

## CHANGES IN THE BIOCHEMICAL PROFILE OF FRUITS OF TWO SPECIES OF *AVERRHOA* DURING DEVELOPMENT

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### ABSTRACT

**Objectives:** The species of the genus *Averrhoa* are well known for their economic as well as medicinal importance. Nutritional and anti-nutritional characterization of the two available species, *A.bilimbi* L. and *A.carambola* L. (sweet and sour) were carried out in the present study.

**Methods:** Fruit quality was assessed at two maturity stages using ten nutritional parameters, five antinutritional parameters and HPLC profiling of six organic acids according to standard procedures were done.

**Results:** *Averrhoa carambola* sweet ripe fruits showed high values for eight out of ten nutritional parameters studied and relatively low values for the antinutritional parameters. Anti-nutritional parameters such as phytates, saponins, oxalates, tannins and phenols were well below the permissible levels in all the samples. HPLC analysis clearly indicates that oxalic acid is the predominant organic acid in all the samples (both stages), the only exception being *A. carambola* sweet ripe, where malic acid dominates.

**Conclusion:** All the fruit samples of *Averrhoa* taken into consideration for the study showed that they are nutritious. Since the antinutritional factors studied were well below the permissible levels in all the samples they appear to be safe for consumption.

**Keywords:** *Averrhoa*, Nutritional, Antinutritional, Organic acids, HPLC analysis

### INTRODUCTION

Fruits are considered nature's gift to mankind. They are important sources of essential dietary nutrients. Recently, fruits are also being recognized as rich sources of bioactive compounds including antioxidants, natural sugars and organic acids [1]. Evidences gathered from many clinical studies support the fact that consumption of fruits and vegetables ameliorate age-related diseases, cancers and heart diseases [2,3]. Thus, the popularity and acceptability of fruits among consumers is not only due to their high nutritive value and characteristic taste but also due to their known health promoting properties.

Information on the nutrient composition of fruits is considered essential since the data provides a measure of the fruit quality and is utilized by government and public agencies and also agricultural industries to promote fresh produce. In addition, the development of nutraceuticals and functional foods from nutritionally rich fruits is a promising research area. For this reason, the assessment of chemical constituents and organic acid contents of fruits is of interest to agriculturalists, industrialists and nutritionists.

The characteristic flavor of fruits is usually attained as the fruit ripens. During fruit ripening, the biochemistry, physiology and structure are developmentally altered to influence the appearance, texture, flavour and aroma [3].

The changes in the composition of different chemical constituents and organic acids during fruit ripening play a key role in flavor development and can affect the chemical and sensory characteristics such as pH, total acidity, microbial stability and sweetness [5]. Chemical composition is one of the most important quality criteria for fruits and is measured while selecting them in the preparation of various fruit products. The sugar type and content, organic acids, pigments and many other essential constituents enable the ripe fruits to produce many value added products [6,7]. Different types of fruits contain different amounts of organic acids. Organic acids act as intermediates in the metabolic processes and are directly involved in growth, maturation and senescence. Organic acids determine the characteristic fruit flavor and play a major role in quality criteria

such as stability, color and flavor [7,8]. The high amount of acids in ripe fruits influences its palatability and may restrict its use for specific purposes as in the manufacture of beverages, drinking juices and wines [8,9]. Fruit juices have a low pH, because they contain high levels of organic acids. It has been advocated that some principal acids in ripe fruits could be used to enhance beverage flavours such as oxalic, citric, malic, ascorbic and tartaric acids [10]. The edible ripe fruits usually contain citric and malic acid in higher quantities and trace amounts of tartaric, benzoic, oxalic and succinic acids [11].

