



# Dynamics of the loss and emergence of volatile compounds during the concentration of cashew apple juice (*Anacardium occidentale* L.) and the impact on juice sensory quality



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## ABSTRACT

Concentrating cashew apple juice alters the beverage aroma and flavor, compromising consumer acceptability of the concentrated beverage. To understand the mechanisms involved in these changes, this research characterized the dynamics of the loss and emergence of volatile compounds during cashew apple juice concentration, reporting their impact on beverage sensory quality. Fresh cashew apple juice (10.3°Brix) was concentrated in a thermal-siphon type evaporator operating in a closed system. Five samples were taken throughout the concentration process with the following soluble solids contents: 11.8°Brix, 14.9°Brix, 20.2°Brix, 29.6°Brix and 42.1°Brix. Trained judges rated the aroma note intensities, described as “fresh cashew apple” and “cooked” as perceived in the fresh and concentrated beverages. The headspace volatiles of the six samples were identified and quantified by GC–MS. The results indicated the esters as the major component in the fresh juice (226.46  $\mu\text{g kg}^{-1}$ ) representing 45.0% of the total mass of volatiles, followed by the terpenes (118.98  $\mu\text{g kg}^{-1}$ ), acids (45.23  $\mu\text{g kg}^{-1}$ ), aldehydes (39.10  $\mu\text{g kg}^{-1}$ ), alcohols (18.91  $\mu\text{g kg}^{-1}$ ), lactones (19.15  $\mu\text{g kg}^{-1}$ ), hydrocarbons (18.02  $\mu\text{g kg}^{-1}$ ) and ketones (11.05  $\mu\text{g kg}^{-1}$ ). Predictive statistical models ( $R^2 > 0.956$ ,  $p \leq 0.002$ ) revealed that on reaching 14.9°Brix, the ester concentration declined more than 90%, the terpene content almost 100%, alcohols 85%, aldehydes 80% and hydrocarbons 90%. At 14.9°Brix, the intensity of “fresh cashew apple” aroma still predominated in the juice, but the panelists detected the presence of a weak “cooked” aroma. Concentration of the beverage to 20.2°Brix or above expressively increased the cooked aroma intensity and the concentration of hydrocarbons, alcohols and some aldehydes usually associated with off-flavors such as pentanal and decanal. This raises the possibility that some of these compounds might have been formed during juice processing. Juice with better sensory quality could possibly be obtained by concentrating the beverage to levels below 20.2°Brix, recovering the esters that evaporated off the juice until ~15°Brix is reached, and re-adding them to the juice concentrated.

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## 1. Introduction

The annual production of Brazilian concentrated cashew juice is approximately 44 million L. This represents an income above US\$ 70 million, only surpassed by concentrated orange and grape juices (IBGE, 2014). Nevertheless, the sector could still grow considerably, since of the 1.8 million t of pseudo-fruits annually processed to obtain cashew nuts and more than 80% of the peduncles are discarded as industrial residues after removing the nut (Leitão et al., 2011). This

occurs because the nut is a product of greater demand, with an aggregated value much higher than the juice.

An increase in the consumption and demand for concentrated cashew apple juice requires optimization of the technological processing of the beverage, since the current practice alters the exotic and highly appreciated aroma and flavor of the juice (Garruti, Facundo, Neto, & Wagner, 2008).

Changes in the sensory quality of cashew apple juice occurring during concentration are partially attributed to evaporation of the volatile compounds originally present in the juice (Garruti et al., 2008; Sampaio, Garruti, Franco, Janzantti, & Da Silva, 2011). This occurs notably with the esters, which represent 90% of the total mass of volatiles that evaporate off from the cashew apple juice during concentration to 40°Brix (Sampaio, Biasoto, Marques, Batista, & Da Silva, 2013).

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The esters are the major chemical class in cashew apple juice (Bicalho, Pereira, Aquino Neto, Pinto, & Rezende, 2000; Garruti, Franco, Da Silva, Janzantti, & Alves, 2003; Maciel, Hansen, Aldinger, & Labows, 1986; Sampaio, Biasoto, & Da Silva, 2015). They are known to add desirable aromatic notes to the fresh beverage, described as 'fresh cashew apple', 'fruity' and 'sweet' (Garruti et al., 2003; Sampaio et al., 2015). Therefore, it is reasonable to assume that the loss of esters during concentration of the juice would alter its aroma and flavor. Nevertheless, so far, no controlled study has quantified how much the mass of esters evaporated off represents in relation to that originally present in the beverage, or if their loss is the only factor reducing the sensory quality of the concentrated cashew apple juice.

In fruit juices, alterations in the sensory profile during heat processing can also occur due to formation of volatile compounds. Known examples of these are the lactones, formed in pineapple as from esters such as 5-hydroxy-octanoate and 5-ethyl hydroxy-decanoate, adding a marked note of coconut aroma/flavor to the product (Elss et al., 2005; Fisher & Scott, 1997). In turn, the degradation of ascorbic acid can form furans such as 3-hydroxy-2-pyran, furaldehyde and 5-hydroxy-methyl-furfural, amongst other odor volatiles (Robertson & Samaniego, 1986). The aroma described as 'cooked', characteristic of canned pineapple, is attributed to 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF or furaneol), which is liberated from its glycoside during heat processing of the fruit, prejudicing the sensory quality of the processed fruit (Reineccius, 2006). Recently, Zepka, Mercadante, Garruti, Sampaio, and Da Silva (2014), studying volatiles formation in model cashew juice solutions, verified the formation of potential odor volatiles from the heat degradation of carotenoids present in the fruit. Amongst the compounds identified were *p*-xylene, naphthalene, 4-ethyl-1,2-dimethylbenzene and 1,2,3,5-tetramethylbenzene. Although several odor volatiles can be formed during the concentration of cashew apple juice, there is a lack of studies in the academic literature investigating this phenomenon and its impact on the sensory quality of the concentrated beverage.

For the reasons outlined above, the objective of this research was to characterize and quantify the dynamics of the losses and emergences of volatile compounds during the concentration of cashew apple juice, analyzing the impact of these alterations on the beverage sensory quality. Such information is of great importance for the cashew apple juice processing industries in the optimization of their processes.

## 2. Material and methods

### 2.1. Raw material

This study used pseudo-fruits from the clone CCP 76, variety *early dwarf*, developed by the Brazilian Agricultural Research Corporation – EMBRAPA, Brazil, and cultivated in Arthur Nogueira, State of São Paulo, Brazil (22°34'23"S and 47°10'21"W, 588 m altitude). To obtain the whole juice, the nuts were removed, the pseudo-fruits washed with a sodium hypochlorite solution (100 mg L<sup>-1</sup>), triturated in a knife mill and filtered through cotton cloth. The fresh juice, showing a soluble solids content of 10.3°Brix, was immediately concentrated.

### 2.2. Juice concentration

Seven batches of fresh juice, each 24.5 kg, were concentrated in a thermal-siphon type pilot evaporator (Precision Scientific Co., Chicago, USA). It is a closed system operating under vacuum (700 mm Hg), heating with steam at 110 °C as described by Sampaio et al. (2013). The beverage was repeatedly passed through the evaporator until reaching approximately 40°Brix. In the evaporator the juice temperature remained at 38° ± 2 °C throughout the whole process, and it took approximately 15 min for each batch to reach 40°Brix.

During processing, the system was paralyzed after evaporation of approximately 20% of the total amount of water to be removed, and an aliquot of 200 g juice taken for analysis, thus obtaining five distinct

samples with different soluble solids contents (°Brix). After processing the seven batches, the aliquots presenting the same °Brix were mixed, obtaining, in addition to the fresh juice (10.3°Brix), five concentrated cashew juice samples with the following soluble solids contents: 11.8°Brix (juice<sub>11.8°Brix</sub>), 14.9°Brix (juice<sub>14.9°Brix</sub>), 20.2°Brix (juice<sub>20.2°Brix</sub>), 29.6°Brix (juice<sub>29.6°Brix</sub>) and 42.1°Brix (juice<sub>42.1°Brix</sub>). These samples were transferred to amber glass flasks and frozen at -18 °C until analysis. A Carl Zeiss Jena bench refractometer model 844976 (Carl Zeiss Industrial Metrology, Maple Grove, Minnesota, USA) was used to determine the soluble solids contents (°Brix) at 20 °C.

### 2.3. Isolation of the volatiles

The volatiles in the fresh juice (10.3°Brix) and in the five concentrated juice samples (see above) were extracted using the headspace vapor enrichment method described by Sampaio et al. (2015). Porapak® type Q, 80–100 mesh (Bellefonte, PA, USA) was used as the trap.

For this, 300 g of juice was placed in a 1000 mL round glass flask and 90 g NaCl (Merck PA, Darmstadt, Germany) added. The system was stirred using a magnetic stirrer and the volatiles were trapped at 22 ± 2 °C for 2 h under 70 mm Hg of vacuum. Elution was done with 300 µL of acetone (Mallinckrodt Baker, KY, USA).

A gas chromatograph coupled to a mass spectrometer (GC–MS) HP 7890/5975 (Agilent Technologies, Palo Alto, USA) and two fused silica capillary columns: DB-Wax and DB-5 (30 m × 0.25 mm, 0.25 µm – J&W Scientific, Folsom, CA, USA) were used to identify and quantify the volatiles isolated from the fresh juice (10.3°Brix) and from the five concentrated juices. The analytical conditions were similar to those employed by Sampaio et al. (2013) and Sampaio et al. (2015), using an injection volume of 2 µL.

