



Harvest And Postharvest

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Cambuci ripening: Postharvest quality and volatile compounds production implications

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Abstract: Cambuci (*Campomanesia phaea*) is a native fruit from the Atlantic Forest, one of the richest biomes in the world. The cambuci has soft and astringent pulp, green coloration and is characterized as an aromatic fruit. This is the first study that describes the quality evolution of cambucis during their ripening. Quality parameters were evaluated at three harvesting points: weight loss, pulp firmness, skin color, total soluble solids, titratable acidity, ascorbic acid, total soluble sugars, total phenolic compounds, soluble and total tannins, respiratory activity, ethylene production, and volatile compound profiling. Fruit harvested in stages 1 (somewhat rounded equatorial region) and 2 (increasingly rounded equatorial region) of ripening had two additional days of postharvest life. A decrease in firmness was observed over the days, demonstrating desirable pulp softening. No increase in ethylene production associated to respiratory peaks were observed. Volatile profile changed according to ripeness, and early harvested fruit was able to produce partially volatile compounds found in ripe fruit.

Keywords: *Campomanesia phaea*, physicochemical quality, Atlantic Forest.

Amadurecimento de cambucis: qualidade pós-colheita e implicações na produção de compostos voláteis

Resumo: O cambuci (*Campomanesia phaea*) é uma fruta nativa da Mata Atlântica, um dos biomas mais ricos do mundo. Este fruto possui polpa macia e adstringente, coloração verde e caracteriza-se por seu aroma agradável. Este é o primeiro estudo que descreve a evolução da qualidade de cambucis durante o amadurecimento. Os seguintes parâmetros de qualidade foram avaliados em três pontos de colheita: perda de massa, firmeza da polpa, cor da casca, sólidos solúveis totais, acidez titulável, ácido ascórbico, açúcares solúveis totais, compostos fenólicos totais, taninos solúveis e totais, atividade respiratória, produção de etileno e compostos voláteis. Frutos colhidos nos estádios 1 (região equatorial ligeiramente arredondada) e 2 (região equatorial mais arredondada) de maturação tiveram dois dias adicionais de vida pós-colheita. Observou-se diminuição da firmeza ao longo dos dias, demonstrando o desejável amolecimento da polpa. Não foi observado aumento na produção de etileno associado a picos respiratórios. O perfil volátil mudou de acordo com a maturação, e frutos colhidos precocemente foram capazes de produzir compostos parcialmente voláteis encontrados em frutos maduros.

Termos de indexação: *Campomanesia phaea*, qualidade físico-química, Mata Atlântica.

Introduction

The cambuci (*Campomanesia phaea*) is a native Brazilian fruit that grows in the Atlantic Rainforest biome and belongs to the Myrtaceae family. It has a green skin, and a soft, fleshy, juicy pulp, with an acidic and astringent flavor, being a source of ascorbic acid and several phenolic compounds - displaying high antioxidant capacity (TOKAIRIN *et al.*, 2018). Its aroma is very attractive, described as both intense and sweet, and is one of its noteworthy characteristics. As cambucis present certain attributes that favor industrialization, such as a high amount of fibers, pectin and high pulp yield, they present both industrial and commercial potential (VALLILO *et al.*, 2005).

Cambuci consumption is still limited to its production region, as its required fresh sale represents an obstacle for reaching new markets. In addition to displaying a short post-harvest life, cambuci quality is also impaired by collection performed only after the fruit fall from the tree, as producers justify that the ideal harvest point is noted during

natural fruit tree detachment. However, the advanced ripening stage in these conditions favors mechanical fruit injuries, such as bruises and cracks. In addition, soil contact exposes these fruit to a high microbiological loads, which may lead to diseases during the postharvest period (GODOY *et al.*, 2010).

Cambuci harvesting before their natural fall may result in added conservation time and higher fruit commercialization quality. To assess these factors, quality changes that determine cambuci ripeness, i.e. physico-chemical parameters, require further understanding. These determinations have been successfully carried out for other non-conventional fruit, such as *Ziziphus mauritiana* L. (BASTOS *et al.*, 2016), *Myrciaria dubia* (NEVES *et al.*, 2017) and *Eugenia pyriformis* (FREITAS *et al.*, 2019), but no cambuci data is available.

In this context, this study aimed to monitor the physical and chemical quality of cambucis during ripening, providing data to support conservation strategies.

Material and methods

Plant material

Cambucis were harvested in March-April 2011 from a commercial orchard in the municipality of Natividade da Serra, in the state of São Paulo, Brazil (23°31' S and 45°27' W and 78,712 m of altitude). Three maturation stages were defined from the equatorial region rounding of each fruit (Figure 1).

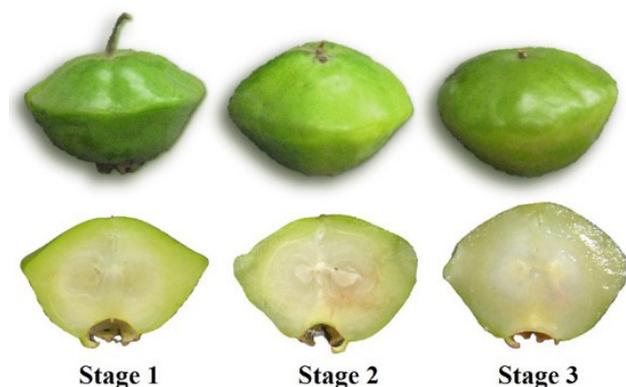


Figure 1. Cambuci maturation stages based on the equatorial region rounding of each fruit. A) S1 - somewhat rounded equatorial region (un-ripe); B) S2 - increasingly rounded equatorial region (intermediate) and C) S3 – fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe).

After harvesting, cambucis were packed in cardboard boxes lined with bubble wrap and transported at $22 \text{ }^{\circ}\text{C} \pm 1 \text{ }^{\circ}\text{C}$ to the Laboratory of Postharvest of Horticultural Products-ESALQ/USP in Piracicaba, SP, Brazil. At the laboratory, fruit were homogenized, separated by maturation stage and stored in refrigerated chambers at $22 \text{ }^{\circ}\text{C} \pm 1 \text{ }^{\circ}\text{C}$ and at 85 % RH for up to 6 days.

Quality analyses

- **Weight loss:** quantified on a semi-analytical scale (Tecnal Mark 210 A, São Paulo, Brazil). Results are expressed as a percentage, using five fruit per repetition. **Pulp firmness:** determined with a digital penetrometer (Tr-Turoni, Forli-Italy) with an 8 mm diameter tip. Fruit were broken longitudinally, and two readings were taken at opposite ends. Results are expressed as Newtons (N).

- **Skin color:** determined with a colorimeter (Minolta CR-300, Osaka, Japan) through the $L^* a^* b^*$ color system, with two readings per fruit taken at opposite ends, on the upper side.

- **Total soluble solids:** determined with a digital refractometer (Atago Palette 101, Tokyo, Japan). Two readings were performed per repetition and the results are expressed as percentage (AOAC, 2012).

- **Titrateable acidity:** determined by neutralization titration. Results are expressed as % citric acid (CARVALHO *et al.*, 1990).

- **Ascorbic acid:** determined by neutralization titration, based on the reduction of the 2,6-diclorophenol indolphenol-sodium indicator (DCFI) by ascorbic acid and expressed as $\text{mg } 100 \text{ g}^{-1}$ (CARVALHO *et al.*, 1990).

