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Cashew wine and volatile compounds produced during fermentation by non-*Saccharomyces* and *Saccharomyces* yeast

Ester Seixas Bullé Rêgo[a], Carlos Augusto Rosa[b], Ana Luiza Freire[b], Ana Maria de Resende Machado[c], Fátima de Cássia Oliveira Gomes[c], Ayslan Santos Pereira da Costa[d], Marcelo da Costa Mendonça[a,e], Maria Lucila Hernández-Macedo[a,e,*], Francine Ferreira Padilha[a,e,**]

[a] Postgraduate Program in Industrial Biotechnology, University Tiradentes, CEP, 49032-490, Aracaju, Brazil
[b] Departament of Microbiology, Institute of Biological Sciences, Federal University of Minas Gerais, CEP, 31270-901, Brazil
[c] Department of Chemistry, Federal Center of Technological Education of Minas Gerais, CEP: 30421-169, Brazil
[d] Postgraduate Program in Engineer of Process, University Tiradentes, CEP 49032-490, Aracaju, Brazil
[e] Institute of Technology and Research, CEP 49032-490, Aracaju, Brazil

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ABSTRACT

Making wines from tropical fruits with pleasant sensory characteristics has gradually gained potential as a new product within the beverages market. In this study, the ability to ferment cashew apple juice of non-*Saccharomyces* strains previously isolated from tropical fruits and *Saccharomyces* were evaluated, as well as their production of desirable volatile compounds. The isolates *Torulaspora delbrueckii* and *Hanseniaspora opuntiae* showed higher fermentative capacities in co-fermentations with *Saccharomyces cerevisiae*. The simple and co-fermentative process of the cashew must with these yeasts leads to a highly desirable production of volatile compounds such as phenethyl alcohol, 2-phenethyl acetate and 3-methyl-1-pentanol, and these favoured the organoleptic properties of the product. The concentrations of acetic acid during fermentation were in the range of 0.2–0.5 g/L, which is considered desirable to contribute towards the aromas and flavours of fruit wines. These results highlighted the role of yeast isolates from fruits on cashew juice fermentation and its production of desirable volatile compounds to produce cashew wine.

1. Introduction

Besides grape must, tropical fruits are viable alternatives for making new wines with desirable organoleptic properties for the beverage market (Cakar et al., 2019; Dellacassa et al., 2017; Lu, Chan, Li, & Liu, 2018). In addition, to prepare beverages, tropical fruits are an important source of wild yeast strains, which have high desirable flavouring production potentials for alcoholic beverage industries (Grondin et al., 2015).

In the beverage fermentative process, yeast plays an important role due to its high performance in the conversion of sugar into ethanol as well as aromatic esters and other metabolic products. The efficiency of the ethanol production is associated with the fermentative potential of the yeasts as well as the fermentation process optimization to improve the sensorial quality of the alcoholic beverages (Azhar et al., 2017).

The “conventional” yeast extensively used in alcoholic beverage industries is *S. cerevisiae* due to its tolerance to harsh conditions during alcoholic wine fermentation. However, the commercially available use of *Saccharomyces* yeasts limits the sensory characteristics of the fruit wine production leading to the search for new yeast strains to increase the flavour diversities and the final alcohol content (Liu, Laaksonen, Kortesniemi, Kalpio, & Yang, 2018). In this sense, there is a strong interest in the use of non-*Saccharomyces* yeasts (otherwise called non-conventional yeasts) in the alcoholic beverage industries, since these yeasts contribute towards aroma complexity and increased yields of desirable compounds (Canonico, Comitini, & Ciani, 2018; Jolly, Augustyn, & Pretorius, 2006).

Recently, mixed fermentations between *S. cerevisiae* and non-*Saccharomyces*, such as *Torulaspora delbrueckii* and *Hanseniaspora vineae*, have been proposed as a promising way to increase the flavour diversities of fruit wines (Liu, Laaksonen, & Yang, 2019; Lorenzini, Simonato, Slaghenaufi, Ugliano, & Zapparoli, 2019). Furthermore,

* Corresponding author. Postgraduate Program in Industrial Biotechnology, University Tiradentes, CEP, 49032-490, Aracaju, Brazil.
** Corresponding author. Postgraduate Program in Industrial Biotechnology, University Tiradentes, CEP, 49032-490, Aracaju, Brazil.
E-mail addresses: lucyherma@gmail.com, maria_lucila@itp.org.br (M.L. Hernández-Macedo).