#### 2.3.1. Identification of the volatiles

The volatiles extracted from the fresh juice (10.3°Brix) and from the five concentrated juices were identified as follows: i) by comparing the experimentally obtained mass spectra with those available in the GC–MS library (NIST/EPA/NIH Mass Spectral Library, version 2.0, 2008) and with the spectra of pure standards analyzed using the same equipment under the same conditions described above; and ii) by comparison of the retention indexes (RI) obtained experimentally on the DB-Wax and DB-5 columns with the RI of the pure standards and of compounds described in the literature, obtained on columns with the same polarity (Acree & Arn, 2014; Cacho, Moncayo, Palma, Ferreira, & Culleré, 2012; El-Sayed, 2014; Garruti et al., 2003; Sampaio et al., 2011, 2013, 2015; Valim, Rouseff, & Lin, 2003). The RI were calculated using the retention times of C7–C30 linear chain alkanes (Polyscience 211 C kit, Chicago, USA) present in a solution analyzed on the GC–MS under the same conditions described above.

Compounds were considered *positively* identified when presenting mass spectra and retention indexes compatible with those of pure standards injected and analyzed under the same conditions. Compounds were considered *identified* when presenting mass spectra compatible with those provided by the equipment library and retention indexes similar to those found in the literature. Compounds were considered *tentatively* identified when the identification was based only on information generated by the mass spectrometer.

#### 2.3.2. Quantification of the volatiles

The volatiles present in the fresh juice (10.3°Brix) and in the five concentrated juices were quantified by external standardization. For this, twelve pure standards belonging to the chemical classes considered important for cashew apple aroma and flavor in previous research (Garruti et al., 2003; Garruti et al., 2008; Maciel et al., 1986; MacLeod & Troconis, 1982; Sampaio et al., 2011; Sampaio et al., 2013; Sampaio et al., 2015; Valim et al., 2003) were used. They were: esters (ethyl isovalerate to esters with RI ≤ 1400, and ethyl cinnamate to esters with RI > 1400), alcohols (*cis*-3-hexenol to alcohols with a linear

chain, and benzyl alcohol to alcohols with a benzene ring), aldehydes (hexanal to aldehydes with a linear chain, and benzyl alcohol to aldehydes with a benzene ring), ketones (2,3-butanedione to ketones with  $RI \leq 1300$ , 4-hydroxy-4-methyl-2-pentanone to ketones with  $RI > 1300$ , and benzyl alcohol to ketones with a benzene ring), lactones ( $\gamma$ -nonalactone), hydrocarbons ( $\beta$ -myrcene to hydrocarbons with a linear chain, and benzyl alcohol to aromatic hydrocarbons), acids (2-methyl-butanoic acid), terpenes (carveol to alcoholic terpenes, and  $\beta$ -myrcene to the other terpenes) and sulfur compounds (dimethyl sulfide). All the standards presented a purity grade  $> 98\%$  (Sigma-Aldrich, St. Louis, MO, USA). Each standard was prepared with nine concentrations from 0.0025 to 50.00  $\text{mg kg}^{-1}$ . The standard solutions were prepared by dissolving the pure standards in acetone (Mallincrodt Baker, KY, USA) and injecting three replications into the GC–MS under the same conditions described above. Predictive statistical models ( $R^2 \geq 0.990$ ,  $p < 0.001$ ) between the concentration of the standard ( $\text{mg kg}^{-1}$ ) and the corresponding unit area obtained by GC–MS were generated for each standard. These models were used to quantify the volatiles identified in the juice.

#### 2.4. Sensory evaluation

A sensory panel consisting of eight trained judges evaluated the intensities of “characteristic fresh cashew apple aroma” and “cooked aroma” in the fresh juice (10.3°Brix) and in the five concentrated juices. All the judges had extensive experience in the sensory evaluation of food and beverages, and were trained according to the procedures detailed in Sampaio et al. (2013). Tests occurred in individual booths of the Sensory Analysis Laboratory, Department of Food and Nutrition, Food Engineering Faculty, UNICAMP, Brazil. The study was previously approved by the Ethics Committee in Human Research of the University of Campinas (protocol number 1058/2008).

The samples were served at a temperature close to 18 °C in tulip-shaped glasses coded with three-digit numbers and covered with a watch glass. The intensity of the two descriptors was evaluated using a 9 cm non-structured scale anchored at the extreme left and right by the terms ‘weak/none’ and ‘strong’, respectively. All the judges evaluated all the samples with three repetitions.

#### 2.5. Dynamics of the sensory and volatiles changes during juice concentration

The dynamics of the losses and emergence of esters, terpenes, alcohols, aldehydes, ketones and hydrocarbons during concentration to 42.1°Brix was evaluated using predictive models constructed between the independent variable ‘°Brix of the juice’ (10.3°Brix, 11.8°Brix, 14.9°Brix, 20.2°Brix, 29.6°Brix and 42.1°Brix) and the dependent variable ‘concentration of the chemical class’ ( $\mu\text{g kg}^{-1}$ ). In addition, differences between the concentrations of the volatiles present in the fresh juice (10.3°Brix) and in the most concentrated beverage (42.1°Brix) were investigated using the Student *t*-test ( $p \leq 0.05$ ).

Predictive models were also generated between the independent variable ‘°Brix of the juice’ and the variable ‘aroma intensity’ for the aromatic notes of ‘fresh cashew apple juice’ and ‘cooked’. The models were constructed using the statistical software OriginPro 8.0® (OriginLab Corporation, Northampton, MA, USA).

#### 2.6. Impact of the loss and emergence of volatiles on juice sensory quality

Initially a Pearson correlation analysis ( $p \leq 0.05$ ) was applied to the concentration of the volatiles in the six juice samples (10.3°Brix, 11.8°Brix, 14.9°Brix, 20.2°Brix, 29.6°Brix and 42.1°Brix) and the corresponding intensity of fresh cashew apple and cooked aromas perceived in the beverages. This procedure was carried out using the version 9.3 of SAS (SAS® Institute Inc., Cary, N.C., 2011). It allowed for an initial examination of the relationship between the changes in the juice volatiles

composition and the changes in the beverage sensory quality during its concentration.

Volatiles showing a significant Pearson's correlation ( $p \leq 0.05$ ) with the fresh or cooked aroma of the juice were selected for further analysis using Extended Mapping (Biasoto, Netto, Marques, & Da Silva, 2014; McEwan, 1998). This is a multivariate statistical analysis allowing the regression of the sensory data (such as intensity of cashew apple aroma) against the co-ordinates of chemical data (e.g. concentration of each chemical class in the juices) obtained from a dimension reduction multivariate analysis (such as Principal Component Analysis). This analysis was carried out using the XLSTAT Sensory software (Addinsoft Inc., Paris, FR, 2014).

### 3. Results and discussion

#### 3.1. Profiles of the volatiles in the juices

Table 1 shows the concentrations of the volatiles identified in the fresh cashew juice (10.3°Brix) and in the juices concentrated to 11.8°Brix, 14.9°Brix, 20.2°Brix, 29.6°Brix and 42.1°Brix. A total of 108 volatiles were identified in the fresh juice, whereas only 62 were found in that concentrated to 42.1°Brix, indicating a loss of volatiles during concentration. Of the 108 volatiles identified in the fresh juice, 29 were esters, 30 terpenes, 9 ketones, 9 hydrocarbons, 10 aldehydes, 9 alcohols, 3 acids and 2 lactones, amongst others. On the other hand, 9 esters, 2 terpenes, 11 ketones, 14 hydrocarbons, 8 aldehydes, 1 acid, 5 alcohols and 1 lactone, amongst others, were identified in the juice concentrated to 42.1°Brix.

Table 1 also shows a great loss in the total mass of volatiles initially present in the fresh juice during its concentration to 42.1°Brix, from 503.27  $\mu\text{g kg}^{-1}$  in the fresh juice to 34.90  $\mu\text{g kg}^{-1}$  in the most concentrated juice ( $p = 0.001$ ). The concentration of the majority of the volatiles was significantly reduced ( $p \leq 0.050$ ) when the beverage was concentrated to 42.1°Brix, except the concentration of octanal, nonanal, tetradecane, *o*-xylene, 2,3-butanedione, 3-hydroxy-2-butanone, 6-methyl-5-hepten-2-one, methyl *trans*-2-butanoate and *trans*-geranyl acetone.

A great change in the juice volatiles profile was observed when the beverage was concentrated. In the fresh juice, the esters were present in the greatest concentration (226.46  $\mu\text{g kg}^{-1}$ ), representing 45.0% of the total mass of volatiles, followed by the terpenes (23.6%), acids (9.0%), aldehydes (7.8%), alcohols (3.8%), lactones (3.8%), hydrocarbons (3.6%) and ketones (2.2%). On the other hand, the hydrocarbons represented the largest group of volatiles present in the juice concentrated to 42.1°Brix (10.27  $\mu\text{g L}^{-1}$ ), representing 29.4% of the total mass of volatiles, followed by the aldehydes (25.7%), acids (16.0%), alcohols (14.1%), esters (10.2%), ketones (1.8%), terpenes (1.6%) and lactones (0.4%).

There was an enormous decrease in esters during concentration of the juice to 42.1°Brix (Table 1), from 226.46  $\mu\text{g kg}^{-1}$  in the fresh juice to 3.56  $\mu\text{g kg}^{-1}$  in the juice concentrated to 42.1°Brix ( $p = 0.001$ ). The concentrations of the major esters in the fresh juice, such as ethyl isovalerate, ethyl *trans*-2-butenate, ethyl hexanoate, ethyl butanoate, ethyl propanoate, ethyl 2-methyl-butanoate, methyl isovalerate and isoamyl acetate, were greatly reduced ( $p \leq 0.005$ ) when beverage was concentrated to 42.1°Brix. In fact, only four esters were found in this juice, namely isoamyl acetate, ethyl propanoate, ethyl hexanoate and ethyl butanoate, and in concentrations greatly reduced as compared to the fresh juice.

All esters lost during the concentration of the juice (Table 1) added desirable aromatic notes to the beverage, usually described as ‘fruity’, ‘ripe fruit’, ‘cashew apple’ and ‘sweet’ (Garruti et al., 2003; Sampaio et al., 2015). Taking the studies of Sampaio et al. (2013) as a basis, their loss was mostly due to evaporation, since esters were the major chemical class identified by the authors in the material evaporated from cashew apple juice during its concentration to 40°Brix.