- **Total soluble sugars:** analyzed by HPLC-PAD (Dionex, Sunnyvale - California USA), using an amperometric pulse detector, CarboPac PA1 column (4 × 250 mm) (Dionex), using an isocratic flow of a 1 mL min^{-1} flow of 18 mM NaOH, for 25 min (GOMEZ; LAJOLO; CORDENUNSI, 2002). Sucrose, fructose, and glucose standards were determined (Sigma-Aldrich, St. Louis, MO, USA).

- **Total phenolic compounds:** assessed by the Folin-Ciocalteu spectrophotometric (Biochrom Libra S22, Massachusetts, USA) method (WOISKY; SALATINO, 1998). Results are expressed as gallic acid equivalents ($\text{mg } 100 \text{ g}^{-1}$).

- **Soluble and total tannins:** determined by spectrophotometry, using Folin-Ciocalteu (TAIRA, 1995). Results are expressed in $\text{mg } 100 \text{ g}^{-1}$.

- **Respiratory activity and ethylene production:** Four fruit were placed individually in airtight glass containers (600 mL) for 1 h. At this time, 1 mL of the headspace of each vial was collected using a silicone septum and a syringe (Hamilton, Gastight, USA) and injected in a chromatograph (ThermoFinnigan Trace 2000GC, USA). Respiratory activity and ethylene produc-

tion determinations were performed daily and expressed as $\text{nmol kg}^{-1} \text{s}^{-1}$.

- **Volatile compound profiling:** performed through the solid phase microextraction method (SPME) for four replicates (PESIS *et al.*, 2009). Approximately 3 g of each sample (from the pulp of four fruit) and 7 mL of a 30% NaCl solution were mixed in 20 mL vials with a silicone septum cap and frozen at $-20\text{ }^{\circ}\text{C}$. A model 6890 Hewlett-Packard chromatograph was used coupled to a selective model 5973 Hewlett-Packard mass detector. A chromatographic Supelcowax 10 column (30 m, 0.25 mm internal diameter, 0.25 μm film thickness) was used. The mass spectra obtained for each replicate were compared to those contained in the NIST version 1.6 library. Only those with a similarity index above 70 were % considered.

Pulp firmness analysis, skin color, soluble solid content, titratable acidity and ascorbic acid, loss of fresh weight, total soluble sugars, total phenolic compounds, soluble and total tannins and organic acid determinations were performed at the beginning and at the end of each maturation stage. Respiratory activity and ethylene production were assessed daily. Volatile compounds were determined from samples frozen in liquid N_2 and preserved in an ultra-freezer at $-80\text{ }^{\circ}\text{C}$ and analyzed at 4 time points (days) (D0, D2, D4 and D6).

Statistical analyses

The experimental design consisted in a completely randomized 3x4 factorial scheme, where the three cambuci maturation stages were combined with four evaluation days (days 0, 2, 4 and 6), comprising four repetitions consisting of five fruit each, with three repetitions for each frozen sample. The results were submitted to an analysis of variance by the F test ($p < 0.05$). Volatile compound profiles were analyzed by a Principal Component Analysis (ACP) and Hierarchical Cluster Analysis visualized by Heatmaps using the Metaboanalyst 3.0 software (CHONG; WISHART; XIA, 2019).

Results and discussion

Cambucis in the S1 and S2 stages had a postharvest life of six days, while S3 fruits presented a postharvest life of four days. Therefore, early harvest allowed for an extra two days between the harvest and consumption stages. CO_2 production in cambucis during this period varied according to fruit maturation stage (Fig 2 - A). S1 fruit displayed an average CO_2 production of $0.19\text{ nmol kg}^{-1} \text{s}^{-1}$, followed by 0.26 and $0.34\text{ nmol kg}^{-1} \text{s}^{-1}$ for S2 and S3, respectively. The high respiratory rate observed in cambucis is an important factor concerning its highly perishable characteristics. The initial increase in respiratory fruit activity is related to the need for energy production to maintain metabolic processes, since this is the main physiological process that fruit undergo after harvest (TONUTTI; BONGHI, 2014). Respiratory activity in cambucis resembled the activity reported for 'Golden' papayas (FAÇANHA *et al.*, 2019), while ethylene production was low and with no observable differences between maturation stages (Figure 2 - B). No production increase associated with respiratory peaks that would characterize these fruits as climacteric was observed.

Fresh weight loss was not influenced by fruit maturation stage, as similar losses of $4.26\% \pm 2.57$ for S1, $4.24\% \pm 2.54$ for S2 and $4.40\% \pm 3.17$ for S3 were observed at the end of the storage period (Figure 3). Differences in pulp firmness were observed between the three stages at harvest (Figure 3). Therefore, this parameter can be applied as a maturity index for cambuci fruits. Firmer cambucis were harvested at the S1 stage ($4.95 \pm 0.45\text{ N}$), representing more than twice the initial firmness of S2 fruits. Despite a noticeable initial firmness difference in S1 fruit, cambuci pulps softened over time, signaling the possibility of early cambuci harvesting. This greater firmness represents an advantage concerning fruit postharvest life, ensuring transport resistance and allowing for the arrival of fresh fruits over long distances.

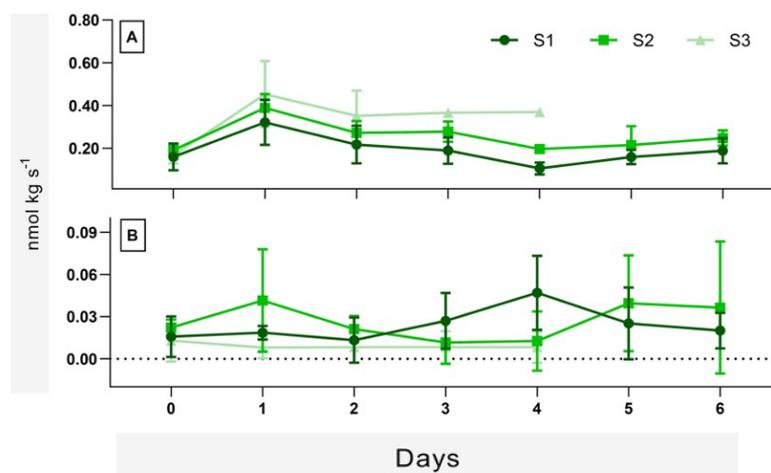


Figure 2. CO₂ (A) and ethylene (B) production in cambucis harvested at the S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 – fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages and stored at 22 ± 1 °C and 85 ± 5% RH. The bars represent the standard deviation of the means (n = 4).

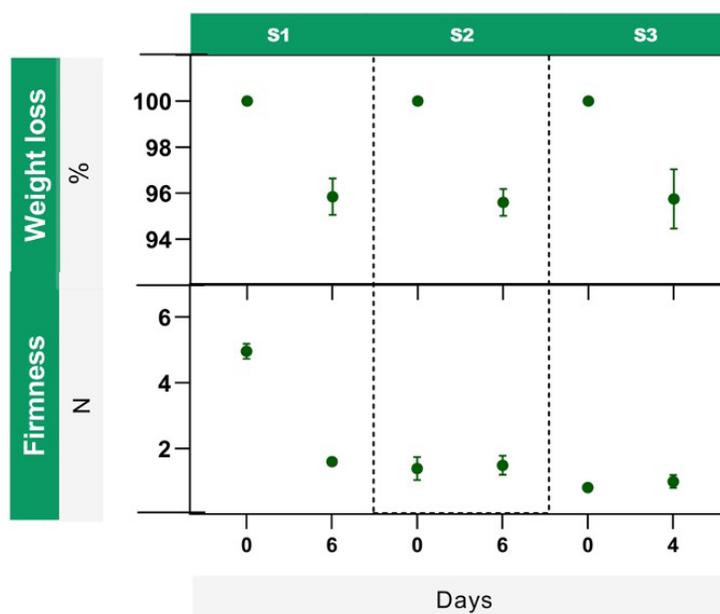


Figure 3. Fresh weight and firmness loss in cambucis harvested at the S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 – fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages and stored at 22 ± 1 °C and 85 ± 5% RH. Bars represent the standard deviations of the means (n = 4).

