

Impact of season and maturity stage on biochemical parameters of *Averrhoa carambola* fruits

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Abstract

The nutritional quality of fruits can be affected by changes in physiological indicators during fruit ripening, which is why it is important to identify the stage of maturity and the appropriate season for harvesting. Based on this observation, this study was carried out to evaluate the influence of the two different seasons and the stage of maturity on the nutritional parameters of *Averrhoa Carambola*.

The results showed a significant effect ($p < 0.0001$) of the two seasons and maturity stage on the biochemical parameters of *A. carambola* fruits. Ash contents and pH were significantly high in rainy seasons and at the ripe stage. Their values were respectively ($6.80 \pm 0.59\%$) and (3.54 ± 0.27). Moisture and titratable acidity concentrations decreased significantly during maturation while total sugars, reducing and non-reducing sugars, and total soluble solids increased significantly during maturation.

vitamin C and phenolic compounds were more abundant in the dry season and at the green stage. The values were Vitamin C ($15,869.64 \pm 0.03$ mg/g), polyphenols (30.92 ± 0.03 mg GAE/mg), flavonoids (30.59 ± 0.28 mg EQ/g) and tannins (21.02 ± 0.01 mg GAE/g).

This study suggests that there are differences in the biochemical parameters of starfruit between seasons depending on the maturity stage. The data provided useful information on the season and ideal stage of maturity for harvesting and their use in food products.

Keywords: Season; Biochemistry; Maturity stage; Carambola's fruits

1. Introduction

The diversity of fruit species that are underutilized or neglected can help promote agro-biodiversity. Such fruit species have strong potential to challenge food security, nutrition, nutritional and medicinal health. They also participate in income generation, environmental services and the fight against hidden hunger caused by micronutrient deficiencies. However, these fruit species are less known by people, less appetizing on the market demand [1].

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Averrhoa carambola, also known as star fruit, is a medicinal fruit plant from Southeast Asia [2]. Today, she is well-established in other parts of the world [3]. Widely consumed for its taste properties, the fruit is a very good source of nutritional compounds and is generally consumed fresh or in the form of fruit juice. The fruit is a very good source of natural antioxidants, phenolic compounds such as gallic acid in the form of gallotannin, catechins and epicatechins [4, 5]. It is therefore a potential functional food [6].

Furthermore, hypotheses have been made regarding the time and season for the collection of different parts of medicinal plants. According to [7, 8]. knowledge of the variation in the phytochemical content of plants depending on the seasons is essential for functional food producers in order to meet consumer demand.

Thus, the variation of seasons has created a new challenge for scientific research. That of exploring its influence on the nutritional quality of fruit plants. The objective of this work is to understand the impact of the seasons (dry and rainy) and the stage of maturity on the nutritional and antioxidant quality of the fruits and leaves of the carambola tree

2. Material and methods

2.1. Material

The material consists of carambola fruits harvested at different stages of maturity over two seasons, namely the dry season which extended from June to August 2022 and the dry season from December to February 2023. The fruits were collected at Attinguié in the south of Côte d'Ivoire, a town belonging in the district of Abidjan. They were transported in closed coolers to the Laboratory of Industrial Processes and Synthesis of the Environment and New Energies (LAPISEN) of the Felix Houphouët-Boigny National Polytechnic Institute.

2.2. Methods

2.2.1. Sample preparation

Each sample was washed with distilled water. The fruits were separated by maturity stage and cut into thin slices with a stainless-steel knife. Some fresh portions were taken to determine moisture, vitamin C, titratable acidity, pH and soluble solids. The remainder was dried in an electric hot air food at 45°C for 48 hours. The dried samples were ground in a MOULINEX brand Blender with stainless steel blades and stored in hermetically sealed bottles in the refrigerator at 6°C.

2.2.2. Analysis

Moisture

Content was determined using [9] method by drying samples in a hot air circulating oven (Mermet ULM 800, Germany) at $105 \pm 2^\circ\text{C}$ until a constant.