Table 1

Volatile compounds and respective concentrations ( $\mu\text{g kg}^{-1}$ ) in fresh and concentrated cashew apple juices.

Compounds identified grouped according to chemical class	RI DB-wax	RI DB-5	Fresh Juice 10.3°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 11.8°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 14.9°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 20.2°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 29.6°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 42.1°Brix ( $\mu\text{g kg}^{-1}$ )
<i>Acids</i>								
Acetic acid <sup>a</sup>	1452	nd	38.47	4.93	3.64	2.89	9.00	5.58
3-Methyl butanoic acid <sup>b</sup> + 2-Methyl butanoic acid <sup>a</sup>	1684	nd	6.76	nd	nd	nd	nd	nd
Total			45.23	4.93	3.64	2.89	9.00	5.58
Total % of the chromatogram			8.99%	2.45%	11.54%	14.60%	26.53%	15.99%
<i>Alcohols</i>								
n-Propanol <sup>b</sup>	1057	nd	nd	nd	nd	nd	nd	0.92
Isobutyl alcohol <sup>b</sup>	1118	nd	nd	1.29	nd	nd	0.93	2.87
n-Butanol <sup>b</sup>	1162	nd	0.96	0.88	0.46	0.55	0.57	nd
2,3-Dimethyl-3-buten-2-ol <sup>c</sup>	1161	894	nd	nd	nd	nd	0.58	0.65
1-Penten-3-ol <sup>b</sup>	1175	nd	0.69	0.73	nd	nd	0.50	nd
3-Methyl-1-butanol <sup>a</sup>	1222	nd	4.93	3.54	1.80	0.57	0.41	nd
2-Hexanol <sup>b</sup>	1237	nd	nd	0.45	nd	nd	nd	nd
n-Pentanol <sup>b</sup>	1263	nd	nd	1.04	nd	nd	nd	nd
4-Methyl-3-penten-2-ol <sup>b</sup>	1276	nd	1.53	nd	nd	nd	nd	nd
n-Hexanol <sup>a</sup>	1377	876	3.10	0.83	nd	nd	nd	nd
2-Ethyl-1-hexanol <sup>b</sup>	1495	1031	1.59	0.73	nd	nd	nd	nd
n-Octanol <sup>a</sup>	1571	nd	1.69	0.82	nd	nd	nd	nd
Benzyl alcohol <sup>a</sup>	1881	nd	1.91	nd	nd	nd	nd	nd
2,6-Di-tert-butyl-p-cresol (BHT) <sup>b</sup>	1917	nd	2.51	1.74	0.18	0.21	0.21	0.22
2,4-Di-tert-butyl phenol <sup>c</sup>	2315	nd	nd	0.96	nd	nd	0.23	0.24
Total			18.91	13.01	2.44	1.33	3.43	4.90
Total % of the chromatogram			3.76%	6.45%	7.74%	6.72%	10.11%	14.04%
<i>Aldehydes</i>								
Pentanal <sup>b</sup>	993	nd	nd	nd	0.80	1.13	1.14	1.47
Hexanal <sup>a</sup>	1093	nd	8.00	5.46	1.79	2.26	3.36	2.11
2-Methyl-2-pentenal <sup>b</sup>	1161	nd	2.73	nd	nd	nd	nd	nd
Heptanal <sup>b</sup>	1187	nd	1.41	2.72	0.58	0.76	0.86	0.93
3-Methyl-2-butenal <sup>b</sup>	1198	nd	11.94	nd	nd	nd	nd	nd
Trans-2-hexenal <sup>a</sup>	1216	nd	1.47	nd	nd	nd	nd	nd
Octanal <sup>a</sup>	1288	1003	1.76	2.11	0.84	0.67	1.16	0.95
Nonanal <sup>b</sup>	1389	1104	2.59	2.48	1.17	1.14	2.01	1.51
Decanal <sup>a</sup>	1492	1207	nd	1.16	0.83	1.08	1.86	1.30
Benzaldehyde <sup>b</sup>	1509	966	2.92	0.87	0.16	0.25	0.57	0.59
Cis-4-decenal <sup>b</sup>	1570	nd	2.16	nd	nd	nd	nd	nd
Trans-2-cis-6-nonadienal <sup>b</sup>	1579	1162	4.12	nd	nd	nd	nd	nd
3-Ethyl-benzaldehyde <sup>b</sup>	1698	1178	nd	1.58	0.19	0.10	0.11	0.11
4-Ethyl-benzaldehyde <sup>a</sup>	1718	nd	nd	0.85	nd	nd	nd	nd
Total			39.10	17.23	6.36	7.39	11.07	8.97
Total % of the chromatogram			7.77%	8.55%	20.17%	37.34%	32.63%	25.70%
<i>Esters</i>								
Ethyl propanoate <sup>b</sup>	980	nd	19.38	11.31	0.78	0.54	0.53	0.56
Ethyl 2-methylpropanoate <sup>b</sup>	983	721	nd	4.17	nd	nd	nd	nd
Propyl acetate <sup>a</sup>	992	799	1.50	0.82	nd	nd	nd	nd
Methyl 2-methylbutanoate <sup>b</sup>	1020	nd	0.79	0.63	nd	nd	nd	nd
Isobutyl acetate <sup>b</sup>	1023	806	0.07	ns	ns	0.46	0.25	0.44
Methyl isovalerate <sup>a</sup>	1028	815	8.58	3.54	nd	nd	nd	nd
Ethyl butanoate <sup>a</sup>	1045	807	32.38	18.99	0.37	0.97	0.16	0.22
Ethyl 2-methylbutanoate <sup>a</sup>	1061	860	13.73	7.35	nd	nd	nd	nd
Ethyl isovalerate <sup>a</sup>	1078	858	56.53	31.94	0.28	nd	nd	nd
Butyl acetate <sup>a</sup>	1082	823	2.59	4.78	nd	0.37	0.10	nd
Propyl isovalerate <sup>b</sup>	1112	nd	0.17	nd	nd	nd	nd	nd
Methyl trans-2-butenate <sup>b</sup>	1117	nd	0.37	0.34	nd	nd	nd	nd
Isoamyl acetate <sup>a</sup>	1133	884	7.13	3.02	9.17	0.35	0.63	0.64
Methyl 3-methylpentanoate <sup>b</sup>	1137	889	1.37	1.64	nd	nd	nd	nd
Ethyl pentanoate <sup>b</sup>	1140	nd	0.60	nd	nd	nd	nd	nd
Propyl 3-methylbutanoate <sup>b</sup>	1161	nd	nd	0.48	nd	nd	nd	nd
Ethyl trans-2-butenate <sup>a</sup>	1169	857	39.84	21.33	2.06	0.30	0.42	nd
Ethyl 3-methylpentanoate <sup>b</sup>	1184	1002	2.23	8.31	nd	nd	nd	nd
Methyl hexanoate <sup>b</sup>	1192	934	0.24	0.49	nd	nd	nd	nd
Methyl 2-methylene-butanoate <sup>b</sup>	1193	nd	0.30	0.32	nd	nd	nd	nd
Ethyl 3-methyl-2-butenate <sup>b</sup>	1225	nd	0.16	nd	nd	nd	nd	nd
Ethyl hexanoate <sup>a</sup>	1243	1000	34.40	15.96	0.19	0.18	0.31	0.21
Ethyl trans-2-pentenoate <sup>b</sup>	1264	959	0.29	0.49	nd	nd	nd	nd
Isopentyl 2-methyl-propanoate <sup>b</sup>	1271	nd	nd	0.15	nd	nd	nd	nd
2-Methyl butyl isovalerate <sup>b</sup>	1296	nd	0.25	0.19	nd	nd	nd	nd
Ethyl cis-3-hexenoate <sup>b</sup> + ethyl trans-3-hexenoate <sup>b</sup>	1301	1004	0.21	0.21	nd	nd	nd	nd
Ethyl trans-2-hexenoate <sup>b</sup>	1345	nd	0.81	0.24	nd	nd	nd	nd
Propenyl 2-hexanoate <sup>b</sup>	1372	1084	0.30	nd	nd	nd	nd	nd

(continued on next page)

Table 1 (continued)

