# Changes in phenolic composition, ascorbic acid and antioxidant capacity in cashew apple (*Anacardium occidentale* L.) during ripening

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**Changes in phenolic composition, ascorbic acid and antioxidant capacity in cashew apple (*Anacardium occidentale* L.) during ripening.**

**Abstract – Introduction.** Cashew apple is a rich source of sugars, vitamin C and polyphenols. In spite of its nutritional value, this pseudo-fruit has been left unexploited to a large extent in the crop-growing areas. Some reports of the chemical characteristics of cashew apple have been published. However, nothing is known about the changes in the composition of its bioactive compounds in the course of ripening. **Materials and methods**. Cashew apples at three different maturity stages were examined with respect to their ascorbic acid content, phenolic compounds and antioxidant capacity. Ascorbic acid was quantified by HPLC. Phenolic compounds were identified and quantified by using HPLC-ESI-MS/MS by comparison with authentic standard compounds. The antioxidant capacity was measured by TOSC assay against peroxyl radicals and peroxynitrite. **Results**. Amounts of identified phenolic compounds were the highest in unripe cashew apple and decreased in the course of ripening. Myricetin 3-O-rhamnoside, quercetin 3-O-galactoside and quercetin 3-O-rhamnoside turned out to be the main flavonoids in all maturity stages. The antioxidant capacity and the concentration of ascorbic acid increased in the course of ripening. The antioxidant activity was considerably influenced by ascorbic acid, more than by the content of phenolic compounds. **Conclusion**. This study provides, for the first time, information on changes in bioactive compounds and the antioxidant capacity in cashew apple during ripening. A dietary or technological exploitation of ascorbic acid is useful in the ripe condition. The unripe pseudo-fruits are a good source for the extraction of polyphenols with regard to possible food technological purposes or the preparation of food supplements.

**Brazil / *Anacardium occidentale* / fruits / maturation / developmental stages / processing quality / physicochemical properties / flavonoids / antioxidants / ascorbic acid**

**Changements de la composition phénolique, de l'acide ascorbique et de la capacité antioxydante de la pomme cajou (*Anacardium occidentale* L.) au cours de sa maturation.**

**Résumé – Introduction**. La pomme cajou est une source riche en sucres, vitamine C et polyphénols. Malgré sa valeur nutritive, ce pseudofruit est resté largement inexploité dans ses aires de production. Certains rapports sur les caractéristiques chimiques de pomme de cajou ont été publiés. Cependant, rien n'est connu sur les changements de la composition de ses composés bioactifs au cours de la maturation. **Matériel et méthodes**. Des pommes cajou récoltées à trois stades de maturité différents ont été examinées quant à leur teneur en acide ascorbique, composés phénoliques et capacité antioxydante. L'acide ascorbique a été quantifié par HPLC. Les composés phénoliques ont été identifiés et quantifiés à l'aide d'une HPLC-ESI-MS/MS par comparaison avec d'authentiques composés standards. La capacité antioxydante a été mesurée par dosage TOSC contre les radicaux peroxyles et la peroxynitrite. **Résultats**. Les quantités de composés phénoliques identifiés ont été les plus élevés dans les pommes cajou non mûres et elles ont diminué au cours de la maturation. Les myricétine 3-O-rhamnoside, quercétine 3-O-galactoside et quercétine 3-O-rhamnoside se sont avérées être les principaux flavonoïdes quel que soit le stade de maturité considéré. La capacité antioxydante et la concentration en acide ascorbique ont augmenté au cours de la maturation. L'activité antioxydante a été plus fortement influencée par la concentration en acide ascorbique que par la teneur en composés phénoliques. **Conclusion**. Notre étude fournit, pour la première fois, des informations sur les changements des composés bioactifs et de la capacité antioxydante dans la pomme cajou pendant sa maturation. L'acide ascorbique de la pomme cajou mûre pourrait être exploité dans l'industrie alimentaire ou technologique. Les pseudofruits non mûrs pourraient être une bonne source pour l'extraction des polyphénols en technologie alimentaire ou pour la préparation de compléments alimentaires.