Ash content

The method used to determine ash was that described by [9]. 5 g of sample, oven-dried (Electric hot air food dryer) for two days at 45°C, then ground, were weighed into a porcelain incineration crucible of known mass. The sample was then placed in a muffle furnace (NABERTHERM gamme FA1203B) and calcined at 600°C for 4 hours until a constant mass was obtained. After the crucible was removed from the muffle furnace and cooled in a desiccator, it was weighed again.

pH

Five grams (5 g) of sample pulp will be suspended in 20ml of distilled water. After vigorous magnetic stirring for 1 hour, the pH will be determined in each extraction solution using a pH meter, which will be calibrated using standardized pH 4 and 7 buffers.

Titratable acidity

After reading the pH a volume of 10ml is taken for the determination of titratable acidity by titrimetric assay with a sodium hydroxide solution (0.1 N) in the presence of a colored indicator (phenolphthalein).

Total soluble solids

Total soluble solids (TSS) of *A. carambola* fruits were determined using a refractometer (DSA E-Scan, Electron Machine Corp., Umatilla, FL, USA with an accuracy of 0.005°Brix). Indeed, a thin portion of crushed fruit was placed in the center of the refractometer blade and the reading was done by receiving the eye at the base of the refractometer.

Total sugars

The determination of total sugars was carried out according to the method of [10] using phenol and sulfuric acid. To determine the concentration of total sugars in the sample, 0.1 mL of the extract will be taken, and 0.9 mL then treated with phenol (1 mL) at 5% (m/v) and sulfuric acid (5 mL). Then the optical density will be read at 490 nm on the spectrophotometer (340-1000nm Single Beam UV Visible Spectrophotometer). The sugar concentration of the test will be determined from the calibration curve.

Reducing sugar

The determination of reducing sugars was carried out according to [11] using 3,5-dinitro-salicylic acid (DNS). To determine the concentration of reducing sugars in the tests, 1 mL of the extract is taken + 1 mL of DNS then heated for 5 minutes by boiling water. Then, 10 mL of distilled water will be added to each tube and the optical density will be read with a spectrophotometer (340-1000nm Single Beam UV Visible Spectrophotometer) at 540 nm against a blank. The assay will be carried out in triplicate for each test.

Non-reducing sugar

Non-reducing sugars content was calculated by difference between total sugar and reducing sugar.

Ascorbic acid

Ascorbic acid content is measured by the 2, 6-dichloroindophenol (2, 6 DCIP) titration method according to [12]. For the standard ascorbic acid solution, 50mg of ascorbic acid are accurately weighed and diluted with the metaphosphoric acid/acetic acid solution in a 50ml volumetric flask protected from light.

To measure the juice, first extract it with the same quantity of metaphosphoric acid/acetic acid. Take 2 mL of this solution and measure out the 2.6 DCIP. Also measure out 2 mL of the ascorbic acid solution.

2.3. Phenolics component

2.3.1. Total polyphenols

Determination of total polyphenols was used [13] method. 2.5 mL of diluted (1/10) Folin-ciocalteu reagent was added to 30 μ L of *A. carambola* fruit. The mixture was kept for 2 min in the dark at ambient temperature, and 2 mL of calcium carbonate solution (75 g/L) was added. Then, the mixture was placed in a water bath at 50°C during 15 min, then rapidly cooled. The absorbance was measured at 760 nm. The tests were performed in triplicate for each sample. A calibration line was performed with gallic acid at different concentrations (1 μ g/mL; 2 μ g/mL; 4 μ g/mL; 6 μ g/mL; 8 μ g/mL; 10 μ g/mL; 12 μ g/mL; 15 μ g/mL; 20 μ g/mL). The concentration of polyphenols was expressed in grams per liter of gallic acid equivalent extract (mg/g, GA Equivalent).

2.3.2. Total flavonoids

The method of [14] was used for the determination of total flavonoids. In a 25 mL flask, 0.75 mL of 5% (w/v) sodium nitrite (NaNO₂) was added to 2.5 mL of extract. 0.75 mL of 10% (w/v) aluminium chloride (AlCl₃) was added to the mixture and incubated for 6 minutes in the dark. After incubation, 5 mL of sodium hydroxide (1N NaOH) was added and the volume was made up to 25 mL. The mixture was shaken vigorously before being assayed using a UV-visible spectrophotometer. The reading was taken at 510 nm. Trials were carried out in triplicate. Flavonoid content was expressed in grams per liter of quercetin equivalent extract.