The abrupt reduction in S1 firmness can be attributed to two processes observed during the ripening process, namely water loss, which decreases turgor tissue pressure and hydrolytic enzyme activities, such as polygalacturonase and pectinamethylesterase, leading to constituent cell wall pectin solubilization and consequent pulp softening (WANG *et al.*, 2018).

Cambuci is a green colored fruit that becomes slightly yellow due to chlorophyll degradation during storage. Although variations in hue angle values between S1 and S3 were evident, only slight cambuci skin color alterations were noted (Figure 4). Furthermore, skin color faded over time, as demonstrated by the chromaticity assessments (Figure 4).

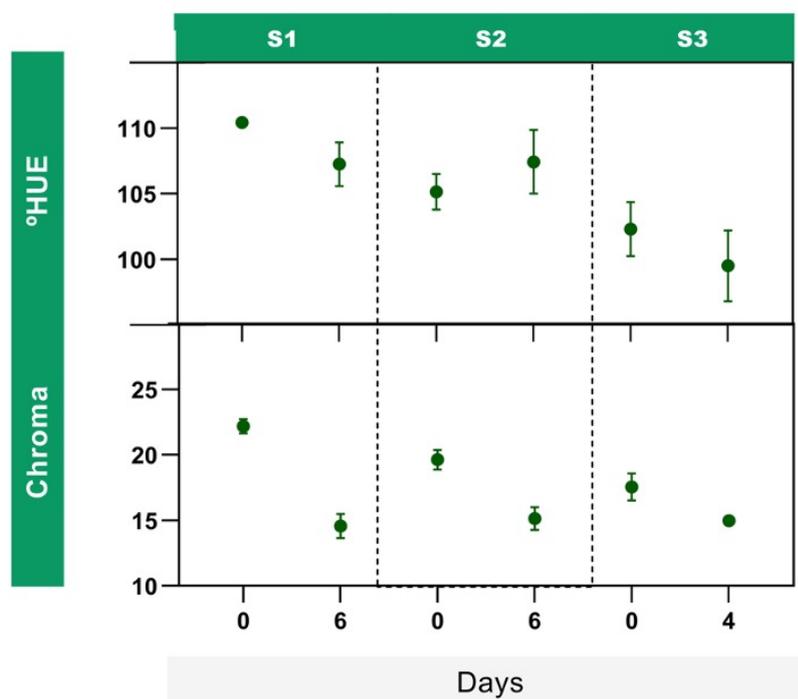


Figure 4. Coloring represented by Hue angle and chromaticity in cambucis harvested at S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 – fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages stored at 22 ± 1 °C and $85 \pm 5\%$ RH. Bars represent the standard deviations of the means ($n = 4$).

Cambucis harvested at the S3 stage presented a mean soluble solid value of 10.40% on the first day, while S1 and S2 fruits presented 9.96% and 9.93% values, respectively (Figure 5). No significant increase in soluble solids during the postharvest period was observed between maturation stages. A slight decrease was noted in relation to total titratable acidity, although it was not statistically significant (Figure 5). TSS and AT, generally used as maturation indices, did not discriminate cambuci maturation stages. This has also been reported for guavas, another Myrtaceae fruit, by Azzolini et al. (2004).

The cambuci sugar composition profiling indicates that glucose is not as present in these fruits, reaching maximum expression in greener fruits (Figure 6). Maximum sucrose content also occurs during S1, while fructose production intensifies with ripening. Sweetness intensity in fruits depends both on total sugar content and the relative content of each individual sugar, as fructose and

glucose contribute to approximately 1.7- and 0.8-fold the sweetening power of sucrose (MARIOTTI; LUCISANO, 2014). In ripe uvaías, another native Atlantic Rainforest fruit, sucrose was the predominant sugar in the ‘Rugosa Doce’, ‘Doce de Patos de Minas’ and ‘Common’ accessions, while fructose was the predominant sugar in the ‘Rugosa’, Pêra’ and ‘Dura’ accessions (SILVA *et al.*, 2019). In ripe peaches, sucrose is the predominant mesocarp sugar, representing about 40 to 85% of total sugar content, followed by glucose and fructose, at varying ratios (CIRILLI; BASSI; CIACCIULLI, 2016).

Succinic acid is the predominant organic acid in cambucis harvested at S1 and S2 stages, alongside citric acid in S3 fruits (Figure 7). This acid is characterized as a tricarboxylic acid (TCA) cycle intermediate and an anaerobic metabolism product, and its predominance in fruits is unusual. In 2004 and 2010, the US Department of Energy indicated succinic acid as one of five promising biochem-

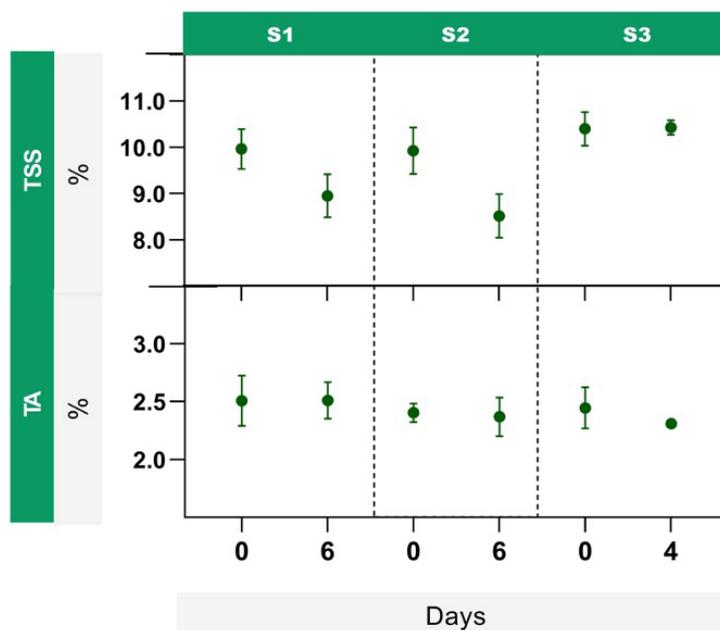


Figure 5. Soluble solids (TSS) and titratable acidity (AT) in cambucis harvested at S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 - fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages and stored at 22 ± 1 °C and $85 \pm 5\%$ RH. Bars represent the standard deviations of the means ($n = 4$).