Besides the nutritional advantages of fruits, they may also contain certain antinutritional factors (ANFs). The ANFs are substances that are generated in fruits during the course of normal metabolism. They may be produced by different mechanisms (e.g., inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed) which exert effects contrary to optimum nutrition. In the present study, ANFs such as phytates, oxalates, tannins, phenols and saponins were taken into consideration. The present study is concerned with the fruits of two species of *Averrhoa* belongs to the family Oxalidaceae. The genus is represented by two cultivated species, namely *A.bilimbi* L. (Syn: *Averrhoa obtusangula* Stokes) and *A.carambola* L. (Syn: *Averrhoa acutangula* Stokes). Both these species of *Averrhoa* are widely cultivated throughout the tropical countries for their fruits and are grown in the backyards of most homesteads. The genus invites special attention on account of its economic and medicinal importance but it still remains unexploited to a certain extent. Most of the studies till date are restricted to the physicochemical properties of the sour *A.carambola* [12,13,14,15].

The aim of the present study is

- i) To determine the nutritional and antinutritional content of the fruits of *A. bilimbi* and *A. carambola* (Sour and Sweet) in two different stages of development.
- ii) To determine the organic acid (oxalic, tartaric, malic, citric, ascorbic and  $\alpha$ -keto glutaric acid) composition of fruits in two different stages of development.

## MATERIALS AND METHODS

### Collection of Sample

Fruit samples of *A. bilimbi* and *A. carambola* (Sour and Sweet type) at two different developmental stages, collected from Thiruvananthapuram district of Kerala state during the period February-May, 2013 were utilized for the present study. After the harvest, the fruits were washed in running tap water, blotted dry and classified into two apparent maturities (unripe and ripe) according to their age calculated after flowering as:

- A. bilimbi* unripe (2 weeks after flowering - BU)
- A. bilimbi* ripe (5 weeks after flowering - BR)
- A. carambola* sour unripe (3 weeks after flowering- CSU)
- A. carambola* sour ripe (8 weeks after flowering - CSR)
- A. carambola* sweet unripe (3 weeks after flowering - CTU)
- A. carambola* sweet ripe (8 weeks after flowering - CTR)

### Nutritional analysis

The edible portion of the fruit was homogenized using a mortar and pestle. The biochemical analysis like reducing sugar content [16], total carbohydrates [17], starch [18], proteins [19], lipids [20], vitamin C [21], Phenols [22], Tannins [23], Chlorophyll [24] and  $\beta$ -carotene [25] were estimated in the pulp at two stages of maturity. Six samples of each maturity group were analyzed in triplicate. The pH of juices was determined with a pH meter (Oakion pH tester 10, Singapore) and the titratable acidity (TA) by titrating 10 ml sample with 0.1 N NaOH to pH 8.1. TA was expressed as 'g' oxalic acid/l [26].

### Anti nutritional analysis

The antinutritional factors considered for the study include oxalates [26], phytates [26], saponins [26], phenols [26] and tannins [26]. All the ANFs were estimated in the fruit pulp at two stages of maturity and six samples of each maturity group were analyzed in triplicate.

### Statistical Analysis

Univariate analysis of variance (ANOVA) was performed in each individual fruit trait using Minitab 2000 Version 13.2 statistical software. A probability value of  $p < 0.05$  was considered to denote a statistically significant difference.

### HPLC Conditions Used for Organic Acid Analysis

HPLC analysis of organic acids in fruit juices were performed on HPLC system equipped with a Waters 600 series pump, 7725 Rheodyne injector and 2487UV Detector.

The separation was carried out using a C 18 column (250 mm  $\times$  4.6mm). The mobile phase consisted of 0.1% phosphoric acid ( $H_3PO_4$ ) in distilled water, and was delivered at a flow rate of 1.0 ml/min. The sample juices, standards and mobile phase were filtered through Nylon membrane filter (0.45 mm) before use. Target compounds were detected at 214 nm and 254 nm.

### Organic acid standards

The organic acid standards used for the analysis included, tartaric acid, oxalic acid, malic acid, ascorbic acid,  $\alpha$ - keto glutaric acid and citric acid. Organic acids were obtained from ALDRICH Co. (Sigma-Aldrich Chemie, Steinheim). The organic acid standards were diluted at different concentrations to prepare the standard curve (peak area versus concentration in mg/l).