**Brésil / *Anacardium occidentale* / fruits / maturation / stade de développement / qualité technologique / propriété physicochimique / flavonoïde / antioxydant / acide ascorbique**

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## 1. Introduction

*Anacardium occidentale* L. (cashew) is an evergreen shrub or tree up to 15 m in height that originates from the coastal strip of northern and north-eastern Brazil [1]. Nowadays, cashew is distributed across tropical America, the West Indies, India and Africa [2]. The cashew tree bears two food products, the 'cashew nut' and the 'cashew apple'. The cashew nut is in demand on international markets due to its sweet flavor. Botanically, the cashew nut is the embryo of the kidney-shaped drupe, which has a length of 3–5 cm. The cashew apple is attached as an enlarged peduncle to the drupe. This false fruit has a yellow to red skin and a juicy flesh. It is 6–8 cm long and approximately 4.5 cm in diameter [1].

Despite their promising economic potential, cashew apples are still underutilized. Only 10% of the production is used in either fresh or processed form as ice cream and jellies [3]. Most of it rots in the crop-growing areas, although cashew apple juice is palatable because of its strong exotic flavor. In addition, cashew apples are nutritive due to the high content of vitamin C and sugars [4]. A reason for the low attention to cashew apple is the astringent taste. Cashew apple juice has to be prepared technologically prior to consumption due to the content of tannins [5]. Both the clarified product 'cajuina' and fresh cashew apple juice have been reported to be antimicrobially active because of the bioactive constituents such as flavonols, tannins, carotenoids and ascorbic acid [6].

Polyphenols have been ascribed to contribute to the prevention of degenerative diseases due to their health-promoting properties [7, 8]. On the other hand, polyphenols have also become interesting for food technological purposes as they can be applied as antioxidants or antimicrobial agents [9, 10].

Vitamin C is regarded to be the most important vitamin in human nutrition. Approximately 90% of vitamin C in the human diet is supplied by fruits and vegetables [11]. Besides a number of important physiological functions it acts *inter alia* as a dietary antioxidant [11] and is used on a large scale as an antioxidant agent in foods and drinks [12].

The aim of this study was to evaluate the ascorbic acid content, phenolic composition and antioxidant capacity in the course of ripening of *Anacardium occidentale* pseudo-fruits in order to assess the bioactive potential for either dietary or food technological applications.

## 2. Materials and methods

### 2.1. Chemicals

Ultrahigh-quality (UHQ) water was prepared with a Direct-Q 3 system (Millipore, Billerica, USA). Gallic acid (≥ 97.5%), quercetin (≥ 98%), myricetin (≥ 96%), diethylenetriaminepentaacetic acid (DTPA) (≥ 99%), α-keto-γ-methiolbutyric acid (KMBA) (≥ 97%), 2,2′-azobis(2-methylpropionamidine) dichloride (ABAP) (≥ 97%), 3-morpholino-sydnonimine N-ethylcarbamide (SIN-1), (–)-epigallocatechin (≥ 95%), and (–)-epigallocatechin gallate (≥ 95%) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Myricetin 3-O-rhamnoside (≥ 95%) was obtained from Extrasynthése (Genay, France). Ascorbic acid (≥ 95%) was purchased from Kraemer & Martin GmbH (Sankt Augustin, Germany). Standards of quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-arabinoside, quercetin 3-O-rhamnoside, quercetin 4'-O-glucoside and kaempferol 3-O-glucoside were a gift from Professor Dr. Galensa, University of Bonn, Germany. These standards were self-isolated and of different purity grades (≥ 42% in the case of quercetin 3-O-arabinoside, ≥ 90% for the remaining flavonols). Purity grades were taken into account for the quantification.

### 2.2. Sampling

Cashew apple pseudo-fruits of the commercial variety CCP 76 were harvested at three maturity stages by visual analysis according to the classification described by Figueiredo



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[13]. Unripe fruits grew for 33–36 days, medium-ripe fruits 45–50 days, and ripe fruits 52 days. Fruits were collected at Embrapa's experimental station in Pacajus, Ceará, Brazil. The harvested fruits of each ripening stage were randomly divided into three sub-sets (each representing an independent replicate) of at least five fruits. Fruits were lyophilized immediately after harvest. Freeze-dried samples were air-shipped to Germany and ground by ball milling (MM2000, Retsch, Haan, Germany) prior to storage at –30 °C.