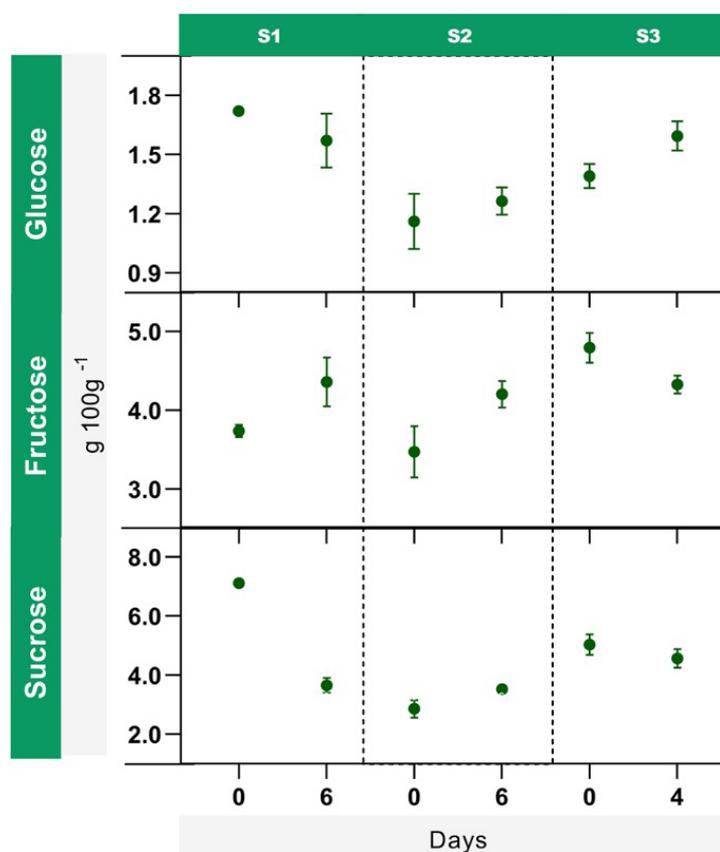


Figure 6. Cambuci sugars harvested at S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 - fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages and stored at 22 ± 1 °C and $85 \pm 5\%$ RH. Bars represent the standard deviations of the means ($n = 4$).

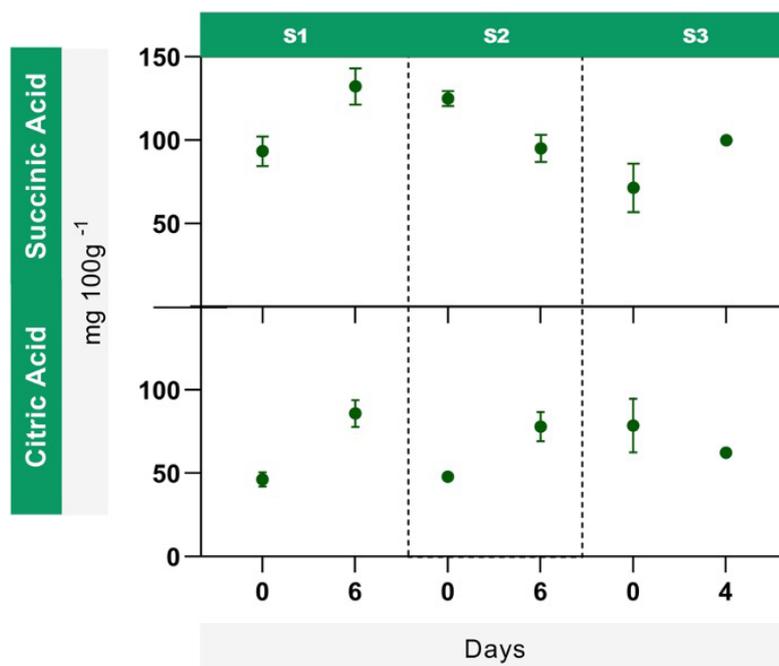


Figure 7. Organic acids - succinic acid and citric acid in cambucis harvested at S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 - fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages and stored at $22 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH. Bars represent the standard deviations of the means ($n = 4$).

ical platforms, and its current uses include applications as a surfactant, ion chelator and as an additive in agriculture and food and in the pharmaceutical industries. Efforts have been made to develop succinic acid production processes using fungal/yeast strains (AHN; JANG; LEE, 2016).

Cambuci vitamin C content declined progressively with advancing maturation stages (Figure 8). This decrease during the ripening process is common, and is reduced during senescence, probably due to organic acid oxidation. The name “vitamin C” is common to two biologically active compounds: ascorbic acid (AsA) and its first oxidation product, dehydroascorbic acid (DHA) (NOLLET; TOLDRÁ, 2012). The current recommended daily allowance (RDA) for AsA intake is 100-120 mg day⁻¹ to meet cellular needs and reduce the risk of cardiovascular and neurodegenerative diseases, cancer and stroke in healthy humans (NAIDU, 2003). Vitamin C content ranged from 244.9 mg 100g⁻¹ to 111.2 mg 100g⁻¹ in the three cambuci ripening stages.

The lowest average phenolic compound value detected in cambucis was 612.1 mg 100⁻¹ of GAE (Figure 8 - B), above values described for 62 other fruits (FU *et al.*, 2011), where total phenolic content ranged from 11.88 ± 0.11 mg 100 g⁻¹, to 585.52 ± 18.59 mg 100 g⁻¹, with the maximum value belonging to the Chinese date fruit. Therefore, cambuci is a rich source of phenolic compounds, an important phytochemical category displaying high antioxidant capacity and the ability to eliminate free radicals (FU *et al.*, 2011). Some levels evaluated in conventional fruits support this statement, such as in ‘Red Delicious’ apples (73.96 ± 3.52 mg 100 g⁻¹ GAE), bananas (57.13 ± 3.64 mg 100 g⁻¹ GAE), guavas (194.11 ± 7.01 mg 100 g⁻¹ GAE), peaches (27.58 ± 1.57 mg 100 g⁻¹ GAE) and pomegranates ($146.94 \pm 0,04$ mg 100 g⁻¹ GAE) (FU *et al.*, 2011).

Tannins are polyphenols that display high antioxidant potential due to their molecular weight and high hydroxylation degree of their aromatic rings (KOLECKAR *et al.*, 2008). These compounds play an essential role in the formation of sensory fruit properties,

