Bantè Cultivar 2 color Yelow .

The variations in the physico-chemical characteristics of cashew apple juices from differents locations can be associated with changes in soil conditions, cultural practices and other climatic conditions such as temperature and humidity (Egbekun and Otiri, 1999).

Otherwise, the small significant difference between the cultivars could be explained by the fact that these varieties could have the same genotypes and that the morphological and color differences are induced by the phenotypic plasticity phenomenon. Indeed, phenotypic plasticity describes the "property given to genotype to produce different phenotypes response in distinct environmental conditions" (Pigliucci, 2001). Phenotypic plasticity is widespread in nature and many examples can be observed in both animals and plants (Pfennig *et al.* 2010).

Conclusion

Physico-chemical compositions of red and yellow cashew apple juice of both agro-ecological zones were globally similar. They are rich in vitamin C and have high antioxidant capacity. The cashew apple is rich in nutritional composition but is ignored because of its astringency. The cashew apple juice can be a good alternative to daily supplementation with vitamin C for children and adults. Moreover, the levels of sugars in the juice of cashew apples are a good substrate for fermentation for the production of wine, alcohol, and vinegar.

Acknowledgements

The authors are thankful to the University of Abomey-Calavi through the project *Biodiversity and valorization of cashew products in Benin (PROANAC)* for providing the funds to investigate this research.

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