## 2.3. Analysis of phenolic compounds

### 2.3.1. Extraction of phenolic compounds

Extraction of phenolic compounds was carried out by using a modified pressurized liquid extraction (PLE) method previously described by Papagiannopoulos *et al.* [14]. A freeze-dried sample (250 mg) of each ripening stage was extracted in triplicate with acetone-water-formic acid (70 + 29 + 1; v/v/v) in an Accelerated Solvent Extractor (ASE 200, Dionex, Idstein, Germany) at room temperature, for 20 min, in two cycles. The subsequent solid-phase extraction (SPE) was performed by using a Gilson ASPEC XL system (Automated Sample Preparation with Extraction Cartridges, Abimed, Langenfeld, Germany) following a modified method described by Papagiannopoulos *et al.* [14]. Polyamide (PA) SPE cartridges (500 mg PA, 3 mL cartridge, Macherey-Nagel, Düren, Germany) were conditioned with 3 mL of dimethyl sulfoxide-formic acid-trifluoroacetic acid (DMSO-FAc-TFA) (98.7 + 1 + 0.3; v/v/v) and washed with 5 mL of UHQ water. Prior to loading the cartridge, the sample extract was diluted to contain less than 15% (v/v) of organic solvent. The cartridge was loaded with sample extract in volumetric steps of 20 mL until exhaustion and washed with 10 mL of water after each load. While eluting with DMSO-FAc-TFA solvent, the first 0.5 mL were discarded and the next 1.25 mL collected. Prior to application to HPLC-MS/MS, the samples were filtered through a 1.0/0.45-µm syringe filter (Chromafil GF/PET-45/25, Macherey-Nagel, Düren, Germany).

### 2.3.2. Identification and quantification of phenolic compounds

Quantification of the phenolic compounds was performed following a previously described method [15]. The HPLC instruments consisted of a pump system and a UV-detector of the HP 1050 series (Hewlett Packard, Waldbronn, Germany), a degasser: Degasys Populair DP3010 (Uniflows, Tokyo, Japan) and an analytical column: Aqua 3 µm C18, 150 mm, 2 mm i.d., combined with a guard column: Security Guard, C18, 4 mm, 2 mm i.d. (both Phenomenex, Aschaffenburg, Germany). The solvents were UHQ water with 1% (v/v) formic acid (mobile phase A) and 1% (v/v) formic acid in acetonitrile (mobile phase B). The HPLC gradient using a flow rate of 0.3 mL·min$^{-1}$ started at 5% B, was held isocratic for 10 min, and rose to 40% B after 60 min. Subsequently, the column was flushed for 10 min at 100% B and re-equilibrated for 25 min at 5% B. Twenty µL were injected for analysis. Each sample extract was analyzed in duplicate ($n$ = 6). The coupled API 2000 HPLC-ESI-MS/MS system was controlled with Analyst 1.5 Software (both Applied Biosystems, Darmstadt, Germany). Mass spectra for the determination of phenolic compounds were generated in negative ionization mode.

Identification was performed by comparing retention times and fragmentation patterns of phenolic compounds in multiple reaction monitoring modes with those of authentic standard substances. Standards were also used to create calibration curves for quantification. Results are presented in mg·100 g$^{-1}$ dry matter (DM).

## 2.4. Antioxidant capacity by TOSC

For Total Oxidant Scavenging Capacity (TOSC) analysis, a freeze-dried sample of each ripening stage was reconstituted with UHQ water under consideration of the dry matter content of 12.9% (w/w). The suspension was sonicated for 5 min and centrifuged for 10 min at 12,000 rpm with a Heraeus Biofuge Stratos (Kendro, Langenselbold, Germany). The aqueous supernatant (WE) was stored for further analysis at –30 °C. The extraction procedure was performed in duplicate for each ripening stage.