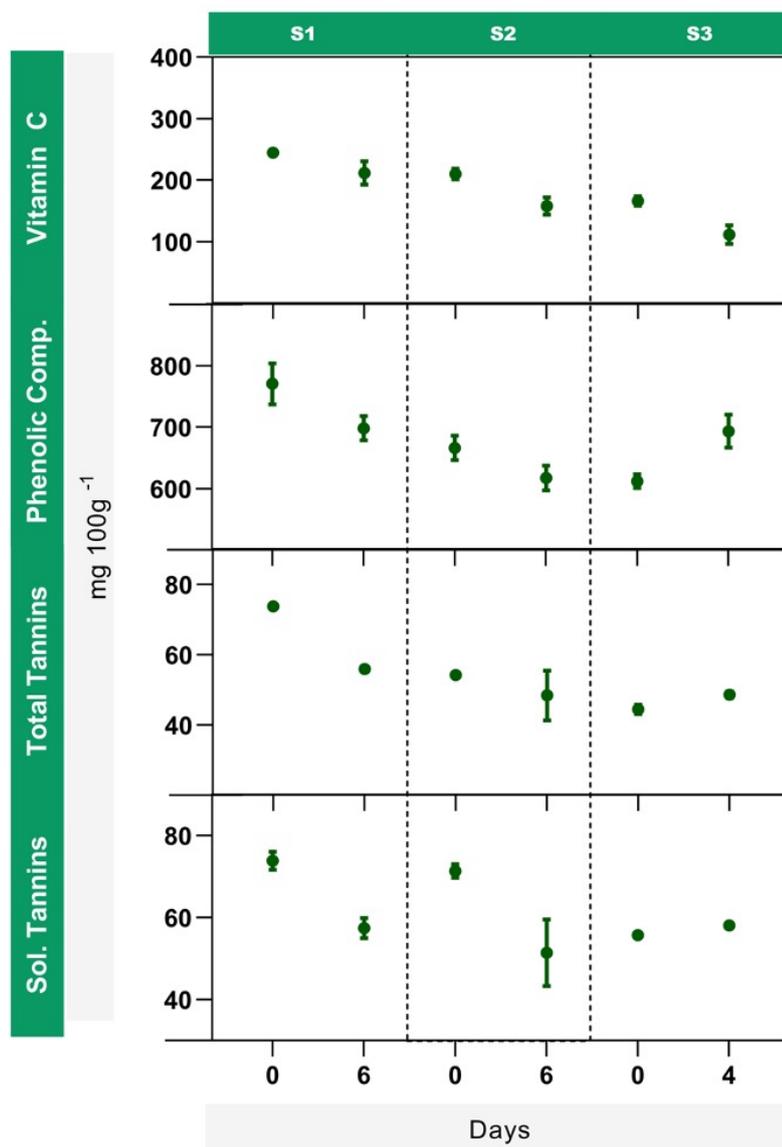


Figure 8. Vitamin C, phenolic compounds and total and soluble tannins in cambucis harvested at the S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 - fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages and stored at $22 \pm 1^\circ \text{C}$ and $85 \pm 5\% \text{RH}$. Bars represent the standard deviations of the means ($n = 4$).

leading to astringency. In cambucis, a decrease in total and soluble tannin content was observed during ripening at stages S1 and S2, closer to those observed for stage S3 (Figure 8), due to tannin polymerization during maturation, triggering astringency reduction, which is desirable for consumption.

Volatile compounds

The volatile compound profile analysis identified 27 compounds distributed throughout the three fruit maturation stages during the four assessment days. Terpenes (59.26%)

and esters (37.04%) were the most abundant classes concerning cambuci aroma, and only 3.70% were derived from aldehydes (Table 1).

Concerning day 0, volatile compounds in the most advanced maturation stage (S3) identified at higher concentrations were butanoic acid, linalool, eucalyptol and α -terpineol, which are responsible for the formation of a complex aroma with floral, herbaceous and menthol notes (Figure 9). In contrast, fewer volatile compounds were identified in less mature cambucis (S1), with a predominantly

Table 1. Description of volatile cambuci compounds emitted during the storage period.

CN	Name	CAS	Aroma	Classification
1	α -Cubebene	17699-14-8	Herbal	Terpene
2	α -Phellandrene	99-83-2	Citrus	Terpene
3	α -Terpineol	98-55-5	Pine-like	Terpene
4	β -Phenylethyl butyrate	103-52-6	Floral	Ester
5	γ -Elemene	29873-99-2	Green	Terpene
6	Linalool	78-70-6	Citrus	Terpene
7	β -cubebene	13744-15-5	Citrus	Terpene
8	Ethyl Z-4-decenoate	7367-84-2	NF	Ester
9	Ethyl Z-4-octenoate	34495-71-1	Fresh, pineapple	Ester
10	Alloaromadendrene	25246-27-9	Woody	Terpene
11	Diethyl succinate	123-25-1	Mild fruity cooked, apple, ylang	Ester
12	Butanoic acid, hexyl ester	2639-63-6	Green	Ester
13	β -Terpinene	99-84-3	NF	Terpene
14	D-Limonene	5989-27-5	Sweet, citrus, and peely	Terpene
15	Ethyl methylthioacetate	4455-13-4	Sulfurous, green, and fruity	Ester
16	Ethyl E-4-decenoate	76649-16-6	Green fruity	Ester
17	Eucalyptol	470-82-6	Eucalyptus	Terpene
18	Ethyl hexanoate	123-66-0	Sweet, fruity, pineapple	Ester
19	Humulene	6753-98-6	Woody	Terpene
20	d-Cadinene	483-76-1	Thyme, herbal, woody	Terpene
21	β -Selinene	17066-67-0	Herbal	Terpene
22	Ethyl octanoate	106-32-1	Fruity, wine, waxy	Ester
23	o-Cymene	527-84-4	NF	Terpene
24	Propanal	123-38-6	Earthy, alcohol, wine	Aldehydes
25	Isobutyl propionate	540-42-1	Fruity, green, ether	Ester
26	Terpinen-4-ol	562-74-3	Pepper, woody, earth	Terpene
27	E- β -Ocimene	3779-61-1	Sweet, herbal	Terpene

* Compound number (CN), unique record number at the Chemical Abstracts Service (CAS) database, an American Chemical Society division, characteristic flavor, chemical classification, precursor, and stage in which the compound was identified. Information not found in the literature was identified as NA.

herbaceous profile characterized by the presence of d-cadinene and α -cubebene with an earthy nuance due to propanal. Ethyl methylthioacetate, ethyl hexanoate and ethyl octanoate were detected in S2, resulting in ripe and sweet fruit aromatic notes, such as pineapple (RODA *et al.*, 2017; STEINGASS; CARLE; SCHMARR, 2015; ZHENG *et al.*, 2012). The volatile compound profile characterization of the three maturation three stages indicate that compound abundance is greater in more mature fruits (S2 and S3), consistent with one of the main aroma functions for ripe fruits, i.e. the need for seed dispersal (NEVO *et al.*, 2018; RODRÍGUEZ; ALQUÉZAR; PEÑA, 2012).

On the second day, a significant increase in the variety of volatile compounds in S1 cambucis was noted, when cambuci aroma became fruitier, with citrus, fresh and sweet notes, similar to S2, due to the presence of linalool, ethyl octanoate, ethyl Z-4-decenoate, eucalyptol and β -cubebene (Figure 10).

The aroma evolution observed in S1 and S2 may be associated to amino acid and carbohydrate degradation and fatty acid oxidation, both common during ripening (FREITAS *et al.*, 2019; SCHWAB; DAVIDOVICH-RIKANATI; LEWINSOHN, 2008; ZHU *et al.*, 2018). In S3, decreased volatile emissions were observed compared to day 0. Some of the most fre-