Compounds identified grouped according to chemical class	RI DB-wax	RI DB-5	Fresh Juice 10.3°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 11.8°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 14.9°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 20.2°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 29.6°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 42.1°Brix ( $\mu\text{g kg}^{-1}$ )
Ethyl octanoate <sup>a</sup>	<b>1435</b>	<b>1196</b>	0.84	0.38	0.16	0.12	nd	nd
<i>Esters</i>								
Ethyl 3-hydroxy-butanoate <sup>b</sup>	<b>1522</b>	<b>944</b>	ns	0.40	nd	0.10	0.24	0.32
1-Methyl hexyl acetate <sup>c</sup>	<b>1533</b>	<b>nd</b>	nd	nd	0.03	0.13	0.28	0.27
Isopropyl hexadecanoate <sup>b</sup>	<b>2242</b>	<b>nd</b>	nd	1.46	0.24	nd	nd	0.41
homomenthyl salicylate <sup>c</sup>	<b>2464</b>	<b>1894</b>	nd	nd	0.30	0.33	0.33	0.49
<b>Total</b>			<b>226.46</b>	<b>140.30</b>	<b>13.58</b>	<b>3.85</b>	<b>3.25</b>	<b>3.56</b>
<i>Total % of the chromatogram</i>			45.00%	69.61%	43.07%	19.45%	9.58%	10.20%
<i>Hydrocarbons</i>								
4-Ethyl octane <sup>b</sup>	<b>969</b>	<b>962</b>	nd	nd	nd	nd	nd	0.50
5-Methyl nonane <sup>c</sup>	<b>972</b>	<b>nd</b>	nd	nd	nd	nd	nd	0.68
3-Methyl nonane <sup>b</sup>	<b>981</b>	<b>977</b>	nd	nd	nd	nd	nd	0.86
Decane <sup>a</sup>	<b>1005</b>	<b>1000</b>	1.04	1.44	0.60	0.74	0.87	2.61
Cis-3-decene <sup>b</sup>	<b>1061</b>	<b>nd</b>	nd	nd	nd	nd	nd	0.58
Undecane <sup>a</sup>	<b>1097</b>	<b>nd</b>	0.51	1.01	nd	nd	nd	nd
Dodecane <sup>a</sup>	<b>1205</b>	<b>1200</b>	nd	nd	nd	nd	nd	0.88
Tridecane <sup>a</sup>	<b>1299</b>	<b>nd</b>	2.64	nd	nd	nd	nd	nd
Tetradecane <sup>a</sup>	<b>1398</b>	<b>1400</b>	0.81	0.41	0.42	0.40	nd	0.65
Pentadecane <sup>a</sup>	<b>1500</b>	<b>1500</b>	nd	1.04	nd	nd	nd	nd
Hexadecane <sup>a</sup>	<b>1601</b>	<b>1600</b>	nd	0.76	nd	nd	nd	nd
Heptadecane <sup>a</sup>	<b>1702</b>	<b>1701</b>	nd	1.19	nd	nd	nd	nd
Eicosane <sup>a</sup>	<b>2003</b>	<b>nd</b>	nd	1.38	nd	nd	nd	nd
Toluene <sup>b</sup>	<b>1045</b>	<b>804</b>	nd	nd	0.01	0.10	2.31	1.57
Ethylbenzene <sup>b</sup>	<b>1125</b>	<b>870</b>	7.91	0.75	0.37	0.41	nd	nd
$\rho$ -Xylene <sup>b</sup>	<b>1130</b>	<b>877</b>	0.91	0.53	nd	0.37	0.43	0.52
m-Xylene <sup>b</sup>	<b>1144</b>	<b>nd</b>	2.18	1.94	nd	nd	nd	nd
o-Xylene <sup>b</sup>	<b>1182</b>	<b>nd</b>	1.05	0.19	nd	nd	nd	0.40
m-Ethyltoluene <sup>b</sup>	<b>1224</b>	<b>nd</b>	nd	nd	nd	nd	nd	0.22
$\rho$ -Ethyltoluene <sup>b</sup>	<b>1259</b>	<b>nd</b>	nd	nd	nd	nd	nd	0.20
Styrene <sup>b</sup>	<b>1260</b>	<b>891</b>	0.97	0.96	0.31	0.29	0.49	0.32
Psi-cumene <sup>b</sup>	<b>1275</b>	<b>nd</b>	nd	nd	nd	nd	0.30	0.28
<b>Total</b>			<b>18.02</b>	<b>11.60</b>	<b>11.71</b>	<b>2.31</b>	<b>4.40</b>	<b>10.27</b>
<i>Total % of the chromatogram</i>			3.58%	5.76%	5.42%	11.67%	12.97%	29.43%
<i>Ketones</i>								
2,3-Butanedione <sup>a</sup>	<b>993</b>	<b>723</b>	0.13	0.24	0.08	0.06	0.10	0.08
2-Pentanone <sup>b</sup>	<b>995</b>	<b>nd</b>	0.06	nd	nd	nd	nd	nd
3-Hexanone <sup>b</sup>	<b>1069</b>	<b>nd</b>	0.06	nd	nd	nd	nd	nd
3-Methyl-3-buten-2-one <sup>c</sup>	<b>1003</b>	<b>nd</b>	nd	nd	nd	nd	nd	ns
4-Methylpentan-2-one <sup>b</sup>	<b>1017</b>	<b>nd</b>	nd	nd	nd	nd	nd	0.04
1-Penten-3-one <sup>b</sup>	<b>1029</b>	<b>nd</b>	nd	0.26	0.02	0.03	0.03	0.03
5-Hexen-2-one <sup>c</sup>	<b>1101</b>	<b>828</b>	nd	nd	0.04	0.04	0.03	0.04
3-Penten-2-one <sup>b</sup>	<b>1135</b>	<b>nd</b>	7.31	ns	0.02	nd	nd	nd
2,3-Hexanedione <sup>b</sup>	<b>1137</b>	<b>811</b>	nd	nd	ns	0.01	0.01	ns
3-Hydroxy-2-butanone <sup>a</sup>	<b>1283</b>	<b>nd</b>	0.07	0.81	0.02	0.08	0.10	0.10
2-Octanone <sup>b</sup>	<b>1284</b>	<b>nd</b>	nd	1.06	nd	nd	nd	nd
1-Hydroxy-2-propanone <sup>b</sup>	<b>1294</b>	<b>988</b>	0.11	nd	nd	nd	nd	nd
6-Methyl-5-heptene-2-one <sup>b</sup>	<b>1336</b>	<b>nd</b>	ns	0.02	0.01	0.02	0.02	ns
4-Hydroxy-2-methyl-2-pentanone <sup>a</sup>	<b>1363</b>	<b>851</b>	nq	nq	nq	nq	nq	nq
4-Hydroxy-2-butanone <sup>b</sup>	<b>1531</b>	<b>809</b>	3.31	ns	0.17	0.10	ns	ns
Acetophenone <sup>b</sup>	<b>1621</b>	<b>1066</b>	nd	0.73	0.53	0.15	0.14	0.34
2,4-Dimethylacetophenone <sup>c</sup>	<b>1779</b>	<b>nd</b>	nd	0.72	nd	nd	nd	nd
3,4-Dimethylacetophenone <sup>c</sup>	<b>1788</b>	<b>1195</b>	nd	0.64	nd	nd	nd	nd
4-Ethylacetophenone <sup>c</sup>	<b>2250</b>	<b>1286</b>	nd	1.02	0.02	0.14	0.22	nd
<b>Total</b>			<b>11.05</b>	<b>5.50</b>	<b>0.91</b>	<b>0.63</b>	<b>0.65</b>	<b>0.63</b>
<i>Total % of the chromatogram</i>			2.20%	2.73%	2.89%	3.18%	1.92%	1.81%
<i>Lactones</i>								
5,5-Dimethyl-2(5H)-furanone <sup>b</sup>	<b>1597</b>	<b>956</b>	4.12	0.54	0.16	0.14	0.34	0.15
3,5-Dimethyl-2(5H)-furanone <sup>b</sup>	<b>1643</b>	<b>995</b>	15.03	nd	nd	nd	nd	nd
<b>Total</b>			<b>19.15</b>	<b>0.54</b>	<b>0.16</b>	<b>0.14</b>	<b>0.34</b>	<b>0.15</b>
<i>Total % of the chromatogram</i>			3.81%	0.27%	0.51%	0.71%	1.00%	0.43%
<i>Terpenes</i>								
$\alpha$ -Pinene <sup>b</sup>	<b>1026</b>	<b>937</b>	ns	ns	ns	nd	nd	nd
$\beta$ -Pinene <sup>b</sup>	<b>1102</b>	<b>976</b>	0.71	nd	nd	nd	nd	nd
$\rho$ -Limonene <sup>a</sup>	<b>1194</b>	<b>1030</b>	3.80	1.06	0.40	0.40	0.49	nd
$\rho$ -Cineole <sup>b</sup>	<b>1208</b>	<b>1032</b>	1.39	0.77	nd	nd	nd	nd
o-Cymene <sup>b</sup>	<b>1224</b>	<b>1024</b>	0.45	nd	nd	nd	nd	nd
$\rho$ -Cymene <sup>b</sup>	<b>1269</b>	<b>nd</b>	0.41	nd	nd	nd	nd	nd
Cis-linalool oxide <sup>b</sup>	<b>1443</b>	<b>nd</b>	17.61	nd	nd	nd	nd	nd
Trans-linalool oxide <sup>b</sup>	<b>1470</b>	<b>nd</b>	17.84	nd	nd	nd	nd	nd
$\alpha$ -Cubebene <sup>b</sup>	<b>1477</b>	<b>nd</b>	2.11	nd	nd	nd	nd	nd
$\alpha$ -Copaene <sup>b</sup>	<b>1479</b>	<b>nd</b>	2.01	1.02	0.44	nd	nd	nd

Table 1 (continued)