2.3.3. Tannins content

The tannins were determined by Folin-Ciocalteu method of [15]. 0.1 mL of the extract was added to a flask containing 7.5 mL distilled water. 0.5 mL Folin-Ciocalteu reagent (Sigma-Aldrich) 1 mL Na₂CO₃ solution (Sigma-Aldrich) and diluted to 10 mL with distilled water. The mixture was shaken vigorously and kept at room temperature for 30 minutes. Absorbance was measured against the blank at 725 nm with an UV/Visible spectrometer and was compared according to a standard curve OD Gallic acid, the results being expressed mg GAE/g.

2.4. Statistical analysis

All experiments in this study were performed in triplicate ($n= 3$) and results were expressed as mean \pm standard deviation. Two-way analysis of variance ANOVA was performed on the complete data set to test the main effects of seasons and maturity stage on the biochemical parameters of *A. carambola*. Means with significant differences were separated using Student Newman-Keuls post hoc test. Analyses were carried out using the Statistica 7.1 software.

3. Results

3.1. Physicochemical parameters

The effect of maturity stage and seasons (dry and rainy) on physicochemical parameters was presented in Tables 1 and 2. Humidity was higher in the dry season, ranging from 93.01 ± 0.22 to 92.09 ± 0.31 %. The pH is higher in the rainy season (2.2 ± 0 to 3.41 ± 0.03) than in the dry season (1.86 ± 0.00 to 2.83 ± 0.00). Ash concentration was higher in the rainy season (6.84 ± 0.39 to 6.80 ± 0.59). Titratable acidity values were low in the rainy season (5.33 ± 0.23 to 2.63 ± 0.23 meq. g/100g) and high in the dry season (13.45 ± 0.05 to 6.44 ± 0.06 g/l). A high level of total sugars (10.4 ± 0.1 to 14.4 ± 0.1 mg/g), reducing sugars (0.86 ± 0.01 to 1.56 ± 0.02 mg/g), non-reducing sugars (9.54 ± 0.01 to 12.84 ± 0.02 mg/g) and soluble dry extract (5 ± 1 to 8 ± 1) during the dry season. The pH, total sugars, reducing sugars, non-reducing sugars and soluble dry extract increased during ripening. According to the results of the analysis of variance, the factors season and ripening stage had significant effects ($p<0.05$) on the physicochemical parameters of *A. carambola* fruits.

Table 1 Moisture, pH, titratable acidity and ash concentration of *A. carambola* according to maturity stage and seasons

Seasons	Stage of maturity	Moisture (%)	pH	titratable acidity meq. g/100g	Ash (%)
Ds	FV	$93.01\pm 0.22a$	$1.86\pm 0.00f$	$13.45\pm 0.05a$	$4.63\pm 0.29d$
Ds	FVC	$92.67\pm 0.32a$	$1.95\pm 0.00f$	$12.36\pm 0.07b$	$4.57\pm 0.25d$
Ds	FJV	$92.73\pm 0.13a$	$2.11\pm 0.01e$	$10.951\pm 0.00c$	$5.25\pm 0.45cd$
Ds	FJO	$92.87\pm 0.12a$	$2.34\pm 0.00d$	$6.60\pm 0.27d$	$5.87\pm 0.2bc$
Ds	FO	$92.09\pm 0.31b$	$2.83\pm 0.00c$	$6.44\pm 0.06d$	$5.03\pm 0.32cd$
Rs	FV	$91.54\pm 0.06c$	$2.2\pm 0.00de$	$5.33\pm 0.23e$	$6.84\pm 0.39ab$
Rs	FVC	$91.82\pm 0.05bc$	$2.76\pm 0.05c$	$5.57\pm 0.06e$	$6.95\pm 0.79ab$
Rs	FJV	$91.64\pm 0.11d$	$3.043\pm 0.13b$	$4.56\pm 0.23f$	$7.17\pm 0.52a$
Rs	FJO	$91.72\pm 0.17d$	$3.54\pm 0.27a$	$3.59\pm 0.00g$	$6.88\pm 0.42ab$
Rs	FO	$91.26\pm 0.37e$	$3.41\pm 0.03a$	$2.63\pm 0.23h$	$6.80\pm 0.59ab$
Statistical significance of the sources of variation (probability>F) from ANOVA					
Seasons (S)		<0.0001	<0.0001	<0.0001	<0.0001
Maturity (M)		<0.05	<0.0001	<0.0001	<0.5
S*M		<0.0001	<0.0001	<0.0001	<0.5