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other non-*Saccharomyces* species could be used to diversify flavour and aroma profiles in wine, making it necessary to search for new desirable volatile compounds that have been produced by new yeast strains to increase the sensory profiles of alcoholic beverages (Escribano-Viana et al., 2018; Liu & Quek, 2016).

Fruits have been the potential sources to isolate new non-*Saccharomyces* species with desirable flavours and aroma characteristics in biotechnological processes for applications in the food industry (Lu et al., 2018; Wei, Zhang, Yuan, Dai, & Yue, 2019). In this context, tropical fruits such as cashews (*Anacardium occidentale* L.) have the potential environments to promote the search for new yeasts for making new beverages. Furthermore, cashew apple juice can absolutely be used in winemaking musts; it offers a high fermentative potential due to its ability to reduce sugar contents (glucose, fructose and sucrose). Moreover, cashew apple juice is very rich in minerals, vitamin C, salts, amino acids, flavours and aromas, offering desirable characteristics to produce cashew wine (Das & Arora, 2017; Priya & Setty, 2019).

The aromatic esters, methyl 3-methyl butyrate and ethyl 3-methyl butyrate, are the main volatile compounds associated with the sweet, fruity aroma produced during cashew juice fermentation with *S. cerevisiae* (Garruti, Franco, da Silva, Janzantti, & Alves, 2006). However, other desired volatile compounds can be produced during co-fermentation using non-Saccharomyces and Saccharomyces yeast. In this sense, the present work aims to evaluate the potential use of the non-*Saccharomyces* yeasts, *T. delbrueckii* and *H. opuntiae*, that are isolated from tropical fruits to produce cashew wine using a co-fermentative processes with *S. cereviseae*.

2. Materials and methods

2.1. Yeast strains

The yeast strains *T. delbrueckii* (FRU5) and *H. opuntiae* (FRU10) used in this study were obtained from the yeast collection of the Molecular Biology Laboratory of the Institute of Technology and Research (Aracaju, Brazil). These had been isolated from cashew apple fruit (FRU5) and mango peel (FRU10), and they were previously identified by sequencing the D1/D2 domains of the 26S rDNA gene using a methodology reported by Las Heras-Vazquez, Mingorance-Cazorla, Clemente-Jimenez, and Rodriguez-Vico (2003).

The *S. cerevisiae* commercial strain, CAY 1007 (CanaMax®, Brazil), and *S. cerevisiae*, V249 (Collection of Microorganisms and Cells of UFMG), were used in co-fermentation to prepare cashew wine. All of the yeast strains were maintained within a YPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 18 g/L agar) (Oxoid, Basingstoke, UK) at 4 °C for short-term storage and in a YPD broth supplemented with 80% (w/v) glycerol at −80 °C for long-term storage.

2.2. Fermentation assays

Cashew apple juice (Cajuína Nordestina, Ceará, Brazil) with a brix adjusted to 18 by adding sucrose (must), was used to the fermentation analysis. Eight fermentative groups constituted by the simple fermentation (SF) of *S. cerevisiae* CAY1007, *S. cerevisiae* V249, *T. delbrueckii* FRU5 and *H. opuntiae* FRU10 and by the co-fermentation (CF) of the *T. delbrueckii* FRU5 + CAY1007, *T. delbrueckii* FRU5 + V249, *H. opuntiae* FRU10 + CAY1007 and *H. opuntiae* FRU10 + V249 were evaluated.

The fermentation was carried out in 250 mL Erlenmeyer flask, using 10 mL of yeast suspension containing a final concentration of 1×10^8 cells in cashew apple juice, inoculated in 90 mL of cashew juice must at 28 °C for 48 h. Mixed yeast fermentation assays were simultaneously inoculated with *S. cerevisiae* and non-Saccharomyces cultures at 1:1 ratio. Samples were collected at 0, 6, 12, 18, 24, 36 and 48 h intervals to determine the sugar, acetic acid and ethanol concentrations by HPLC. Volatile compounds analyses were performed using headspace vials with the sample of the final fermentation time (48 h).