Standard solutions and juice samples were filtered through a 0.45 mm millipore membrane filter (HAWP Millipore Co., Bedford) and then 20  $\mu$ l aliquots of standard or sample solutions were injected into the HPLC system.

## RESULTS AND DISCUSSION

### Effect of Ripening on pH and Titratable Acidity (TA)

The relatively lower pH values observed for fruits of species of *Averrhoa* are probably responsible for their acidic flavour. The pH values increased gradually with the advancement in maturity (Table 1). As expected, the ripe fruits of both species were significantly less acidic than the unripe fruits. Among the tested fruit samples, the highest pH value (3.7) was recorded for CTR and lowest pH value (1.57) recorded for BU. Thus *A. bilimbi* is more acidic and the ripe sweet *A. carambola* the least acidic. Concomitant with the pH values, the titratable acidity decreased with advancement in maturity. Significant increase in pH and decrease in titratable acidity (TA) could be noticed in the fruit samples in relation to advancement in maturity. A similar trend was also reported by Narain et al [15] in *A. carambola* fruits. All the sample juices showed a slight increase in pH, while the TA levels decreased as the fruits matured. Generally higher values of TA in a typical fruit are negatively linked with the flavor and consumer acceptance [27].

**Table 1: pH and titratable acidity (g l<sup>-1</sup>oxalic acid equivalent) of fruit samples of *Averrhoa* at different maturity stages**

Parameter	BU	BR	CSU	CSR	CTU	CTR
pH	1.57 $\pm$ 0.19 <sup>a</sup>	1.8 $\pm$ 0.16 <sup>b</sup>	2.18 $\pm$ 0.41 <sup>c</sup>	2.43 $\pm$ 0.32 <sup>d</sup>	3.43 $\pm$ 0.21 <sup>e</sup>	3.7 $\pm$ 0.25 <sup>f</sup>
TA (g l <sup>-1</sup> )	0.569 $\pm$ 0.008 <sup>f</sup>	0.434 $\pm$ 0.012 <sup>e</sup>	0.378 $\pm$ 0.005 <sup>d</sup>	0.309 $\pm$ 0.11 <sup>c</sup>	0.296 $\pm$ 0.05 <sup>b</sup>	0.253 $\pm$ 0.09 <sup>a</sup>

\*Values (mean  $\pm$  SE) are average of three samples of each fruit, analyzed individually in triplicate ( $p < 0.05$ ). Different superscripts within the same column represent significant differences for the pH and TA between samples

### Effect of Ripening on other nutritional constituents

The chemical composition of edible fruits is likely to differ as they mature. The chemical composition of *Averrhoa* fruits were studied at different stages of maturity and the results are presented in Table 2. Detectable variation in the chemical parameters could be noticed for both species with advancement in maturity. Total carbohydrate content increased on maturation in all the samples and CTR had the highest value. The study clearly showed that in both sour as well as sweet type of *A. carambola*, starch content decreased on maturation. This is possibly because of the degradation of starch into sugars during development. In the fruits of ripe *A. bilimbi* however, there was an increase in starch content upon ripening. At the same time the reducing sugar level in all the samples increased on maturation, but only a marginal hike could be seen in *A. bilimbi*. In comparison with total carbohydrate content, the protein and lipid levels were very little in both the species. However an increase could be noticed

in all the samples under study upon maturation. CTR possessed the highest amount of proteins.

An appreciable increase in Vitamin C content could be noticed in all samples on maturation. The maximum Vitamin C content was noticed in CTR. In *A. bilimbi* the values were almost stable for both ripe and unripe suggesting that both samples are equally beneficial for consumption. This result is quite interesting as Vitamin C is well known for its curative properties from cold to cancer. A significant decrease in chlorophyll content could be noticed upon ripening while, the  $\beta$ -carotene content showed an increase.