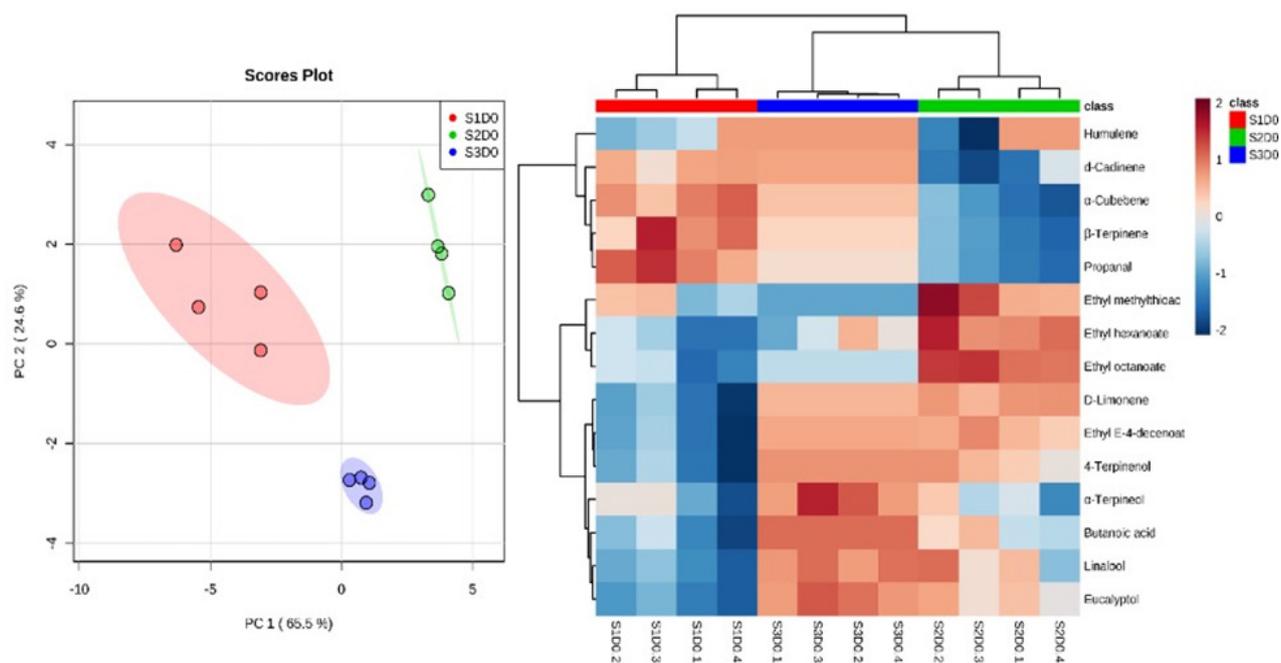


Figure 9. Principal component analysis and heatmap of volatile cambuci compounds during the three maturation stages on the first postharvest day: D0. Where: S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 - fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages (n=4).

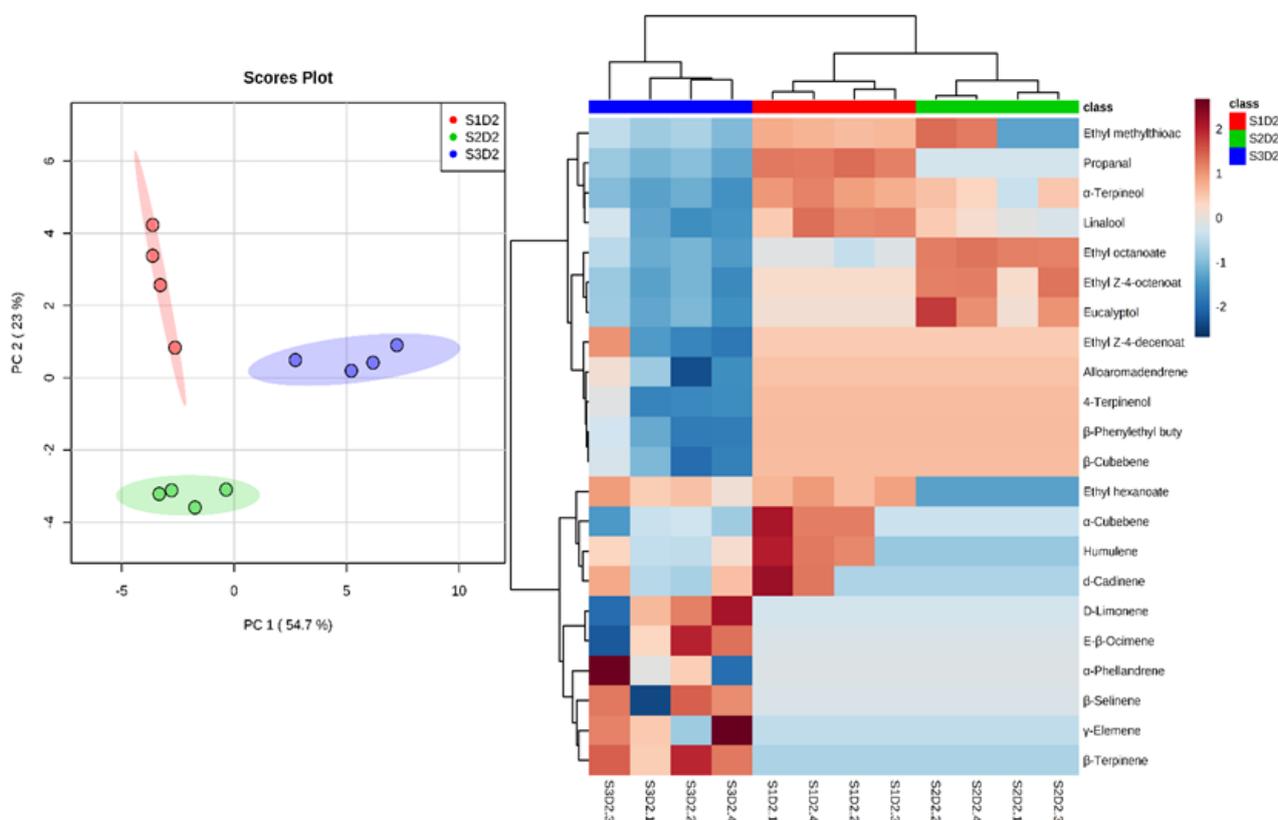


Figure 10. Principal component analysis and heatmap of volatile cambuci compounds during the three maturation stages on the second postharvest day: D2. Where: S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 - fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages (n=4).

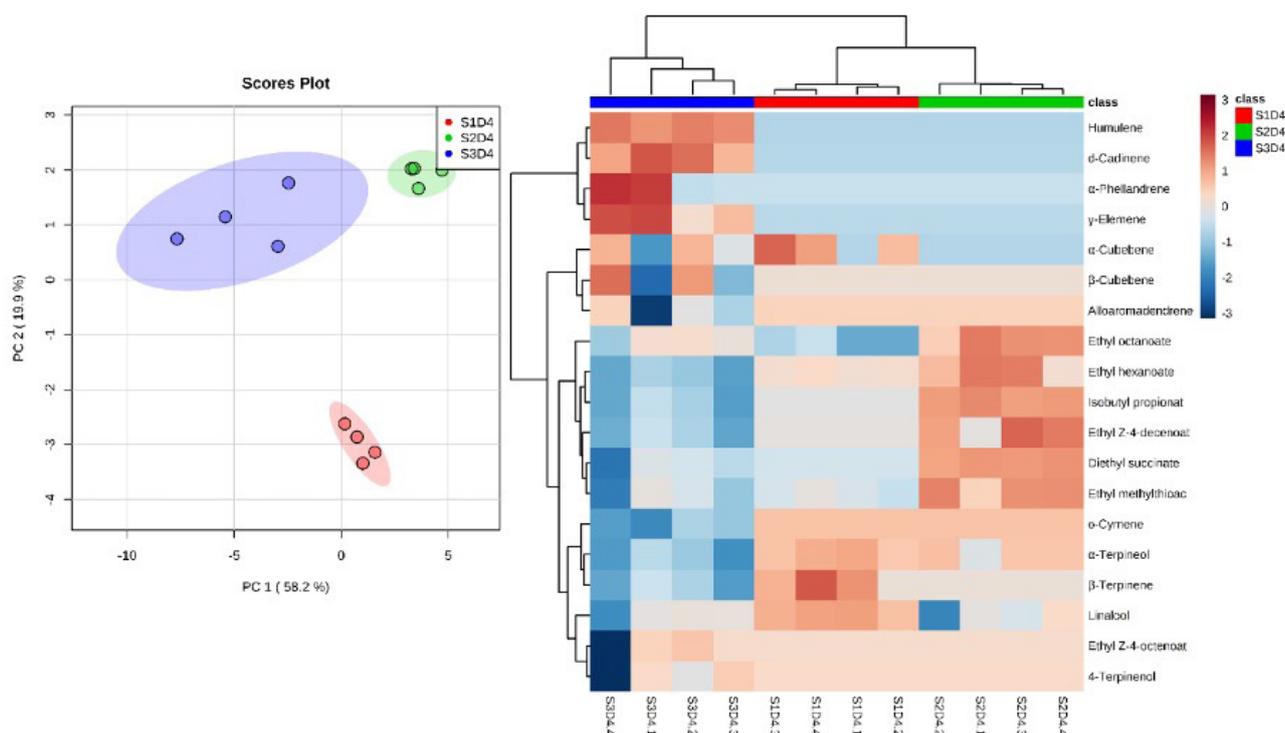


Figure 11. Principal component analysis and heatmap of volatile cambuci compounds during the three maturation stages on the fourth postharvest day: D4. Where: S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 - fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages ($n = 4$).

quently detected compounds comprised D-limonene, E- β -Ocimene, α -Phellandrene, β -Selinene and γ -Elemene, collaborating to the formation of aromas displaying sweet/citrus/peely, sweet/herbal, citrus, sweet/herbal, green notes.