Compounds identified grouped according to chemical class	RI DB-wax	RI DB-5	Fresh Juice 10.3°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 11.8°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 14.9°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 20.2°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 29.6°Brix ( $\mu\text{g kg}^{-1}$ )	Juice 42.1°Brix ( $\mu\text{g kg}^{-1}$ )
<i>Terpenes</i>								
$\alpha$ -Gurjunene <sup>b</sup>	1516	nd	0.48	nd	nd	nd	nd	nd
$\beta$ -Linalool <sup>a</sup>	1557	nd	nd	0.38	nd	nd	nd	nd
$\beta$ -Caryophellene <sup>b</sup>	1582	nd	1.22	nd	nd	nd	nd	nd
$\beta$ -Elemene <sup>b</sup>	1587	nd	3.55	nd	nd	nd	nd	nd
4-Terpineol <sup>b</sup>	1600	1178	0.95	nd	nd	nd	nd	nd
Hotrienol <sup>b</sup>	1615	nd	0.56	nd	nd	nd	nd	nd
$\beta$ -Terpineol <sup>b</sup>	1641	nd	ns	nd	nd	nd	nd	nd
Dihydro citronellol <sup>b</sup>	1683	nd	ns	nd	nd	nd	nd	nd
$\delta$ -Elemene <sup>b</sup>	1686	nd	1.21	nd	nd	nd	nd	nd
<i>Trans</i> - $\alpha$ -farnesane <sup>b</sup>	1691	nd	1.15	nd	nd	nd	nd	nd
$\alpha$ -Terpineol <sup>b</sup>	1699	nd	32.20	nd	nd	nd	nd	nd
$\beta$ -Selinene <sup>b</sup>	1707	nd	2.68	nd	nd	nd	nd	nd
$\alpha$ -Selinene <sup>b</sup>	1711	nd	1.66	nd	nd	nd	nd	nd
Pyran <i>trans</i> -linalool oxide <sup>b</sup>	1726	nd	3.31	nd	nd	nd	nd	nd
$\delta$ -Cadinene <sup>b</sup>	1732	nd	nd	nd	0.43	0.30	0.41	0.21
$\beta$ -Citronellol <sup>a</sup>	1742	nd	ns	nd	nd	nd	nd	nd
<i>Cis</i> -geraniol <sup>a</sup>	1759	nd	2.71	nd	nd	nd	nd	nd
<i>Trans</i> -geraniol <sup>b</sup>	1780	nd	4.36	nd	nd	nd	nd	nd
<i>Trans</i> -geranylacetone <sup>b</sup>	1782	nd	0.80	0.65	0.31	0.29	0.37	0.36
$\alpha$ -Ionone <sup>b</sup>	1809	nd	nd	nd	nd	nd	ns	nd
2,6-Dimethyl-3,7-octadiene-2,6-diol <sup>b</sup>	1953	nd	6.94	nd	nd	nd	nd	nd
2,6-Dimethyl-1,7-octadiene-3,6-diol <sup>b</sup>	2133	nd	4.38	nd	nd	nd	nd	nd
<b>Total</b>			<b>118.98</b>	<b>3.88</b>	<b>1.58</b>	<b>0.99</b>	<b>1.27</b>	<b>0.57</b>
Total % of the chromatogram			23.64%	1.92%	5.01%	5.00%	3.74%	1.63
<i>Other compounds</i>								
Dimethyl sulfide <sup>a</sup>	706	nd	2.88	nd	nd	nd	nd	nd
2,4-Dimethylfuran <sup>b</sup>	984	nd	nd	nd	0.07	nd	nd	nd
1-Ethoxy-1-propoxyetane <sup>c</sup>	985	nd	nd	nd	nd	nd	nd	nq
2,5-Dimethylfuran <sup>b</sup>	998	nd	2.22	3.85	0.53	0.26	0.50	0.06
3,3-Diethoxypropene <sup>c</sup>	1010	nd	nd	nd	nd	nd	nd	nq
Dimethyl disulfide <sup>a</sup>	1079	nd	1.24	0.72	0.55	nd	0.02	nd
1,1-Diethoxy-3-methyl-butane <sup>b</sup>	1090	961	nd	nd	nd	nd	nd	nq
2-Ethoxy ethanol <sup>b</sup>	1230	nd	nd	nq	nd	nq	nd	nd
3,4-Epoxy-2-pentanone <sup>b</sup>	1271	nd	nq	nd	nd	nd	nd	nd
2-Butoxy ethanol <sup>b</sup>	1401	nd	nq	nq	nd	nd	nq	nq
Dimethyl sulfoxide <sup>b</sup>	1576	nd	nd	nd	nd	nd	nd	0.12
Ethyl1-(acetyloxy) acetate <sup>c</sup>	1787	1005	nq	nq	nq	nq	nq	nq
Dimethyl sulfone <sup>b</sup>	1904	nd	nd	nd	nd	nd	nd	0.09
Diethylene glycol <sup>b</sup>	1985	nd	nd	nd	nd	nd	nd	nq
2-Phenoxy ethanol <sup>b</sup>	2155	nd	nd	nq	nd	nd	nd	nd
Ethyl 4-ethoxybenzoate <sup>b</sup>	2184	1526	nq	nd	nq	nd	nd	nd
4-Sec-butoxy-2-butanone <sup>c</sup>	2275	nd	nd	nd	nd	nd	nd	nq
<b>TOTAL</b>			<b>503.24</b>	<b>201.56</b>	<b>31.53</b>	<b>19.79</b>	<b>33.93</b>	<b>3490</b>

RI: retention index; nd: not detected; nq: not quantified; ns: amount not significative, in trace (concentration < 0.01  $\mu\text{g kg}^{-1}$ ).

<sup>a</sup> Positively identified compounds.

<sup>b</sup> Compounds identified by MS and the linear retention index.

<sup>c</sup> Tentatively identified compounds by MS.

In the present study, terpenes, another chemical class important to the characteristic cashew apple aroma (Sampaio et al., 2015), represented the second largest chemical class in terms of concentration in the fresh juice (118.98  $\mu\text{g kg}^{-1}$ ). The major terpenes identified were  $\alpha$ -terpineol (32.20  $\mu\text{g kg}^{-1}$ ) and a mixture of the *cis* and *trans* isomers of linalool oxide (35.45  $\mu\text{g kg}^{-1}$ ). The juice processing caused an expressive reduction in the terpene concentration (Table 1), decreasing from 118.98  $\mu\text{g kg}^{-1}$  in the fresh juice to 0.57  $\mu\text{g kg}^{-1}$  in the juice concentrated to 42.1°Brix ( $p = 0.010$ ). Of the 30 terpenes originally present in the fresh juice, only *trans*-geranylacetone was identified in the juice concentrated to 42.1°Brix; all the others being lost during concentration of the beverage. Paradoxically, the recovery of terpenes by condensation carried out by Sampaio et al. (2013) during the concentration of cashew apple juice to 40°Brix was negligible, giving concentrations below 0.01  $\mu\text{g kg}^{-1}$  in the material recovered. Only 4-terpineol,  $\gamma$ -terpinene and *trans*-geranylacetone were identified in the material recovered by Sampaio et al. (2013). Terpenes also represented minor components in the water phase recovered by Brazilian cashew juice processors and analyzed by Sampaio et al. (2011).

According to Clark and Chamblee (1992), terpenes can be converted into other terpenes during heat processing under acid conditions by way of hydration, dehydration, rearrangements and cyclization reactions. By heating a model solution containing terpenes for 30 min at 90 °C in an acidified medium (pH = 5), Varming, Andersen, and Poll (2006) reported the conversion of limonene into  $\alpha$ -terpineol, of linalool into  $\alpha$ -terpineol, of geraniol into nerol, of 4-terpineol into 1,4-cineole and  $\gamma$ -terpinene, and of  $\alpha$ -terpineol into 1,4-cineole. These results explain the appearance of  $\beta$ -linalool in juice concentrated to 11.8°Brix and of  $\delta$ -cadinene in more concentrated juices (Table 1). Terpenes can also be transformed into terpenic dioles such as 1,8- $\rho$ -mentanodiol by the thermal degradation of  $\alpha$ -terpineol and 4-terpineol, or by hydration reactions in an acid medium (Clark & Chamblee, 1992; Varming et al., 2006). For their part, terpenic dioles are often inadequately recovered by dynamic headspace techniques due to their low volatility and elevated polarity (Varming et al., 2006). This justifies the non-appearance and identification of terpenic dioles such as 1,8- $\rho$ -mentanodiol and 1,8- $\rho$ -mentanodiol in the concentrated juices of the current study (Table 1). Thus the significant decrease in terpenes observed in the

present study when the juice was concentrated to 42.1°Brix ( $p = 0.001$ ), associated with the low recovery of terpenes obtained by Sampaio et al. (2013) during the concentration of cashew juice to 40°Brix, suggests that part of the terpenes present in fresh cashew apple juice are highly heat labile, and degrade during concentration.

Table 1 shows that the acids represented the third most abundant class of volatiles both in the fresh juice ( $45.23 \mu\text{g kg}^{-1}$ ) and in that concentrated to 42.1°Brix ( $5.58 \mu\text{g kg}^{-1}$ ). Three carboxylic acids were identified in the fresh juice: acetic acid, 3-methyl-butanoic acid and 2-methyl-butanoic acid, whereas only acetic acid was found in the juice concentrated to 42.1°Brix. These acids were probably lost by evaporation, since they were identified by Sampaio et al. (2011) in the sample of cashew water phase generated by a Brazilian concentrated cashew juice industry, and also by Sampaio et al. (2013) in the material recovered by condensation during the concentration of cashew juice to approximately 40°Brix. As 3-methyl-butanoic acid and 2-methyl-butanoic acid have disagreeable odors, described as “sweaty”, “sweaty feet”, “stinky” and “cheesy” (Garruti et al., 2003; Garruti et al., 2008; Sampaio et al., 2011; Sampaio et al., 2015), their loss when the juice was concentrated to 42.1°Brix ( $p = 0.002$ ) was not necessarily undesirable.

### 3.2. Dynamics of the loss and emergence of volatiles during concentration

Figs. 1 and 2 show the dynamics of the loss and emergence of the chemical classes of esters, terpenes, ketones, aldehydes, alcohols and hydrocarbons from the juice during concentration. For each chemical class, the predictive models produced high determination coefficients ( $R^2$ ), which varied from 0.956 (for hydrocarbons) to 0.990 (for terpenes) and high levels of significance ( $p \leq 0.002$ ). This guarantees

the validity for the use of these models as predictors of the loss of each chemical class during concentration.

The model present in Fig. 1A ( $R^2 = 0.990$ ,  $p < 0.001$ ) shows that soon after the first step in heat processing, when the juice was concentrated from 10.3°Brix to 11.8°Brix, the terpene concentration ( $\mu\text{g kg}^{-1}$ ) fell more than 95% when compared to those initially present in the fresh juice. For esters ( $R^2 = 0.970$ ,  $p < 0.001$ ) the loss was almost 40% compared to the concentration initially present (Fig. 1B), 50% for ketones ( $R^2 = 0.983$ ,  $p < 0.001$ ) (Fig. 1C), 55% for aldehydes ( $R^2 = 0.966$ ,  $p = 0.002$ ) (Fig. 1D), 35% for hydrocarbons ( $R^2 = 0.956$ ,  $p = 0.002$ ) (Fig. 2B) and more than 30% for alcohols ( $R^2 = 0.971$ ,  $p = 0.002$ ) (Fig. 2A). In this step the most drastic loss was that of the terpenes, which fell from 30 compounds in the fresh juice to just five in the juice concentrated to 11.8°Brix (Table 1).