Vitamin C and chlorophyll content obtained in the present study was almost similar to the values reported in sour *A. carambola* by Patil et al [14]. Higher values have been recorded for starch, reducing sugar, protein and lipid contents by Narain et al [15] in CSR. Vitamin C content reported by Oliveira et al [28] and Narain et al [15] in sour *A.*

*carambola* was much lower than the present report. Among the two species of *Averrhoa*, *A.carambola* appears to be more nutritious as most of the nutritional factors analyzed, such as total carbohydrates, reducing sugars, vitamin C and  $\beta$ -carotene exhibit higher values compared to the other samples. On comparing the sour and sweet *A. carambola*, the sweet *A.carambola* appears to possess a greater nutritive potential.

#### Effect of Ripening on other antinutritional constituents

Fruit samples generally vary in their ANF's and the results are presented in table 3. The current study showed that the ANF's viz oxalate, phytate and tannin levels in all samples studied become less upon ripening. This is a good indication since fruits of *Averrhoa* are usually consumed after ripening. Among all the ANF's studied presently, CTR showed the lowest level for all the factors except tannin. Oxalate and phytate content was observed to be high in BU while saponin and tannin content in CSU. Phytic acid has the capability to chelate divalent elements like calcium thus decreasing

their bioavailability [29]. Saponins are naturally occurring oily glycosides occurring in wide variety of plants when eaten, they are dangerous when injected into the blood stream and quickly haemolyse red blood corpuscles [30].

Tannins exhibit antinutritional potentials by precipitating dietary proteins and digestive enzymes to form complexes that are not readily digestible [31]. Tannin is reported to possess anti-oxidant and anticancer activities [32]. However, reports also show that tannin has anti-nutritive activity [33]. Tannin content in all the samples showed a decrease on maturation especially in both sour and sweet type *A. carambola*. Amount of phenols in both the species showed a characteristic increase on maturation. CSR has the maximum phenol content, though it is still below the permissible levels. Phenolic compounds are plant secondary metabolites, which have numerous health promotion effects especially due to its antioxidant potential [34,35]. Several studies have revealed that star fruit contains high amounts of antioxidants and that it is almost similar to that in guava, papaya and banana [36].

**Table 2: Nutritional analysis of fruit samples of *Averrhoa***

Sl.No.	Parameter	BU	BR	CSU	CSR	CTU	CTR
1.	Total Carbohydrates (mg g <sup>-1</sup> tissue)	4.8±0.71 <sup>c</sup>	7.57±0.56 <sup>a</sup>	3.19±0.71 <sup>b</sup>	7.75±0.48 <sup>d</sup>	16.74±0.64 <sup>e</sup>	24.3±0.76 <sup>f</sup>
2.	Starch (mg g <sup>-1</sup> tissue)	3.54±0.16 <sup>f</sup>	3.71±0.420 <sup>e</sup>	3.98±0.493 <sup>d</sup>	3.09±0.752 <sup>a</sup>	2.86±0.479 <sup>b</sup>	2.031±0.831 <sup>c</sup>
3.	Reducing Sugars (mg g <sup>-1</sup> tissue)	0.9±0.07 <sup>a</sup>	2.1±0.13 <sup>b</sup>	4.8±0.19 <sup>c</sup>	7.0±0.24 <sup>e</sup>	6.12±0.15 <sup>d</sup>	11.8±0.31 <sup>f</sup>
4.	Protein (mg/g tissue)	0.20±0.037 <sup>a</sup>	0.50±0.034 <sup>c</sup>	0.47±0.037 <sup>b</sup>	0.62±0.041 <sup>d</sup>	0.49±0.059 <sup>c</sup>	0.79±0.036 <sup>e</sup>
5.	Lipids (mg g <sup>-1</sup> tissue)	0.27±0.03 <sup>a</sup>	0.28±0.04 <sup>a</sup>	0.31±0.06 <sup>b</sup>	0.33±0.03 <sup>c</sup>	0.37±0.07 <sup>d</sup>	0.38±0.04 <sup>d</sup>
6.	Vitamin C (mg g <sup>-1</sup> tissue)	5.23±0.462 <sup>a</sup>	7.69±0.273 <sup>b</sup>	9.13±0.423 <sup>c</sup>	11.72±0.519 <sup>e</sup>	10.38±0.411 <sup>d</sup>	14.98±0.572 <sup>f</sup>
7.	Chlorophylls (mg g <sup>-1</sup> tissue)	0.037±0.004 <sup>f</sup>	0.025±0.007 <sup>d</sup>	0.017±0.002 <sup>c</sup>	0.008±0.001 <sup>b</sup>	0.049±0.006 <sup>a</sup>	0.003±0.001 <sup>e</sup>
	Chlorophyll a	0.056±0.008 <sup>e</sup>	0.035±0.004 <sup>f</sup>	0.059±0.007 <sup>d</sup>	0.036±0.008 <sup>b</sup>	0.034±0.008 <sup>a</sup>	0.034±0.007 <sup>c</sup>
	Chlorophyll b	0.093±0.007 <sup>f</sup>	0.060±0.009 <sup>d</sup>	0.076±0.005 <sup>b</sup>	0.044±0.005 <sup>c</sup>	0.083±0.003 <sup>e</sup>	0.037±0.008 <sup>a</sup>
8.	Total chlorophyll						
	$\beta$ -carotene (mg g <sup>-1</sup> tissue)	0.071±0.003 <sup>a</sup>	0.196±0.08 <sup>c</sup>	0.092±0.004 <sup>e</sup>	0.316±0.003 <sup>b</sup>	0.103±0.009 <sup>d</sup>	0.428±0.06 <sup>f</sup>