On the fourth day, the emission of volatile compounds from S3 fruits decreased and a woody aroma was noted, marked by the presence of humulene, d-Cadinene, α -Phellandrene, γ -Elemene in relation to the sweet and fruity aromas detected during the previous days (Figure 11). S1 and S2 fruits still presented an aromatic fruity and sweet character, more prominent in S2.

On the sixth day, S1 and S2 fruits were evaluated, as they were still apt for consumption. The number of volatile compounds also decreased, similar to what was observed on the last evaluation day for S3. Ten volatile organic compounds were detected, with only β -terpinene and ethyl-methylthioacetate common for both ripening stages. Volatiles

with citrus and fruity aromas, like α -terpinol and linalool, were released during the S1 stage, while ethyl-hexanoate (sweet, fruity, pineapple) and eucalyptol (eucalyptus) were detected in S2 fruits (Figure 12).

Dynamic changes in volatile compound profiles were observed during all cambuci storage maturation stages, both concerning the amount and the variety of compounds produced and emitted. Volatiles increased in the initial maturation period during all evaluated stages and decreased at the end of the ripening process.

Maturation stage has a direct impact on the formation of the volatile profile, as cambucis collected after their natural fall (S3) contained more volatile compounds on the first analysis day compared to the other stages. During storage, the emission of volatile compounds at stages S1 and S2 increased and was chemically altered, resembling the volatile profile of fruits harvested during the S3 stage.

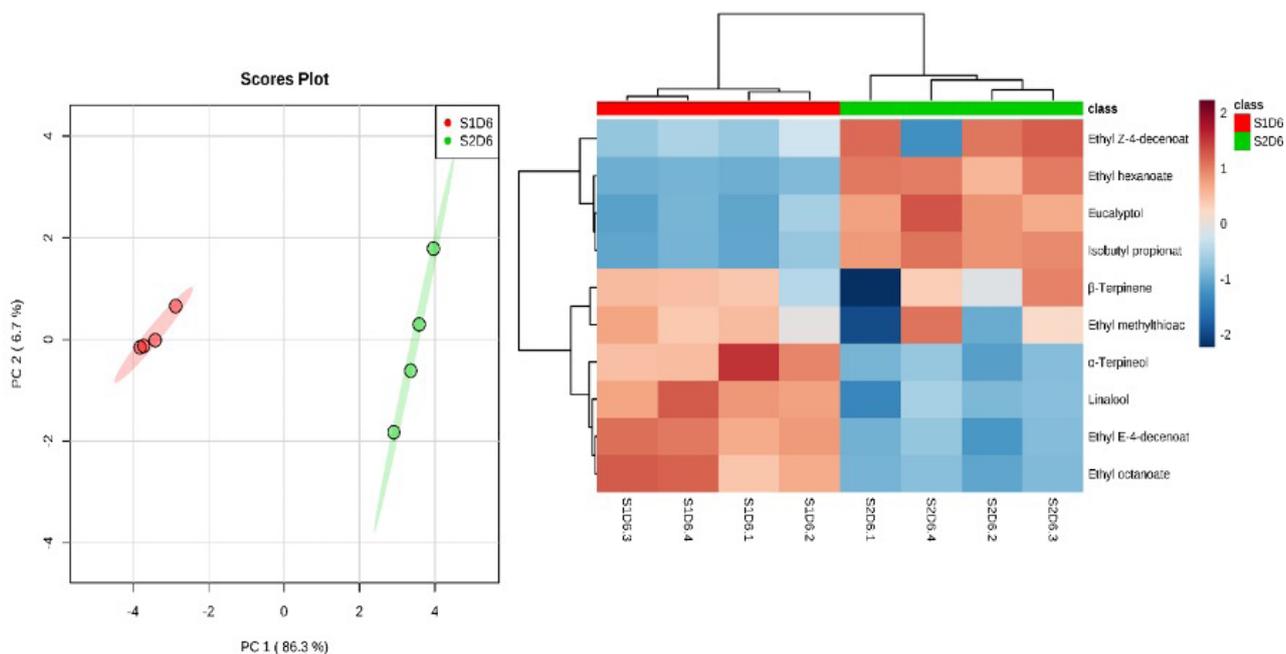


Figure 12. Principal component analysis and heatmap of volatile cambuci compounds during the three maturation stages on the sixth postharvest day: D6. Where: S1 - somewhat rounded equatorial region (unripe); S2 - increasingly rounded equatorial region (intermediate) and S3 - fruit collected from the ground, displaying a significantly rounded equatorial region (fully ripe) maturation stages (n=4).

Conclusions

After 6 days of storage, S1 and S2 fruit achieved quality parameters similar to S3 fruits, with emphasis on vitamin C, tannin, citric acid, fructose and glucose contents. Decreased firmness was also observed, demonstrating desirable pulp softening. Cambuci were proven to be a rich source of phenolic compounds, succinic acid and vitamin C.

During the postharvest period, no increase in ethylene production associated to respiratory peaks that could characterize cambucis as a climacteric fruit were observed.

Cambuci aroma was the quality attribute most affected by maturation stages. Even so, volatile compound synthesis was partially recovered from D2 for stages S1 and S2.

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References

- AHN, J.H.; JANG, Y.S.; LEE, S.Y. Production of succinic acid by metabolically engineered microorganisms. **Current Opinion in Biotechnology**, Lausanne, v.42, p.54-6, 2016.
- AOAC - Association of Official Analytical Chemists. **Official methods of analysis of AOAC international**. 19.ed. Gaithersburg: AOAC, 2012.
- AZZOLINI, M.; JACOMINO, A.P.; BRON, I.U. Índices para avaliar qualidade pós-colheita de goiabas em diferentes estádios de maturação. **Pesquisa Agropecuaria Brasileira**, Brasília, DF, v.39, n.2, 2004.
- BASTOS, V.J.; NEVES, L.C.; DA SILVA, P.M.C.; SHAHAB, M.; COLOMBO, R.C.; ROBERTO, S.R. Harvest point determination of indian jujube fruit (*Ziziphus mauritiana* L.) based on physicochemical and functional parameters. **Scientia Horticulturae**, Amsterdam, v.213, p.392-402, 2016.