2.3. Analysis of sugars, ethanol and acetic acid

Ethanol, acetic acid and sugars (glucose, sucrose and fructose) were analysed using HPLC according to Duarte et al. (2010). Briefly, samples from each fermentation time were filtered through a cellulose acetate membrane (0.22 μm) (Sartorius Stedim Biotech) and injected (20 μL) into a HPLC (Prominence Model LC-20A, Shimadzu Corp., Tokyo, Japan) coupled with a dual detection system consisting of a UV detector and a refractive index detector (RID) RID-10A. The HPLC was performed using a SUPELCOGEL™ C–610H 9 μm × 30 cm × 7.8 mm (Sigma-Aldrich) column, 5 mM of H_2SO_4 as its mobile phase, at a flow rate of 0.6 mL/min, with the column compartment temperature at 45 °C and a run time of 30 min at 65 °C. All samples were analysed in triplicate and standard solutions injected to obtain the retention time for each compound.

2.4. Qualitative analysis of volatile metabolites

The volatile compounds in the cashew wine were analysed in a triplicate using headspace solid phase microextraction and gas chromatography-mass spectrometry (HS-SPME andGC-MS), according to Liu et al. (2019) and Menezes et al. (2019) with some modifications. After fermentation (48 h), the samples were centrifuged (4000 × g), and supernatants (10 mL) with 3.0 g NaCl were placed in a 20 mL headspace vial and. The SPME device was then inserted into the sealed vial by manually penetrating the septum, and the fiber (PDMS-DVB-CAR Supelco, Bellefonte, PA, USA) was exposed to the headspace of the cashew wine at 55 °C for 10 min under stirring. After extraction, the needle on the SPME manual holder was set into the GC injector, and the fiber was directly exposed to the hot injector at 250°C for 10 min in the splitless mode. The samples were analysed on a gas chromatograph (Agilent Technologies 7890A GC System) coupled with a mass spectrometer (Agilent 5975C inert MSD Triple-Axis Detector). An series alkanes (C8–C20, Sigma-Aldrich, St. Louis, MO) were analysed using the same conditions as the samples. Major volatile compounds were separated by GC on a HP-5MS (30 m × 0.25 mm × 25 μm film thickness, Agilent Technologies) column using helium as carrier gas at a flow rate of 1.4 mL/min. The column temperature was maintained at 35 °C for 0.5 min, raised to 150 °C at a rate 5 °C/min and held at 250 °C for 2 min. The retention indices (RIs) of the volatiles were calculated with reference to the n-alkane standards. Volatiles were identified by comparison with mass spectra library (NIST) and RI of pure standard compounds, confirmed by GC-MS (https://webbook.nist.gov/chemistry/).

2.5. Statistical analysis

All statistical values were expressed as mean ± SD from three independent trials and calculated using a one-way analysis of variance (ANOVA) followed by post hoc test (Tukey) using the GraphPad Prism (GraphPad Software Inc., San Diego, USA) software. The significance level was $p \leq 0.05$ throughout the analyses. Principal component analysis (PCA) was employed to evaluate the relationship between different fermentations assays with volatiles that were released using METALAB (METLAB, The MathWorks Inc., Natick, MA, ver. 2015b) software.