The following terpenes were lost during concentration of the juice to 11.8°Brix:  $\alpha$ -selinene,  $\beta$ -caryophyllene,  $\alpha$ -terpineol,  $\beta$ -terpineol, 4-terpineol, *cis*-geraniol, *trans*-geraniol, *cis*-linalool oxide, *trans*-linalool oxide, pyran *cis*-linalool oxide,  $\beta$ -elemene,  $\alpha$ -cubebene and  $p$ -cymene. They are previously associated with the aroma notes described as “green”, “grassy”, “refreshing”, “citric”, “fruity”, “oil”, “sweet” and “floral” (Acree & Arn, 2014; MacLeod & Troconis, 1982; Sampaio et al., 2015). Thus it was to be expected that the juice concentrated to 11.8°Brix should have lost the above mentioned aroma notes to a certain degree.

For their part, when the juice was concentrated to 14.9°Brix, the ester levels in the beverage fell more than 90% when compared with those initially present (Fig. 1B). In this step the terpene concentration fell almost 100%, the hydrocarbons about 90%, the alcohols 85% and the aldehydes 80%. Nevertheless, some esters and aldehydes with odoriferous impact continued present in the juice concentrated up to 14.9°Brix, such as ethyl propanoate, ethyl butanoate, ethyl isovalerate,

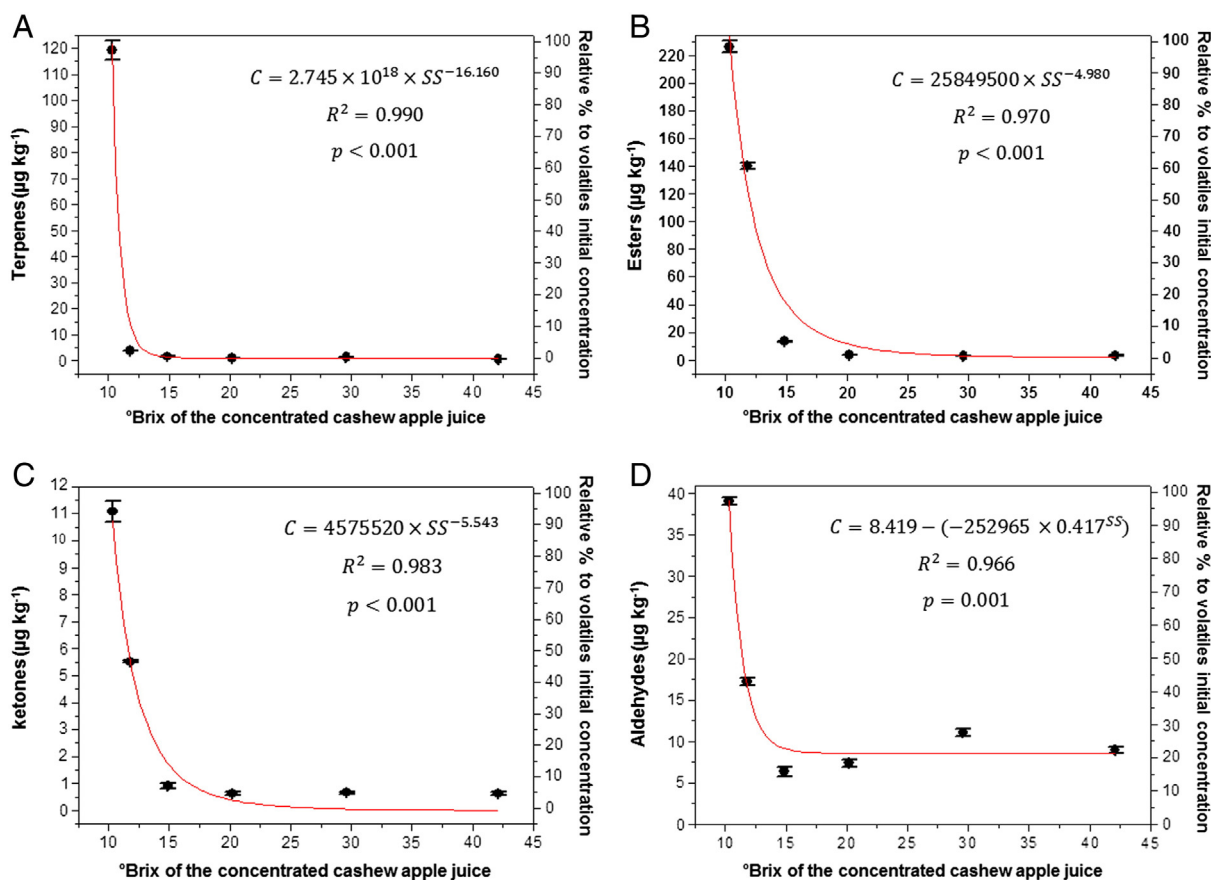


Fig. 1. Concentration ( $\mu\text{g kg}^{-1}$ ) of terpenes (A), esters (B), ketones (C) and aldehydes (D) in the concentrated cashew apple juice as a function of the solids content (°Brix) during the concentration process up to 42.1°Brix, including the standard error values. C = concentration of each chemical class, SS = soluble solids content in °Brix.

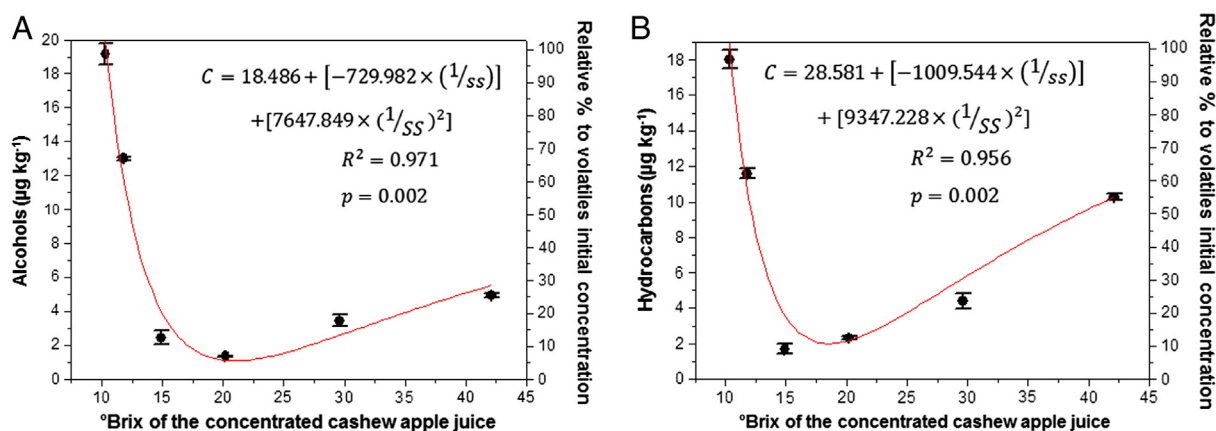


Fig. 2. Concentration ( $\mu\text{g kg}^{-1}$ ) of alcohols (A) and hydrocarbons (B) in the concentrated cashew apple juice as a function of the soluble solids content ( $^{\circ}\text{Brix}$ ) during the concentration process up to  $42.1^{\circ}\text{Brix}$ , including the standard error values. C = concentration of each chemical class, SS = soluble solids content in  $^{\circ}\text{Brix}$ .

isoamyl acetate, ethyl *trans*-2-butenate, ethyl hexanoate, ethyl octanoate, hexanal, octanal and benzaldehyde. These esters add aromatic notes to the cashew apple juice described as “sweet”, “fruity/ripe fruit”, “cashew”, “green/mint/herbal/citric” and “floral” (Garruti et al., 2003; Garruti et al., 2008; Maciel et al., 1986; Sampaio et al., 2011; Sampaio et al., 2013; Sampaio et al., 2015). With the exception of isoamyl acetate, all the esters mentioned above were found in the juice concentrated to  $14.9^{\circ}\text{Brix}$  but at concentrations way below those found in the fresh juice.

The model presented in Fig. 1C ( $R^2 = 0.983$ ,  $p < 0.001$ ) suggests that a great reduction in the levels of ketones also occurred when the juice was concentrated to  $14.9^{\circ}\text{Brix}$ . This can be explained by the loss of 3-penten-2-one, which represented 66% of the ketonic fraction of the fresh juice, but was no longer found as from a juice concentration of  $20.2^{\circ}\text{Brix}$  (Table 1). This loss could be related to its great reactivity, since it is an  $\alpha,\beta$ -unsaturated carbonyl compound (Damasceno, Fernandes, Magalhães, & Brito, 2008).

The decrease in aldehydes suggested by the model shown in Fig. 1D ( $R^2 = 0.966$ ,  $p = 0.001$ ) during juice concentration to  $42.1^{\circ}\text{Brix}$  was much lower than the losses of esters, terpenes and ketones. As shown in Table 1, of the ten aldehydes identified in the fresh juice, five remained in the juice throughout concentration to  $42.1^{\circ}\text{Brix}$ , they were: hexanal, heptanal, octanal, nonanal and benzaldehyde. All of these have odoriferous impact in the fresh cashew apple juice according to MacLeod and Troconis (1982), Maciel et al. (1986), Garruti et al. (2003), Garruti et al. (2008) and Sampaio et al. (2015). On the other hand, some aldehydes not present in the fresh juice, were identified in the concentrated juices, such as decanal, pentanal, 3-ethyl-benzaldehyde and 4-ethyl-benzaldehyde (Table 1). Some of these volatiles could have been formed during heat processing, but may have been present initially at concentrations below the limit detectable by GC–MS, and subsequently concentrated by removal of water from the juice.