The values are averages of the standard deviations of three measures ($n = 3$). Mean values in the same column with different superscript letters were significantly different from each other ($p<0.05$). FV: Green fruit, FVC: Light green fruit, FJV: Yellow green fruit, FJO: Yellow orange fruit, FO: Orange fruit; Ds: Dry season; Rs: Rainy season.

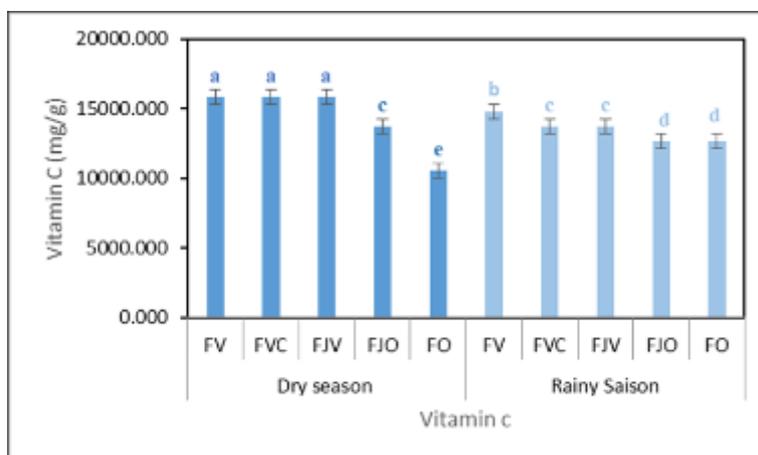
Table 2 Total sugars, reducing sugars, non-reducing sugars (mg/g) and soluble solids concentration of *A. carambola* according to maturity stage and seasons

Seasons	Maturity stage	T. sugars (mg/g)	R. Sugars (mg/g)	NR Sugars (mg/g)	TSS (°Brix)
Ds	FV	10.4±0.1e	0.86±0.01d	9.54±0.01d	5±1bc
Ds	FVC	11.5±0.1d	0.75±0.01e	10.79±0.11c	6±1abc
Ds	FJV	12.9±0.1c	1.02±0.01c	11.91±0.11b	7±1 ab
Ds	FJO	13.43±0.06b	1.52±0.02a	11.88±0.02b	7±1aba
Ds	FO	14.4±0.1a	1.56±0.02a	12.84±0.02a	8±1a
Rs	FV	4.7±0.1j	0.19±0.01j	4.61±0.01d	4±1c
Rs	FVC	5.6±0.1i	0.3±0.1i	5.3±0.1g	4±1c
Rs	FJV	6.2±0.1h	0.37±0.01h	5.82±0.01f	5±1bc
Rs	FJO	9.6±0.1g	0.48±0.01g	9.11±0.01e	6±1abc
Rs	FO	10.2±0.1f	0.63±0.06f	9.60±0.01d	7±1ab a
Statistical significance of the sources of variation (probability>F) from ANOVA					
Seasons (S)		<0.0001	<0.0001	<0.0001	<0.0001
Maturity (M)		<0.0001	<0.0001	<0.0001	<0.0001
S*M		<0.0001	<0.0001	<0.0001	<0.0001

T: total; R: reducing; NR: none reducing. Data are expressed as mean ± standard deviation (n = 3). Mean values in the same column with different superscript letters were significantly different from each other (p<0.05). FV: Green fruit, FVC: Light green fruit, FJV: Yellow green fruit, FJO: Yellow orange fruit, FO: Orange fruit. Ds: Dry season; Rs: Rainy season