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The TOSC assay was performed as described by Lichtenthäler *et al.* [16]. Briefly, the TOSC assay is based on an ethylene-yielding reaction of KMBA (α-keto-γ-methiolbutyric acid) with either peroxyl radicals (px) or peroxynitrite (pn). Antioxidant compounds present in the sample can inhibit the ethylene formation that is recorded in a time course of 1 h using automatically repeated headspace GC analysis (GC-17A, Shimadzu, Tokyo, Japan). Each ripening stage was analyzed in duplicate ($n$ = 4). Quantification of generated ethylene results in a kinetic curve of which the area under the curve (AUC) is calculated. Mean data of a sample are compared with those of an uninhibited reaction with water, which gives rise to the TOSC values. The results of this study indicate the concentration of the sample in grams per liter that is needed to obtain a radical inhibition of 50%.

### 2.5. Determination of ascorbic acid

The ascorbic acid content in the aqueous supernatant (WE) of each maturity stage was determined chromatographically. The HPLC-DAD system of the PRO Star series (Varian, Walnut Creek, USA) was equipped with an analytical column: Synergi 4 µm Hydro RP, 150 mm, 2 mm i.d., and with a guard column: Security Guard, C18, 4 mm, 2 mm i.d. (both Phenomenex, Aschaffenburg, Germany). Separation was performed with acidified UHQ water (2% formic acid, v/v) in isocratic conditions using a flow rate of 0.8 mL·min$^{-1}$. The injection volume was 20 µL. Confirmation of ascorbic acid in the WE was arranged by standard, retention time and doping of the standard to the sample. A five-point calibration curve (5–100 mg·100 mL$^{-1}$, $r^2$ = 0.9996) was created for quantification with an authentic standard. Ascorbic acid was quantified at a wavelength of 260 nm. Sample runs of each WE were performed in duplicate ($n$ = 4).

### 2.6. Statistical analysis

To prove significant differences between maturity stages, statistical analysis of the data was performed by one-way analysis of variance. Means were compared by the Bonferroni test at 95% of probability using PASW Statistics 18.

## 3. Results and discussion

### 3.1. Ascorbic acid

Ascorbic acid was found in all maturity stages of cashew apple. Unripe fruits contained (1038 ± 31) mg·100 g$^{-1}$ dry matter (DM), medium-ripe fruits (1392 ± 52) mg·100 g$^{-1}$ DM, and ripe fruits (1731 ± 45) mg·100 g$^{-1}$ DM. Hence, the results indicate an increase in the ascorbic acid content during ripening. A comparison of the mean values of each ripening stage demonstrated that the maturation process had a significant effect on the ascorbic acid content ($p < 0.05$). Literature studies on ascorbic acid levels at different ripening stages of cashew apple are not known, but the amounts of ascorbic acid in ripe fruits are in accordance with those found by Akinwale [3]. Different cashew apple cultivars determined by Assunção and Mercadante showed approximately 50% lower contents of ascorbic acid [17]. The ascorbic acid content in ripe cashew apples is remarkably high in general. Amounts are 4–5 times higher in comparison with kiwi fruits or oranges [18] and can be ranked at the same level as guavas [19].

Ascorbic acid is generally present in plant tissues that undergo active growth and development [11]. Increasing amounts of ascorbic acid were also observed in ripening guavas [19]. On the contrary, a decrease in the ascorbic acid content was reported by Celik *et al.* [20] during ripening of cranberries. Hence, the ascorbic acid formation during fruit ripening seems to depend in particular on the species.

### 3.2. Phenolic constituents

A total of 14 phenolic constituents were detected in cashew apples by HPLC-MS/MS analysis (*table I*, *figure 1*). With the exception of peaks 7 and 8, all of them could be identified by comparison of their retention times and mass spectrometric data with those of the authentic standard compounds.