The decrease in alcohols shown in Fig. 2A ( $R^2 = 0.971$ ,  $p = 0.002$ ) and in hydrocarbons shown in Fig. 2B ( $R^2 = 0.956$ ,  $p = 0.002$ ) occurred in a way distinct from the other classes of volatiles. There was a great loss of alcohols when the beverage was concentrated from  $11.8$  to  $20.2^{\circ}\text{Brix}$  (Fig. 2A), but when concentrated above  $20.2^{\circ}\text{Brix}$ , the alcohol levels started to increase again. With the exception of 2,6-di-*tert*-butyl- $\rho$ -cresol (BHT), all the alcohols identified in the fresh juice were lost when it was concentrated to  $42.1^{\circ}\text{Brix}$  (Table 1). The studies of Sampaio et al. (2011) and Sampaio et al. (2013) concerning the identification of volatiles recovered during the concentration of cashew apple juice, suggest that the majority of these alcohols, such as 2-methyl-1-propanol, 3-methyl-1-butanol and n-hexanol were lost by evaporation.

The following four alcohols were not present in the fresh juice, but were identified in the most concentrated juices ( $29.6^{\circ}\text{Brix}$  and  $42.1^{\circ}\text{Brix}$ ): isobutyl alcohol, 2,3-dimethyl-3-buten-2-ol, n-propanol

and 2,4-di-*tert*-butyl phenol (Table 1). These compounds may have been present in the fresh juice in concentrations below the detection limits of the GC–MS, and then concentrated due to removal of water. However, it is highly probable that they were formed during heat processing of the juice. One of the mechanisms suggested by some researchers (Ducruet, Fournier, Saillard, Feigenbaum, & Guichard, 2001; Kimball, 1991) for the formation of alcohols during the concentration of fruit juices involves the thermal degradation of esters during heating in an acid medium, such as that of the cashew apple juice.

The increase of hydrocarbons when the juice was concentrated from  $20.2^{\circ}\text{Brix}$  to  $42.1^{\circ}\text{Brix}$ , as suggested by the model shown in Fig. 2B ( $R^2 = 0.956$ ,  $p = 0.002$ ), was mostly due to the emergence of the following hydrocarbons: 4-ethyloctane, 5-methylnonane, 3-methylnonane, *cis*-3-decene, dodecane, toluene, *m*-ethyltoluene,  $\rho$ -ethyltoluene and *psi*-cumene (1,2,4-trimethylbenzene). Based on the studies of Rios, Fernández-García, Mínguez-Mosquera, and Pérez-Gávez (2008) and Zepka et al. (2014), the emergence of these hydrocarbons, notably of dodecane, *m*-ethyltoluene, *psi*-cumene and toluene, can be attributed to their formation from the thermal degradation of carotenoids initially present in the beverage.

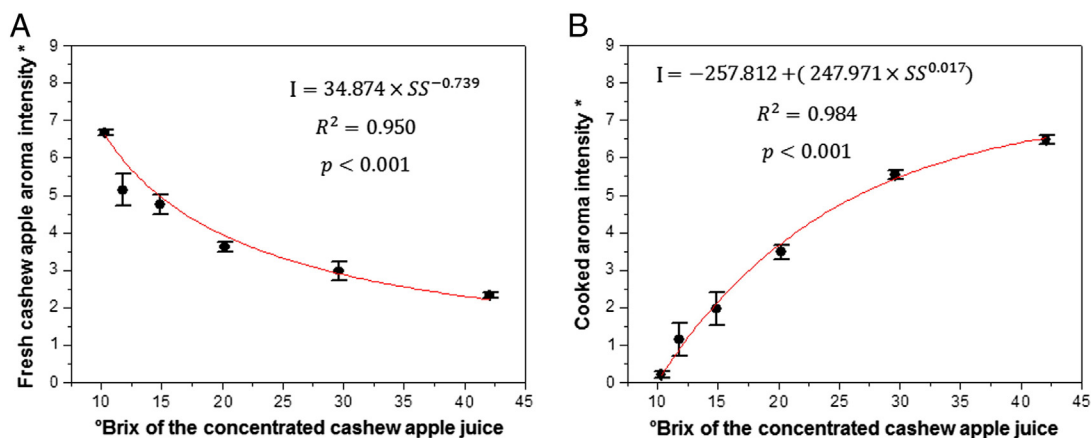
### 3.3. Dynamics of the sensory changes occurring during concentration and their correlation with changes in the volatile profiles

Fig. 3 presents the dynamics of the loss in intensity of the aroma described as ‘fresh cashew apple’ during the juice concentration and the concomitant increase in the ‘cooked’ aroma. The statistical models ( $p < 0.001$ ) were fitted as a power function, since this is the most appropriate to relate a chemical stimulus to a sensory perception (Moskowitz, 1970; Stevens, 1957; Stevens, 1961).

Fig. 3A and B show that the intensity of fresh cashew aroma predominated in the fresh juice and in those concentrated up to  $14.9^{\circ}\text{Brix}$ , being above ‘moderate’ on the intensity scale (score of 4.5 on a 9.0 cm scale), whereas the intensity of cooked aroma was very weak (scores of  $\leq 2.0$ ). However, when the cashew apple juice reached  $20.2^{\circ}\text{Brix}$  the cooked aroma was detected with a similar intensity to that of fresh cashew apple aroma. Finally, in the juices concentrated to  $29.6^{\circ}\text{Brix}$  and  $42.1^{\circ}\text{Brix}$ , the cooked aroma was the predominant sensory note, reaching scores above 4.5 (moderate) on the 9 cm scale.

According to the models shown in Fig. 1 ( $p \leq 0.001$ ) when the juice reached  $20.2^{\circ}\text{Brix}$ , almost 100% of the terpenes and 98% of the esters initially present, had already been lost. However, on concentrating only to  $14.9^{\circ}\text{Brix}$ , although the juice had lost practically 100% of the terpenes, it had lost less of the esters ( $\sim 90\%$ ). At  $14.9^{\circ}\text{Brix}$ , the juice presented a concentration of esters equal to  $13.58 \mu\text{g kg}^{-1}$ , almost four times that found in the juice concentrated to  $20.2^{\circ}\text{Brix}$ , which was  $3.85 \mu\text{g kg}^{-1}$  (Table 1). This difference could explain the slightly





**Fig. 3.** Intensities of the aromas described as “fresh cashew apple” (A) and “cooked” (B), present in the concentrated cashew apple juice, as a function of the soluble solids content (°Brix) in the beverage throughout the concentration process up to 42.1°Brix, including the standard error values. \*Values on the intensity scale: 0 = weak/none; 4.5 = moderate; 9 = strong. I = aroma intensity, SS = soluble solids content in °Brix.

higher intensity of fresh cashew apple aroma in the juice concentrated to 14.9°Brix as compared to that concentrated to 20.2°Brix (Fig. 3A). On the other hand, when the cashew juice was concentrated from 20.2°Brix to 42.1°Brix, according to the model shown in Fig. 2B ( $p = 0.002$ ), the concentration of hydrocarbons increased about five times, from  $2.31 \mu\text{g kg}^{-1}$  to  $10.27 \mu\text{g kg}^{-1}$ . This could be one of the factors responsible for the increase in cooked aroma observed in the cashew apple juices between 20.2°Brix and 42.1°Brix. The concentrations of the following hydrocarbons increased when the juice was concentrated from 20.2°Brix to 42.1°Brix: 4-ethyloctane, 5-methylnonane, 3-methylnonane, decane, cis-3-decene, dodecane toluene, *o*-xylene, *m*-ethyltoluene, *p*-ethyltoluene and *psi*-cumene. Of these, Zepka et al. (2014) identified dodecane, *m*-ethyltoluene and *psi*-cumene as odoriferous volatiles generated by the thermal degradation of cashew apple carotenoids in acidified model solutions. Thus the increase in concentration of these volatiles in the juices above 20.2°Brix, associated with the low concentration of esters, could explain the predominance of cooked aroma in the more concentrated juices (29.6°Brix and 42.1°Brix).

Table 2 shows the Pearson's correlation coefficient between the sensory attributes and the volatiles concentration in the six juice samples. Thirty-one volatile compounds showed correlation ( $p \leq 0.05$ ) with the fresh cashew apple and/or cooked aroma notes in the juices. The hydrocarbons toluene ( $r = 0.857$ ,  $p = 0.029$ ) and *psi*-cumene ( $r = 0.889$ ,  $p = 0.018$ ) showed positive correlation with the cooked aroma intensity of the juices. This suggests that their emergence in the juices promoted a concomitant increase in the cooked aroma note of the beverage. Further GC-olfactometry studies are necessary to check this hypothesis.

On the other hand, 14 of the 28 esters found in the fresh juice showed positive correlation with the intensity of fresh cashew apple aroma in the juice. This suggests that the loss of these compounds in the juice during processing influenced the majority of the reduction in the beverage fresh cashew apple aroma. They were: ethyl octanoate ( $r = 0.934$ ,  $p = 0.006$ ), ethyl *trans*-2-butenate ( $r = 0.884$ ,  $p = 0.020$ ), ethyl butanoate ( $r = 0.867$ ,  $p = 0.025$ ), ethyl propanoate ( $r = 0.869$ ,  $p = 0.025$ ), ethyl isovalerate ( $r = 0.866$ ,  $p = 0.026$ ), ethyl 2-methylbutanoate ( $r = 0.865$ ,  $p = 0.026$ ), propyl acetate ( $r = 0.865$ ,  $p = 0.026$ ), ethyl hexanoate ( $r = 0.862$ ,  $p = 0.027$ ), methyl isovalerate ( $r = 0.859$ ,  $p = 0.028$ ), 2-methyl butyl isovalerate ( $r = 0.848$ ,  $p = 0.033$ ), methyl-2-methylbutanoate ( $r = 0.843$ ,  $p = 0.035$ ), ethyl *trans*-2-hexenoate ( $r = 0.843$ ,  $p = 0.035$ ), methyl butanoate ( $r = 0.814$ ,  $p = 0.049$ ) and the mixture of ethyl *cis*-3-hexenoate and ethyl *trans*-3-hexenoate ( $r = 0.809$ ,  $p = 0.051$ ).