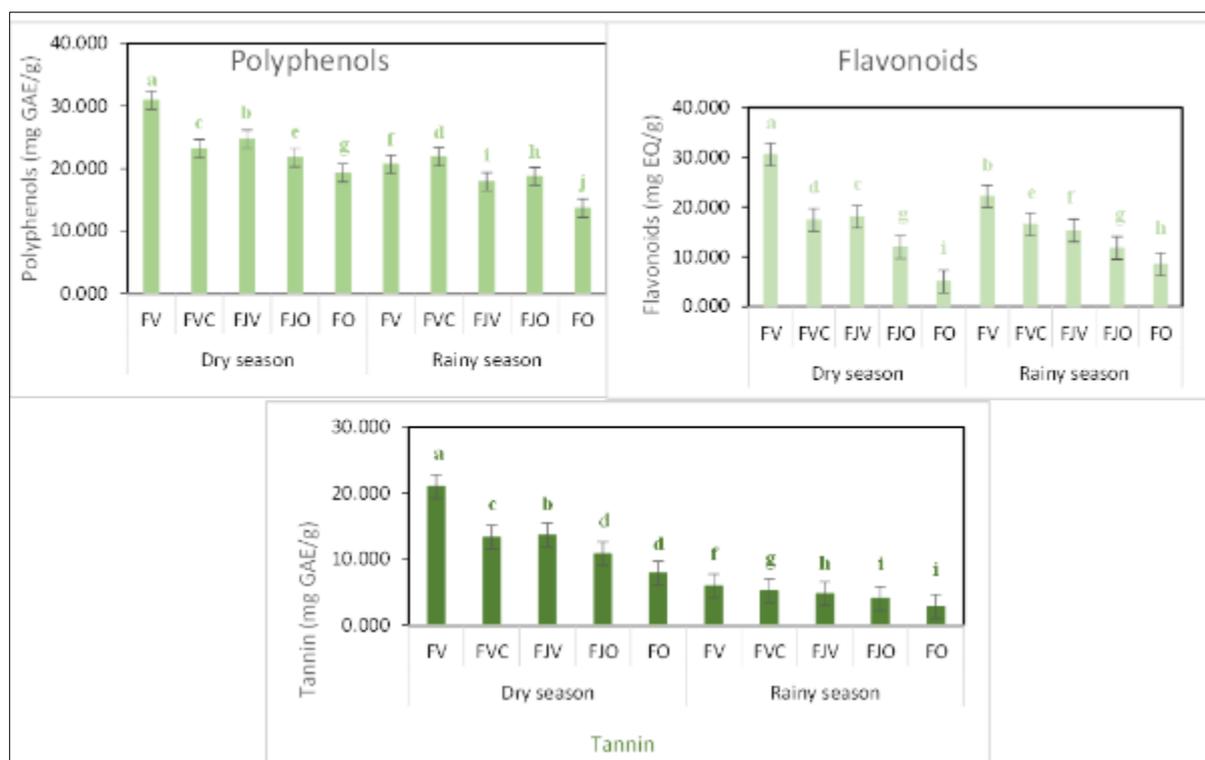
3.2. Antioxidant component

The results for vitamin C, polyphenols, flavonoids and tannins during ripening are shown in Figures 1 and 2. The values for vitamin C, polyphenols, flavonoids and tannins varied significantly with decreasing maturity in both seasons. However, high levels of vitamin C were observed in the dry season, ranging from (10,548.260±0.03 to 15,869.640±0.03 mg/g) with a peak at the FVC stage, Polyphenols (19.29±0.05 to 30.92±0.03 mg GAE/g), flavonoids (5.05±0.01 to 30.59±0.28 mg EQ/g) and tannins (7.88±0.01 to 21.02±0.01 mg GAE/g). Seasons and ripening stage had significant effects (p<0.05) on vitamin C, polyphenols, flavonoids and tannins content in *A. carambola* fruit.



Data are expressed as mean ± standard deviation (n = 3). Mean values in the same column with different superscript letters were significantly different from each other (p<0.05). FV: Green fruit, FVC: Light green fruit, FJV: Yellow green fruit, FJO: Yellow orange fruit, FO: Orange fruit.