\*Values (mean  $\pm$  SE) are average of three samples of each fruit ( $p < 0.05$ ). Different superscripts within the same column represent significant difference for the biochemical parameter considered between the samples.

Of all the ANFs considered in the present study, the most critical one, namely, oxalate showed the lowest value in CTR and highest in BU. A minimum dose of at least 4-5 g of oxalate is to be ingested for causing the death of an adult human being [37]. Also reports have shown that a dosage as high as 10-15g cause fatalities [38].

In the present study the sample that showed the maximum oxalate level was BU, which in turn had only 0.6 g/100g of fresh fruit tissue, which means that it is very much safe for consumption.

Oxalic acid is corrosive to tissue, when ingested; it removes calcium from the blood in the form of calcium oxalate, thereby causing kidney damage. Since the other ANF's included in this study also were present within the dietary permissible levels, it may be suggested that the fruits of both *A. bilimbi* and *A. carambola* are safe for consumption.

#### Effect of Ripening on Organic Acids Composition through HPLC analysis

The fruits of *Averrhoa* vary widely in their organic acid composition during the process of ripening (Table 4, Figures 1-7).

From the HPLC analysis it is clear that oxalic acid is the most prevalent organic acid in all the samples (both stages) except in the sweet *A.carambola* (ripe). In sweet *A.carambola* (ripe) malic acid

level was almost double the amount of oxalic acid. Malic acid level was found to increase on maturation in both sour and sweet type *A.carambola* while in *A.bilimbi* it decreased on maturation. Barker and Solomos [39] observed a marked increase in malic acid during banana ripening also. Malic acid maintains the liver in a healthy condition and helps in the digestion process.

The content of organic acids might be also of interest in that certain acids may lead to a lowering of the postprandial blood glucose and insulin responses [40]. Tartaric acid and ascorbic acid showed an increase in *A. carambola* (both sour and sweet type) on maturation while it decreased or remained stable in *A. bilimbi*. The  $\alpha$ - keto glutaric acid level increased in sweet type *A.carambola* decreased in *A.bilimbi* and the sour type *A.carambola*.

Citric acid content was found to be stable in *A.carambola* (sweet) while slight decrease in both *A.bilimbi* and *A.carambola* (sour) could be noticed. Compared to the other organic acids,  $\alpha$ - keto glutaric acid and citric acid levels could be detected only in trace amounts in the studied samples.