- CARVALHO, C.R.L.; MANTOVANI, D.M.B.; CARVALHO, P.R.N.; MORAES, R.M.M. **Análises químicas de alimentos**. Campinas: ITAL, 1990.
- CHONG, J.; WISHART, D.S.; XIA, J. Using metaboanalyst 4.0 for comprehensive and integrative metabolomics data analysis. **Current Protocols in Bioinformatics**, new York, v.68, n.1, p.e-86, 2019.
- CIRILLI, M.; BASSI, D.; CIACCIULLI, A. Sugars in peach fruit: a breeding perspective. **Horticulture Research**, London, v.3, p.15067, 2016.
- FAÇANHA, R.V.; SPRICIGO, P.C.; PURGATTO, E.; JACOMINO, A.P. Combined application of ethylene and 1-methylcyclopropene on ripening and volatile compound production of “Golden” papaya. **Postharvest Biology and Technology**, Amsterdam, v.161, p.160-9, 2019.
- FREITAS, T.P.; SPRICIGO, P.C.; PURGATTO, E.; JACOMINO, A.P. Aroma and soluble solid contents of the uvaia—a native Atlantic rainforest fruit—are negatively affected by early harvest. **Journal of Food Biochemistry**, Oxford, v.43, n.7, p.1–11, 2019.
- FU, L.; XU, B.T.; XU, X.R.; GAN, R.Y.; ZHANG, Y.; XIA, E.Q.; LI, H. Antioxidant capacities and total phenolic contents of 62 fruits. **Food Chemistry**, Amsterdam, v.129, n.2, p.345-50, 2011.
- GODOY, A.E. de; JACOMINO, A.P.; CERQUEIRA-PEREIRA, E.C.; GUTIERREZ, A.D.S.D.; VIEIRA, C.E.M.; FORATO, L.A. Injúrias mecânicas e seus efeitos na qualidade de mamões golden. **Revista Brasileira de Fruticultura**, Jaboticabal, v.32, n.3, p.682–91, 2010.
- GOMEZ, M.; LAJOLO, F.; CORDENUNSI, B. Evolution of soluble sugars during ripening of papaya fruit and its relation to sweet taste. **Journal of Food Science**, Chicago, v.67, n.1, p.442-7, 2002.
- KOLECKAR, V.; KUBIKOVA, K.; REHAKOVA, Z.; KUČA, K.; JUN, D.; JAHODAR, L.; OPLETAL, L. Condensed and Hydrolysable Tannins as Antioxidants Influencing the Health. **Mini-Reviews in Medicinal Chemistry**, v.8, n.5, p.436-47, 2008.
- MARIOTTI, M.; LUCISANO, M. Sugar and Sweeteners. *In*: ZHOU, W.; HUI, Y. H.; LEYN, I.D.; PAGANI, M.A.; ROSELL, C.M.; SELMAN, J.D.; THERDTHAI, N. **Bakery products science and technology**. 2.ed. Hoboken: John Wiley & Sons, 2014.
- NAIDU, K.A. Vitamin C in human health and disease is still a mystery? An overview. **Nutrition Journal**, London, v.2, n.7, 2003.
- NEVES, L.C.; DE CAMPOS, A.J.; COLOMBO, R.C.; ROBERTO, S.R.; CISNEROS-ZEVALLOS, L. Days after anthesis and postharvest behavior define maturity, harvesting time and nutraceutical content of camu–camu fruit. **Scientia Horticulturae**, Amsterdam, v.224, p.37-47, 2017.
- NEVO, O.; RAZAFIMANDIMBY, D.; JEFFREY, J.A.J.; SCHULZ, S.; AYASSE, M. Fruit scent as an evolved signal to primate seed dispersal. **Science Advances**, Washington, v.4, n.10, 2018.
- NOLLET, L.M.L.; TOLDRÁ, F. **Handbook of analysis of active compounds in functional foods**. Boca Raton: CRC Press. 2012.
- PESIS, E.; IBÁÑEZ, A.M.; PHU, M.L.; MITCHAM, E.J.; EBELER, S.E.; DANDEKAR, A.M. Superficial scald and bitter pit development in cold-stored transgenic apples suppressed for ethylene biosynthesis. **Journal of Agricultural and Food Chemistry**, Washington, v.57, n.7, p.2786–2792, 2009.
- RODA, A.; LUCINI, L.; TORCHIO, F.; DORDONI, R.; DE FAVERI, D.M.; LAMBRI, M. Metabolite profiling and volatiles of pineapple wine and vinegar obtained from pineapple waste. **Food Chemistry**, Amsterdam, v.229, p.734–42, 2017.
- RODRÍGUEZ, A.; ALQUÉZAR, B.; PEÑA, L. Fruit aromas in mature fleshy fruits as signals of readiness for predation and seed dispersal. **New Phytologist**, London, v.197, p.36–48, 2012.
- SCHWAB, W.; DAVIDOVICH-RIKANATI, R.; LEWINSOHN, E. Biosynthesis of plant-derived flavor compounds. **Plant Journal**, Oxford, v.54, n.4, p.712–32, 2008.

- SILVA, A.P.G. da; SPRICIGO, P.C.; PURGATTO, E.; DE ALENCAR, S.M.; SARTORI, S.F.; JACOMINO, A.P. Chemical composition, nutritional value and bioactive compounds in six uvaia accessions. **Food Chemistry**, Amsterdam, v.294, p.547–56, 2019.
- STEINGASS, C.B.; CARLE, R.; SCHMARR, H.G. Ripening-dependent metabolic changes in the volatiles of pineapple (*Ananas comosus* (L.) Merr.) fruit: I.Characterization of pineapple aroma compounds by comprehensive two-dimensional gas chromatography-mass spectrometry. **Analytical and Bioanalytical Chemistry**, Heidelberg, v.407, n.9, p.2591–608, 2015.
- TAIRA, S. Astringency in bersimmon BT . *In*: LINSKENS, H.F.; JACKSON, J.F. (org.). **Fruit analysis**. Heidelberg: Springer Berlin Heidelberg, 1995. p.97–110.
- TOKAIRIN, T.O.; SILVA, A.P.G.; SPRICIGO, P.C.; ALENCAR, S.M.; JACOMINO, A.P. Cambuci: a native fruit from the Brazilian Atlantic forest showed nutraceutical characteristics. **Revista Brasileira de Fruticultura**, Jaboticabal, v.40, n.5, p.e-666, 2018.
- TONUTTI, P.; BONGHI, C. **Postharvest handling**. Amsterdam: Elsevier, 2014.
- VALLILO, M.I.; GARBELOTTI, M.L.; OLIVEIRA, E.de; LAMARDO, L.C.A. Características físicas e químicas dos frutos do cambucizeiro (*Campomanesia phaea*). **Revista Brasileira de Fruticultura**, Jaboticabal, v.27, n2, p.241-4, 2005.
- WANG, D.; YEATS, T.H.; ULUISIK, S.; ROSE, J.K.C.; SEYMOUR, G.B. Fruit softening: revisiting the role of pectin. **Trends in Plant Science**, Oxford, v.23, n.4, p.302-10, 2018.
- WOISKY, R.G.; SALATINO, A. Analysis of propolis : some parameters and procedures for chemical quality control. **Journal of Apicultural Research**, Abingdon, v.37, n.2, p.99–105, 1998.
- ZHENG, L.Y.; SUN, G.M.; LIU, Y.G.; LV, L.L.; YANG, W.X.; ZHAO, W.F.; WEI, C. Aroma volatile compounds from two fresh pineapple varieties in China. **International Journal of Molecular Sciences**, Basel, v.13, n.6, p.7383–7392, 2012. ZHU, X.; LI, Q.; LI, J.; LUO, J.; CHEN, W.; LI, X. Comparative study of volatile compounds in the fruit of two banana cultivars at different ripening stages. **Molecules**, Basel, v.23, n.10, 2018.