Figure 1 Vitamin C concentration of *A. carambola* according to maturity stage and seasons



Data are expressed as mean \pm standard deviation ($n = 3$). Mean values in the same column with different superscript letters were significantly different from each other ($p < 0.05$). FV: Green fruit, FVC: Light green fruit, FJV: Yellow green fruit, FJO: Yellow orange fruit, FO: Orange fruit,

Figure 2 Phenolic compounds concentration of *A. carambola* according to maturity stage and seasons

4. Discussion

The physicochemical and nutritional composition of *A. carambola*, as many other fruits, depends on the variety, the genotype, the maturity of the fruit, the climatic conditions in which it is grown, the type of soil, the nutrients available in the soil, the agronomic management of the plantation and the seasons [16, 17].

The results of the physicochemical parameters of 100 g of fruit showed that it contains a good amount of moisture. Moisture content may be dependent on the habitat and harvest time of the species [18]. Previous studies have reported moisture contents of 87% to 91% [19]. As for pH, it increases during ripening in the two seasons. This is inversely correlated with titratable. This decrease in acids could be due to their use as a substrate during respiration [20]. Ash contents of other carambola varieties have been reported in studies by [21] and [22] respectively. The results obtained were superior to those of these authors.

Total sugars were high compared to studies performed ($2.91 \pm 0.6\%$ to $5.60 \pm 0.73\%$) by [21] as well as reducing sugars content is lower ($2.80 \pm 0.46\%$ to $5.04 \pm 0.44\%$). While non-reducing sugars are much higher than those described in studies (2.04%) on ripe carambola found by authors [23]. According to [24] sweet variety starfruit had a sugar concentration more important than that of sour starfruit varieties which were about 6.25 g/100g. Thus, the starfruit used in the present study could be the sweet variety given its high total sugar content. The total solids soluble value of *A. Carambola* at full maturity is between 8 and 7° Brix. Indeed, at maturity, the increase in TSS could be due to the transformation of metabolic substances into soluble compounds which are mainly sugars. It mainly involves hydrolysis of the starch stored in the fruit during ripening [25].

An appreciable decrease in Vitamin C content could be noticed in all samples on maturation. The use of vitamin C in the fruit's biosynthesis of ethylene, oxalate, and tartrate has resulted in a decrease in vitamin C content [26]. The maximum Vitamin C content was noticed in dry season. This result is quite interesting as Vitamin C is well known for its curative properties from cold to cancer [27]. The vitamin C result obtained are higher than those authors [2].

Seasons, altogether with maturity stage, had a strong impact on the polyphenol, flavonoids and tannin content in *A. carambola*. Also amount of phenols in both the seasons showed a characteristic decrease on maturation. The results of total phenolic content reported in other fruits also support our findings which showed that as fruit ripening continued

from ripe to overripe stage, a decrease is observed in phenolic content total [28, 29]. These authors suggest that the decrease is due to polyphenol oxidase activity. Dry season has the maximum phenol content, Phenolic compounds are plant secondary metabolites, which have numerous health promotion effects especially due to its antioxidant potential [30, 31]. this indicates that phenol production is lowest during the rainy season, when there is heavy rainfall. Also, during the dry season, there is a long period of sunlight for the trees, which favors the accumulation of phenolic compounds in their different parts due to UV rays. [32]. The total polyphenols and flavonoids found are higher than those found in domestic fruits such as apples, with levels ranging from 66.2 and 211.9 mg/100 g [33] and 17.54 to 143.00 mg EC/100 g [34].

Tannin is reported to possess anti-oxidant and anticancer activities [35]. However, reports also show that tannin has anti-nutritive activity [36]. The tannin content obtained at all stages of maturity and whatever the season are higher compared to previous studies. The studies of [23] exhibit tannin contents (0.28 ± 0.01 , 0.22 ± 0.01 and 0.14 ± 0.01 mg/100 g) respect at the green, half-ripe and ripe stage carambola fruits.

5. Conclusion

The present study reveals an interaction between seasons and maturity stage from the mature green stage to ripe fruits of *A. carambola*. The fruits of this study are a good source of vitamin C and phenolic compounds in dry seasons at the green stage. However, the mineral content are important in rainy season at orange stage. With extensive research and promotion these underutilized fruits could become an additional food source for the world.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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