Oxalic acid has been identified as the major organic acid in all the samples of *A.bilimbi* and *A.carambola* studied presently. The study is in agreement with earlier studies in *Carambola* and *Bilimbi*[41,42]. The decrease in oxalic acid content observed during maturation correlates well with the low pH values.

**Table 3: Antinutritional constituents of fruit samples of *Averrhoa***

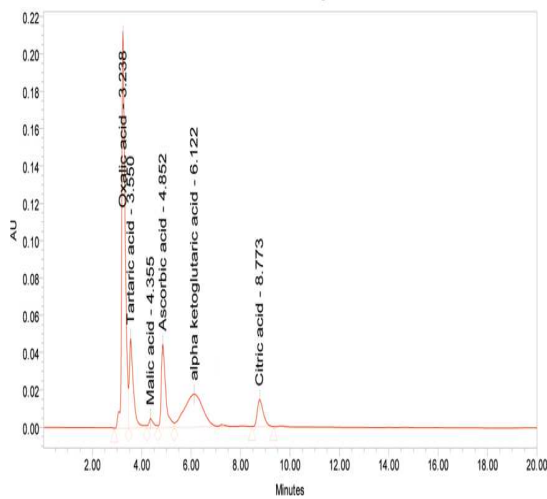
Sl. No.	Parameter (mg g <sup>-1</sup> tissue)	BU	BR	CSU	CSR	CTU	CTR
1.	Oxalate	6.13±0.38 <sup>f</sup>	3.12±0.49 <sup>d</sup>	3.71±0.26 <sup>e</sup>	1.71±0.02 <sup>b</sup>	1.92±0.13 <sup>c</sup>	0.89±0.06 <sup>a</sup>
2.	Saponin	1.20±0.05 <sup>a</sup>	2.34±0.09 <sup>d</sup>	4.82±0.11 <sup>f</sup>	2.12±0.07 <sup>c</sup>	3.91±0.09 <sup>e</sup>	1.90±0.06 <sup>b</sup>
3.	Phenol	2.36±0.49 <sup>a</sup>	4.0±0.23 <sup>b</sup>	5.51±0.28 <sup>d</sup>	7.3±0.58 <sup>f</sup>	4.12±0.68 <sup>c</sup>	6.10±0.87 <sup>e</sup>
4.	Tannin	0.24±0.04 <sup>d</sup>	0.03±0.01 <sup>a</sup>	0.34±0.04 <sup>f</sup>	0.19±0.03 <sup>c</sup>	0.28±0.03 <sup>e</sup>	0.16±0.06 <sup>b</sup>
5.	Phytate	0.079±0.01 <sup>f</sup>	0.06±0.01 <sup>e</sup>	0.05±0.02 <sup>d</sup>	0.03±0.01 <sup>b</sup>	0.04±0.02 <sup>c</sup>	0.03±0.01 <sup>a</sup>

\*Values (mean ± SE) are average of three samples of each fruit (*p* < 0.05). Different superscripts within the same column represent significant difference for the biochemical parameter considered between the samples

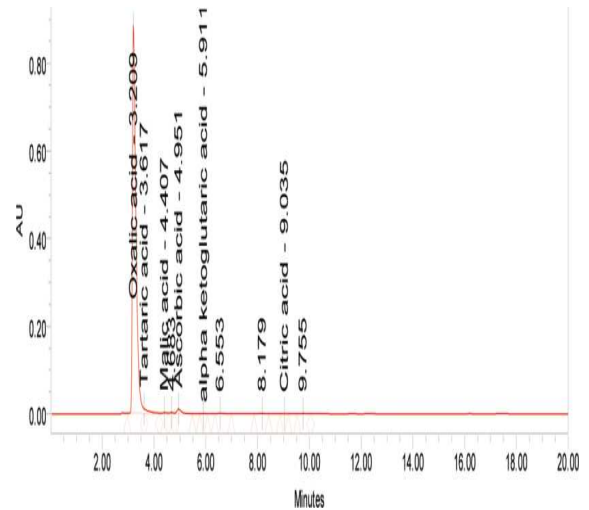
**Table 4: Amount of organic acids found in different samples of *Averrhoa* fruit**