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**Table I.**

Phenolic compounds (mg·100 $g^{-1}$ dry matter) detected by HPLC-ESI-MS/MS, in three maturity stages of cashew apple. Data are mean ± standard deviation.

| Peak | Compound | $[M-H]^-$ / production *m/z* | Maturity stage | | |
|---|---|---|---|---|---|
| | | | Unripe[1] | Medium-ripe[1] | Ripe |
| 1 | Gallic acid | 169 / 125 | 2.22 ± 0.46 | 0.64 ± 0.06 | 0.94 ± 0.15 |
| 2 | Epigallocatechin | 305 / 125 | 0.61 ± 0.13 | 0.11 ± 0.03 | 0.02 ± 0.01 |
| 3 | Epigallocatechin gallate | 457 / 125 | 1.59 ± 0.31 | 0.07 ± 0.01 | 0.04 ± 0.00 |
| 4 | Myrecetin-3-O-rhamnoside | 463 / 316 | 4.48 ± 0.68 | 0.91 ± 0.36 | 0.86 ± 0.14 |
| 5 | Quercetin 3-O-galactoside | 463 / 300 | 3.38 ± 0.53 | 0.99 ± 0.12 | 0.83 ± 0.23 |
| 6 | Quercetin 3-O-glucoside | 463 / 300 | 1.95 ± 0.57 | 0.44 ± 0.07 | 0.31 ± 0.10 |
| 7 | Quercetin pentoside 1 | 433 / 301 | 1.12 ± 0.28[2] | 0.45 ± 0.02[2] | 0.42 ± 0.05[2] |
| 8 | Quercetin pentoside 2 | 433 / 301 | 0.80 ± 0.12[2] | 0.40 ± 0.03[2] | 0.36 ± 0.08[2] |
| 9 | Quercetin 3-O-arabinoside | 433 / 301 | 0.73 ± 0.11 | 0.37 ± 0.05 | 0.38 ± 0.06 |
| 10 | Quercetin 3-O-rhamnoside | 447 / 301 | 2.15 ± 0.28 | 0.65 ± 0.10 | 0.69 ± 0.18 |
| 11 | Quercetin 4-O-glucoside | 463 / 300 | 0.13 ± 0.04 | 0.04 ± 0.01 | Not detected |
| 12 | Kaempferol 3-O-glucoside | 447 / 285 | 0.05 ± 0.02 | Not detected | Not detected |
| 13 | Myricetin | 317 / 151 | 0.64 ± 0.01 | Not detected | Not detected |
| 14 | Quercetin | 301 / 151 | 0.17 ± 0.02 | Not detected | Not detected |
| | Total | | 20.40 ± 3.56 | 5.16 ± 0.86 | 4.92 ± 1.00 |

[1] Means of the compounds 1–11 differed significantly ($p < 0.05$) as assessed by analysis of variance and the Bonferroni test.

[2] Expressed as quercetin 3-O-arabinoside equivalents.