The results and discussion reported above can be visualized better in the Extended Mapping shown in Fig. 4. Fig. 4A presents the volatile compounds, and, as vectors, the aroma descriptors of the “fresh cashew

apple” and “cooked”. Fig. 4B shows the juices in different degrees of concentration. In order to interpret the Extended Mapping, Fig. 4A and B should be superimposed. Interpretation is similar to that of the Principal Component Analysis, well known amongst flavor scientists, and will not therefore be detailed here.

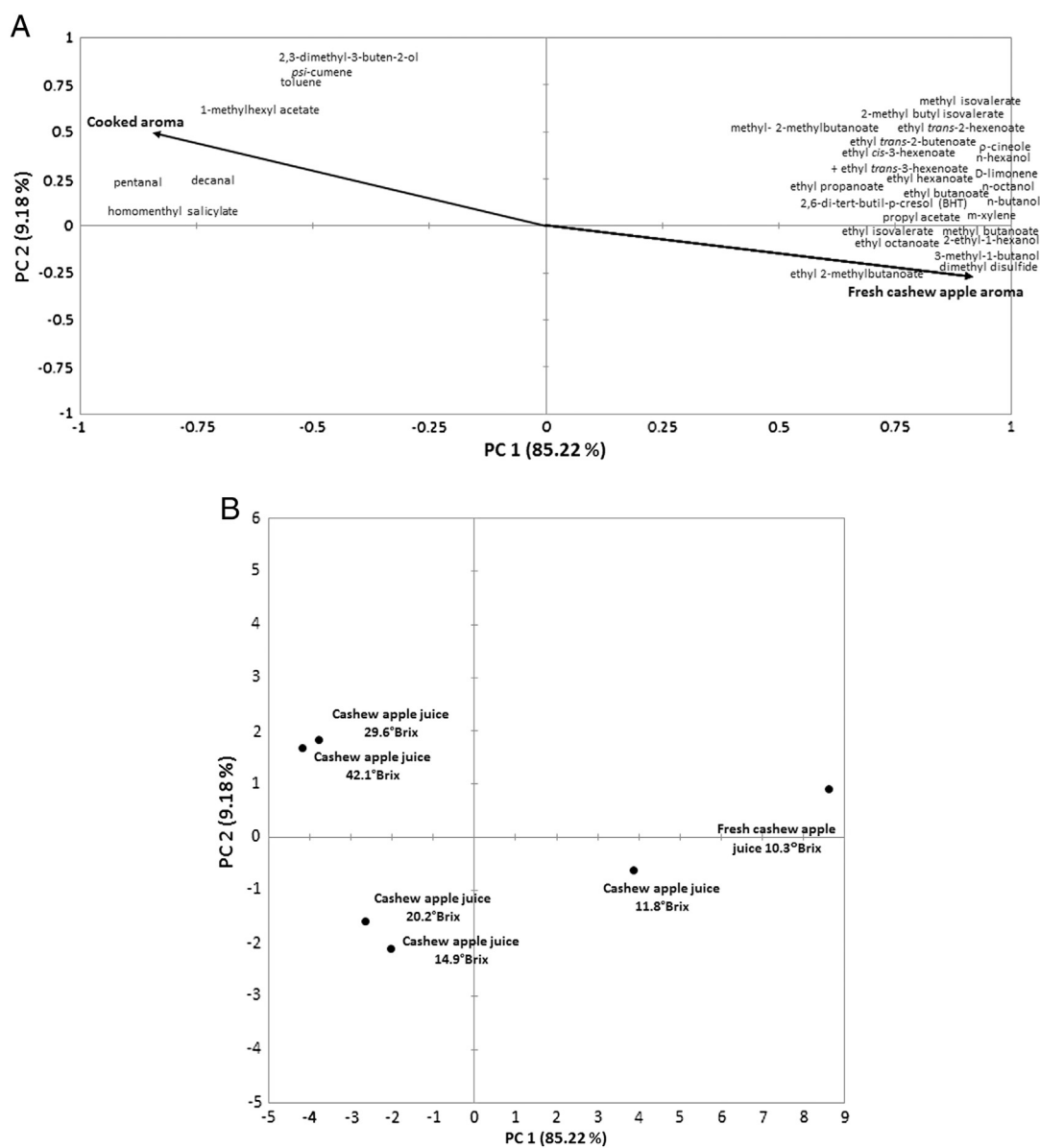
The Extended Mapping presented in Fig. 4 explains approximately 94% of the variation amongst the juices and suggests that the fresh cashew apple aroma is due mainly to the presence of all the volatiles situated more to the right of the PC1 (Fig. 4A), notably those with greater loading on this axis. These compounds represent esters, terpenes and

**Table 2**

Pearson's coefficients ( $r$ ) and respective significance levels ( $p$ -values) for the correlation between the volatiles concentration and the intensity of fresh cashew apple and cooked aroma in cashew apple juice.

Compounds	Fresh cashew apple aroma		Cooked aroma	
	Pearson $r$	$p$ -Values	Pearson $r$	$p$ -Values
n-Butanol	0.836	0.038	-0.831	0.041
2,3-Dimethyl-3-buten-2-ol	ns	ns	0.901	0.014
3-Methyl-1-butanol	0.966	0.002	-0.912	0.011
n-Hexanol	0.837	0.038	ns	ns
2-Ethyl-1-hexanol	0.863	0.027	ns	ns
n-Octanol	0.864	0.027	ns	ns
2,6-Di-tert-butyl- <i>p</i> -cresol (BHT)	0.852	0.031	ns	ns
Pentanal	-0.925	0.008	0.915	0.010
Decanal	-0.839	0.037	ns	ns
Toluene	ns	ns	0.857	0.029
<i>m</i> -Xylene	0.827	0.042	ns	ns
<i>psi</i> -Cumene	ns	ns	0.889	0.018
Ethyl propanoate	0.869	0.025	ns	ns
Propyl acetate	0.865	0.026	ns	ns
Methyl butanoate	0.814	0.049	ns	ns
Methyl-2-methylbutanoate	0.843	0.035	ns	ns
Methyl isovalerate	0.859	0.028	ns	ns
Ethyl butanoate	0.867	0.025	ns	ns
Ethyl 2-methylbutanoate	0.865	0.026	ns	ns
Ethyl isovalerate	0.866	0.026	ns	ns
Ethyl <i>trans</i> -2-butenate	0.884	0.020	ns	ns
Ethyl hexanoate	0.862	0.027	ns	ns
2-Methyl butyl isovalerate	0.848	0.033	ns	ns
Ethyl <i>cis</i> -3-hexenoate + Ethyl <i>trans</i> -3-hexenoate	0.809	0.051	ns	ns
Ethyl <i>trans</i> -2-hexenoate	0.843	0.035	ns	ns
Ethyl octanoate	0.934	0.006	-0.841	0.036
1-Methylhexyl acetate	-0.908	0.012	0.977	0.001
Homomenthyl salicylate	-0.897	0.015	0.886	0.019
$\alpha$ -Limonene	0.852	0.031	ns	ns
$\rho$ -Cineole	0.864	0.026	ns	ns
Dimethyl disulfide	0.966	0.002	-0.894	0.016

ns: correlation not significant at  $p \leq 0.05$ .



**Fig. 4.** (A) Extended Mapping showing the configurations of the volatiles ( $n = 31$ ) and sensory descriptors ( $n = 2$ ), and (B) the juices ( $n = 6$ ), during the concentration of cashew apple juice.

alcohols considered important for the fresh cashew apple aroma in previous GC-olfatometry studies (Garruti et al., 2003; Sampaio et al., 2015). The position of the vector representing the cooked aroma note in the juices suggests that this attribute is associated with the presence of compounds situated to the left in Fig. 4A, notably those with greater loading in the negative part of PC1. Hydrocarbons probably originating from the thermal degradation of carotenoids, such as toluene and *psi*-cumene, are amongst these compounds.

Fig. 4A also indicates that other compounds could be associated with the presence of cooked aroma in the concentrated juices, notably decanal and pentanal. These are odorous volatiles usually associated with off-flavors in foods and beverages, as products of lipid oxidation (Osawa, Gonçalves, & Da Silva, 2013). Fig. 4A also suggests that some esters could be associated with the cooked aroma of the concentrated juices. They are homomenthyl salicylate and 1-methyl hexyl acetate, situated to the left of PC1. The odoriferous importance of homomenthyl salicylate and 1-methyl hexyl acetate requires investigation.

Finally, the location of the juices in Fig. 4B indicates that as the concentration of the juices progressed, the intensity of the fresh cashew apple aroma decreased in the beverages and the cooked aroma

increased. Both results were confirmed in Fig. 3. Since the more concentrated juices (14.9°Brix–42.1°Brix) showed greater loading in the negative portion of PC1, they were differentiated from the others, notably from the fresh juice, for presenting less fresh cashew apple aroma, a greater cooked note and a greater concentration of compounds situated to the left of PC1. These compounds are the hydrocarbons mentioned above and compounds usually associated with off-flavors in foods, such as pentanal and decanal, as confirmed in Table 1.

#### 4. Conclusions

Two factors appear to have a great influence on the loss of sensory quality of cashew apple juice during concentration. The first refers to the loss by evaporation of approximately 90% of the total mass of esters initially present in the beverage, when the juice was concentrated to 14.9°Brix.

The second refers to both the possible formation of hydrocarbons due to the thermal degradation of carotenoids present in the fresh juice, and the emergence of compounds usually associated with off-flavors in foods, such as pentanal and decanal. The odoriferous impact

of these compounds in cashew apple juice has been little studied and deserves attention in future studies, since they could be important markers of sensory quality in concentrated cashew apple juice.

The concentration of cashew apple juice to levels below 20.2°Brix, associated with the recovery and re-addition to the concentrated juice of the esters evaporated off at the beginning of the concentration process, before the beverage reached 14.9°Brix, could generate a juice of better sensory quality and competitiveness on the consumer market.

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