Sl.No.	Organic acids	BU (mg ml <sup>-1</sup> )	BR (mg ml <sup>-1</sup> )	CSU (mg ml <sup>-1</sup> )	CSR (mg ml <sup>-1</sup> )	CTU (mg ml <sup>-1</sup> )	CTR (mg/ml <sup>-1</sup> )
1.	Oxalic acid	7.25±0.42 <sup>f</sup>	6.89±0.42 <sup>f</sup>	8.68±0.54 <sup>f</sup>	7.89±0.37 <sup>f</sup>	6.86±0.41 <sup>f</sup>	2.59±0.13 <sup>e</sup>
2.	Tartaric acid	0.48±0.01 <sup>d</sup>	0.42±0.03 <sup>d</sup>	0.81±0.04 <sup>d</sup>	0.89±0.09 <sup>c</sup>	0.83±0.07 <sup>d</sup>	0.95±0.12 <sup>d</sup>
3.	Malic acid	1.44±0.03 <sup>e</sup>	0.68±0.02 <sup>e</sup>	1.25±0.27 <sup>e</sup>	1.56±0.05 <sup>e</sup>	2.38±0.04 <sup>e</sup>	4.52±0.37 <sup>f</sup>
4.	Ascorbic acid	0.36±0.01 <sup>c</sup>	0.36±0.01 <sup>c</sup>	0.79±0.32 <sup>c</sup>	1.16±0.12 <sup>d</sup>	0.38±0.08 <sup>c</sup>	0.58±0.11 <sup>c</sup>
5.	α- keto glutaric acid	0.06±0.01 <sup>b</sup>	0.01±0.01 <sup>a</sup>	0.02±0.01 <sup>a</sup>	0.01±0.01 <sup>a</sup>	0.05±0.02 <sup>a</sup>	0.09±0.01 <sup>b</sup>
6.	Citric acid	0.03±0.01 <sup>a</sup>	0.01±0.01 <sup>b</sup>	0.01±0.01 <sup>b</sup>	0.01±0.01 <sup>b</sup>	0.14±0.03 <sup>b</sup>	0.06±0.01 <sup>a</sup>

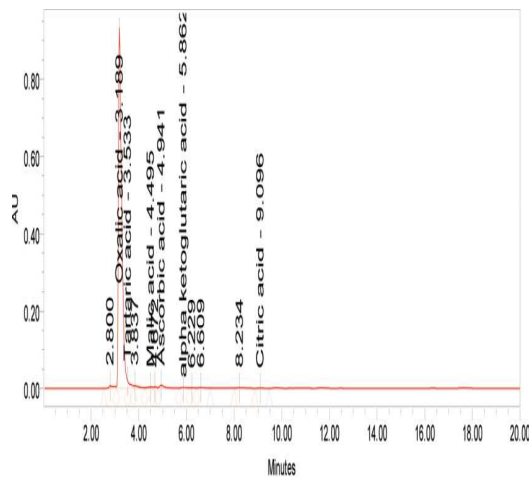
\*Values (mean ± SE) are average of three samples of each fruit (*p* < 0.05). Different superscripts within the same column represent significant difference for the biochemical parameter considered between the samples