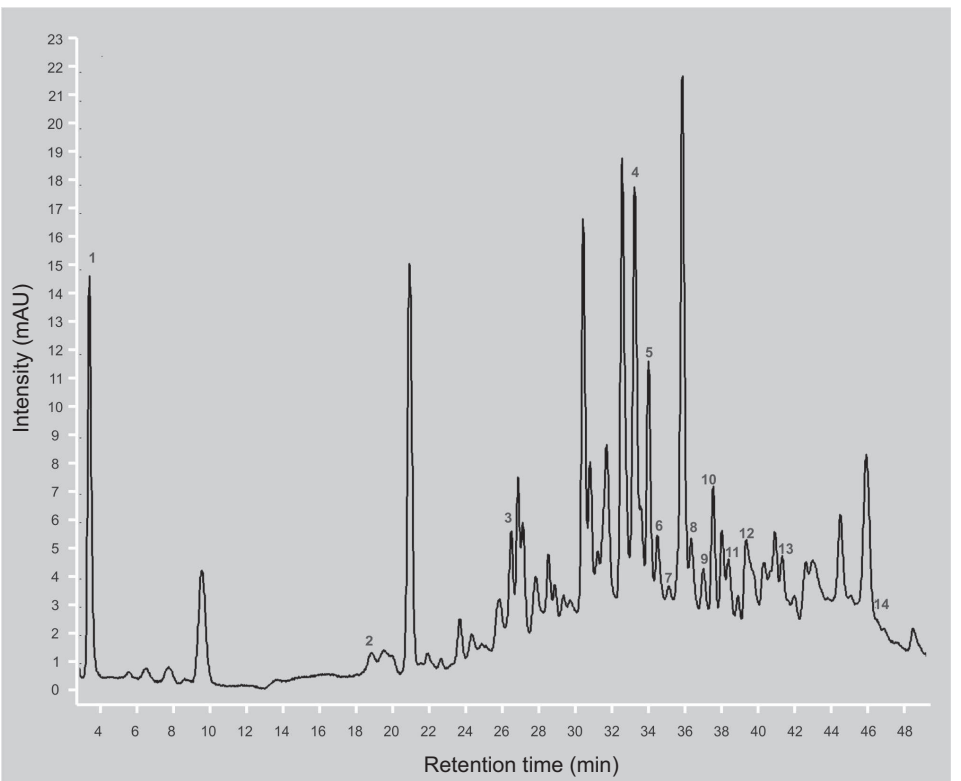



**Figure 1.**
Chromatogram of an aqueous acetone extract of unripe cashew apple recorded at 280 nm. Peaks correspond to those listed in *table I*.



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**Table II.**
Ratio (%) of detected phenolic compounds detected by HPLC-ESI-MS/MS, in three maturity stages of cashew apple.

| Compound | Maturity stage | | |
|---|---|---|---|
| | Unripe | Medium-ripe | Ripe |
| Gallic acid | 11 | 13 | 19 |
| Epigallocatechin | 3 | 2 | < 1 |
| Epigallocatechin gallate | 8 | 1 | 1 |
| Myricetin | 3 | – | – |
| Myricetin 3-O- rhamnoside | 22 | 18 | 18 |
| Quercetin 3-O-galactoside | 17 | 19 | 17 |
| Quercetin 3-O-glucoside | 10 | 9 | 6 |
| Quercetin pentoside 1 | 5 | 9 | 9 |
| Quercetin pentoside 2 | 4 | 8 | 8 |
| Quercetin 3-O-arabinoside | 4 | 7 | 8 |
| Quercetin 3-O-rhamnoside | 11 | 13 | 14 |
| Quercetin 4-O-glucoside | < 1 | < 1 | – |
| Kaempferol 3-O-glucoside | < 1 | – | – |
| Quercetin | < 1 | – | – |

Accordingly, peak 1 consists of a phenolic acid, namely gallic acid. Peaks 2 and 3 correspond to flavanols: epigallocatechin and epigallocatechin gallate, respectively. Nine different flavonols (peaks 4–6, 9–14) were identified (*table I*). Peaks 7 and 8 could only be tentatively assigned to quercetin pentosides. These compounds showed $[M–H]^-$ ions at *m/z* 433 and product ions at *m/z* 301, which is in agreement with the fragmentation pattern of quercetin 3-O-arabinoside. However, the retention times differ.

All detected phenolic compounds were present in unripe fruits. Their amounts decreased significantly from unripe to medium-ripe fruits ($p < 0.05$). Kaempferol 3-O-glucoside (peak 12), myricetin (peak 13) and quercetin (peak 14) vanished completely. From medium-ripe fruits to ripe fruits, a further decrease was observed for flavanols, quercetin pentosides, myricitrin and quercetin hexosides, of which quercetin 4'-O-glucoside (peak 11) was no longer detectable in ripe fruits. In contrast to these compounds, amounts of gallic acid and quercetin 3-O-rhamnoside increased. All changes from medium-ripe fruits to ripe fruits were not found to be significant. The decline in the concentration of the phenolic compounds from the unripe stage to medium-ripe fruits suggests that the biosynthesis becomes less intensive during growth and subsequent maturation, as observed in bitter oranges [21]. Changes in flavonols were also determined in different maturity stages of apricots [22] and common apples [23, 24]. In accordance with the results of cashew apple, the highest values of flavonols were mostly found in the initial maturity stage. Decreasing amounts of total flavonols during ripening were also found in camu camu fruits. In contrast to cashew apple, values of total flavanols did not change remarkably [24]. The decrease in flavanol amounts in cashew apple is in accordance with the report of Almeida *et al.*, who found a higher activity for enzymes involved in the biosynthesis of these flavonoids in the early developmental stage of strawberries [26]. In the case of hydroxybenzoic acids, Gruz *et al.* observed in medlar fruits (*Mespilus germanica* L.) that concentrations of free protocatechuic acid and syringic acid decreased during maturation, as observed for gallic acid in cashew apple from unripe to medium-ripe fruits. The decrease in free phenolic esters in medlar fruits is explained by their integration into cell walls [27].

Interesting results were observed by comparing the ratios of the detected phenolic constituents in each ripening stage of cashew apple (*table II*). Although concentrations of



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quercetin 3-O-galactoside, quercetin 3-O-rhamnoside and myrecetin-3-O-rhamnoside decreased during ripening, these flavonols were present in similar percentages in each ripening stage. The quercetin pentosides and gallic acid increased remarkably, whereas a decrease was found for epigallocatechin, epigallocatechin gallate and quercetin 3-O-glucoside. In regard to flavonols, Awad *et al.* reported that the ratio of the individually identified main quercetin glycosides in different cultivars of common apples (3-O-galactoside, 3-O-rhamnoside, 3-O-glucoside) undergoes a permanent change during ripening [23], which could in cashew apple only be constituted for quercetin 3-O-glucoside.

A determination of phenolic compounds during the ripening process of cashew apple is performed for the first time. Two previously published reports are known on individual phenolic compounds in ripe cashew apple. Compounds 1, 4–6, 10, 12–14 and two quercetin pentosides were identified by Michodjehoun-Mestres *et al.* [2]. In accordance with our results, myricetin-3-O-rhamnoside, quercetin 3-O-galactoside, quercetin 3-O-glucoside and quercetin 3-O-rhamnoside were quantified in similar amounts in the flesh of the cashew apple cultivar CCP 76 [28]. De Brito *et al.* also reported the presence of compounds 4–6, 10 and 12, as well as three different quercetin pentosides [28]. Epigallocatechin and epigallocatechin gallate are reported for the first time to occur in cashew apple.

**Table III.**
Antioxidant capacity of cashew apple in the course of ripening. Total Oxidant Scavenging Capacity (TOSC) values indicate the concentration (g dry matter·$L^{-1}$) of cashew apple that is needed to obtain a radical inhibition of 50%. Comparison of the means was performed by analysis of variance and the Bonferroni test.

| Maturity stage | Peroxyl radicals | Peroxynitrite |
|---|---|---|
| Unripe | 1.38 ± 0.19 a | 1.88 ± 0.23 a |
| Medium-ripe | 1.22 ± 0.16 a | 1.37 ± 0.10 b |
| Ripe | 0.79 ± 0.08 b | 1.00 ± 0.13 c |

a, b, c: within each column, values with the same letters are not significantly different at the level of $p < 0.05$.

## 3.3. Antioxidant capacity

The antioxidant capacity of cashew apple increased during maturity (*table III*). Means were significantly different ($p < 0.05$), with the exception of values between unripe and medium-ripe fruits regarding peroxyl radicals. Ripe cashew apples show approximately a twice as high radical scavenging activity against both radicals in ripe condition when compared with unripe fruits. Cashew apples show high antioxidant properties against both radicals in comparison with other fruits from Latin America. The radical scavenging activity of ripe cashew pseudo-fruits against peroxyl is higher than that of *Clidemia rubra* berries (0.9 g·$L^{-1}$) [15]. However, the antioxidant activity was lower when compared with that of açaí fruits from different harvest years (0.39–0.48 g·$L^{-1}$) [29]. Further, cashew apple turned out to be a good radical scavenger of peroxynitrite, as lower concentrations are needed to obtain a radical inhibition of 50% in comparison with açaí (1.17–1.72 g·$L^{-1}$) [29] and *Clidemia rubra* berries (2.0 g·$L^{-1}$) [15].