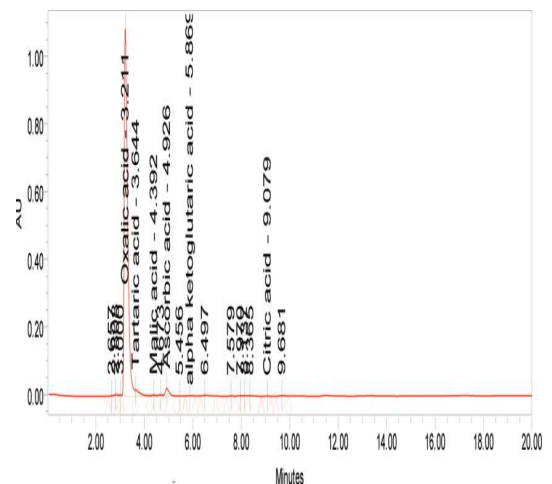
**Fig. 1: Chromatogram of organic acids standard mixture**



**Fig. 3: Chromatogram of BR**



**Fig. 2: Chromatogram of BU**



**Fig. 4: Chromatogram of CSU**

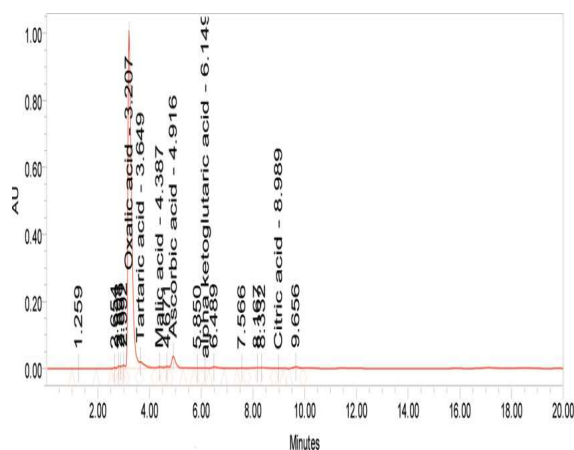


Fig. 5: Chromatogram of CSR

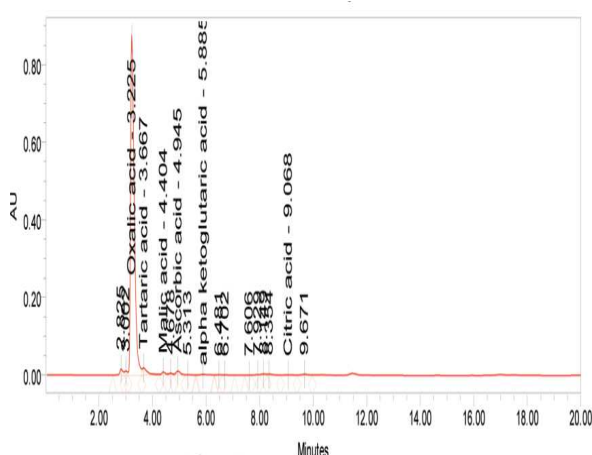


Fig. 6: Chromatogram of CTU

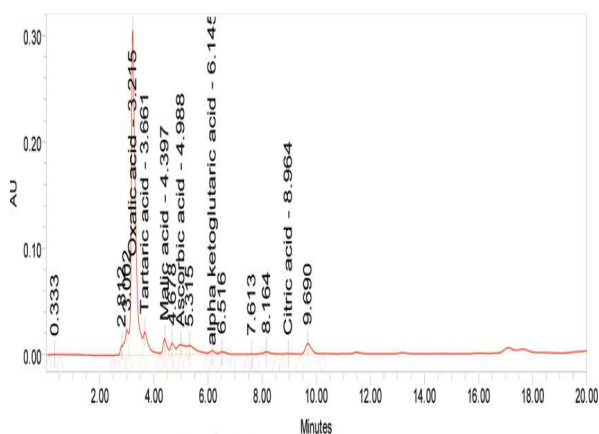


Fig. 7: Chromatogram of CTR

## CONCLUSION

The current study made an attempt to get an insight into the nutritional and antinutritional potential of *Averrhoa* fruits in two different maturity stages. *Averrhoa carambola* sweet ripe fruits appear to be more nutritious with relatively low values for the antinutritional parameters. HPLC profiling of six organic acids revealed oxalic acid to be the major acid. With substantial promotions and research evidences, these underutilized fruits could benefit as a supplementary food source for the world.

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