The report of Lichtenthäler *et al.* [16] might provide an explanation for the antiradical behavior of cashew apple in the course of ripening because of the antioxidant capacity which was determined of different flavonoid standards and ascorbic acid. Briefly, ascorbic acid showed a 4–5 times lower antioxidant activity against peroxyl in comparison with the phenolic standard compounds. Against peroxynitrite, the difference between the polyphenols and ascorbic acid was less distinctive (only 1–2 times). Additionally, the radical scavenging activity of ascorbic acid towards peroxyl and peroxynitrite was nearly identical. In consequence of these results, it becomes obvious that ascorbic acid has considerable influence on the antioxidant capacity of cashew apple. Firstly, the rising ascorbic acid concentration during ripening parallels the increase in the antioxidant activity against both radicals. Amounts of ascorbic acid in each ripening stage are remarkably higher than those of the identified and quantified phenolic compounds (sum given in *table I*).



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Secondly, the high ascorbic acid content is an explanation for the good antioxidant activity of cashew apple, especially against peroxynitrite.

## 4. Conclusion

Cashew apple is an excellent source of ascorbic acid, which contributes the most to the antioxidant capacity of the pseudo-fruit. In total, fourteen phenolic compounds were identified or at least tentatively assigned. This study provides for the first time information on changes in the composition of bioactive compounds and the antioxidant capacity of cashew apples during ripening. A dietary or technological exploitation of ascorbic acid is useful in the ripe condition. Use of polyphenols derived from cashew apple for possible food technological purposes should already be made of unripe fruits.

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## Cambios de la composición fenólica, del ácido ascórbico y de la capacidad antioxidante de la manzana de acajú (*Anacardium occidentale* L.) durante su maduración.

**Resumen – Introducción**. La manzana de acajú es una rica fuente de azúcares, vitamina C y de polifenoles. A pesar de su valor nutritivo, este pseudofruto permaneció poco explotado en sus zonas de producción. Se publicaron algunos informes sobre las características químicas de la manzana de acajú. Sin embargo, no se sabe nada sobre los cambios de la composición de sus compuestos bioactivos durante la maduración. **Material y métodos**. Se examinaron manzanas de acajú cosechadas en tres estados de madurez diferentes, en cuanto a su contenido de ácido ascórbico, compuestos fenólicos y capacidad antioxidante. Se cuantificó el ácido ascórbico por HPLC (cromatografía líquida de alta presión). Se identificaron y cuantificaron los compuestos fenólicos con ayuda de una HPLC-ESI-MS/MS por comparación con auténticos compuestos estándar. Se midió la capacidad antioxidante por dosificación TOSC (capacidad total de atrapamiento de oxiradicales) contra los radicales peroxiles y la peroxinitrita. **Resultados**. Las cantidades más altas de compuestos fenólicos identificados se detectaron en las manzanas de acajú inmaduras y disminuyeron durante la maduración. Las miricetina-3-0-ramnósido, quercetina-3-0-galactósido y quercetina 3-0-ramnósido resultaron ser los principales flavonoides, independientemente del estado de madurez considerado. La capacidad antioxidante y la concentración de ácido ascórbico aumentaron durante la maduración. La actividad antioxidante estuvo más influenciada por la concentración de ácido ascórbico que por el contenido de compuestos fenólicos. **Conclusión**. Nuestro estudio proporciona, por primera vez, informaciones sobre los cambios de los compuestos bioactivos y de la capacidad antioxidante en la manzana de acajú durante su maduración. El ácido ascórbico de la manzana de acajú madura podría explotarse en la industria alimentaria o tecnológica. Los pseudofrutos inmaduros podrían ser una buena fuente para la extracción de los polifenoles en tecnología alimentaria o para la preparación de complementos alimentarios.

**Brasil / *Anacardium occidentale* / frutas / maduración / etapas de desarrollo / calidad de procesamiento / propiedades fisicoquímicas / flavonoides / antioxidantes / ácido ascórbico**



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