3. Results and discussion

3.1. Changes in the sugar consumption and ethanol production during cashew must fermentation

The cashew must fermentation was began with a pH of 4.4 and

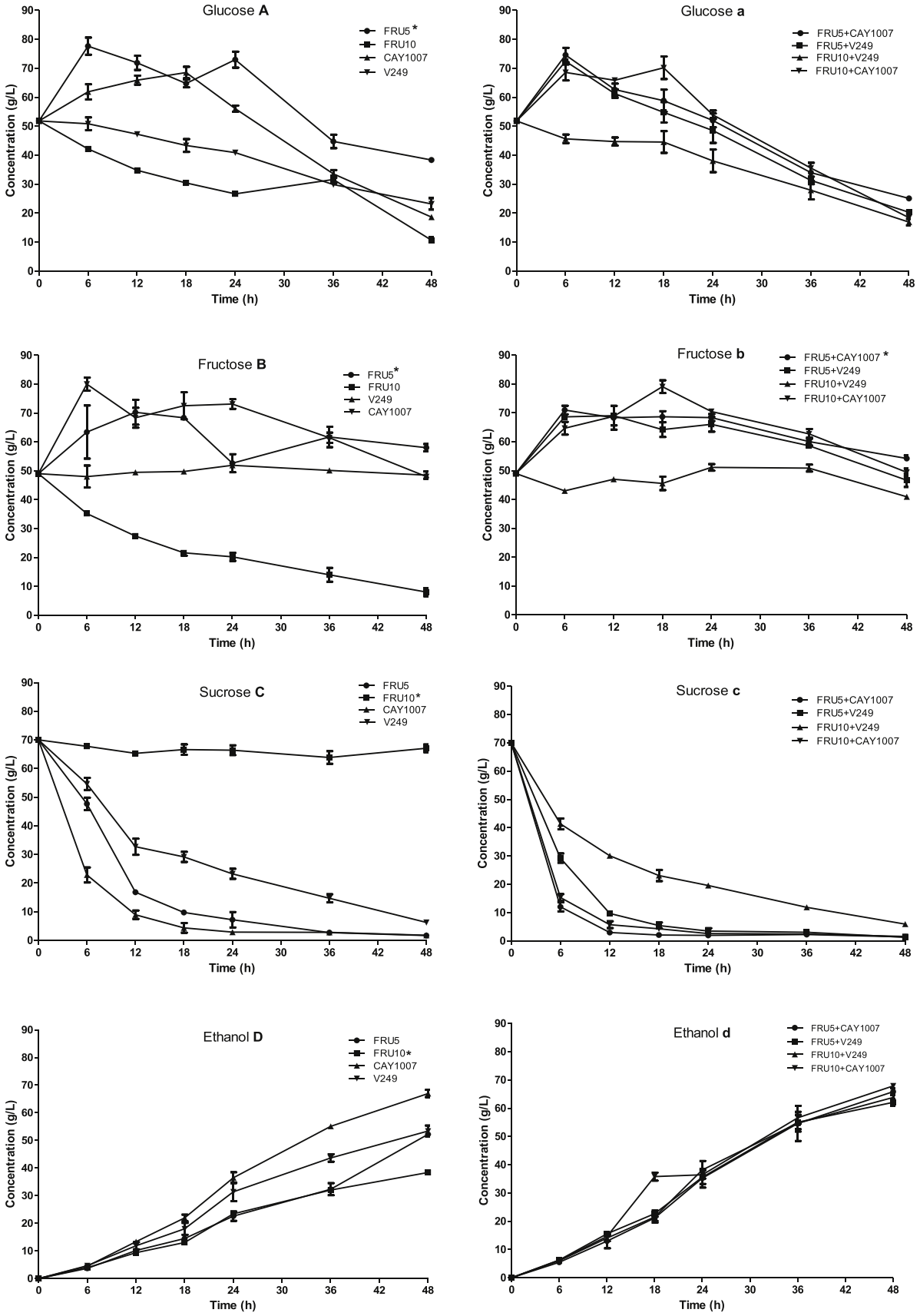

Fig. 1. Changes in the concentrations of glucose, sucrose, fructose, and ethanol during fermentation of cashew must. Asterisks (*) in the legend denote statistically significant difference ($p < 0.05$) between the fermentation date up to 48 h.

Table 1
Principal parameters of the sugar, ethanol and acetic acid HPLC analysis of the cashew must fermentation at 48 h carried out by *Saccharomyces cerevisiae* (CAY1007 and V249), *Torulaspora delbrueckii* (FRU5) and *Hanseniaspora opuntiae* (FRU10).

	Cashew must	Simple fermentation (SF)				Co- fermentation (CF)			
		FRU5	FRU10	CAY1007	V249	FRU5 + CAY1007	FRU5+V249	FRU10 + V249	FRU10 + CAY1007
Glucose (g/L)	51.8 ± 0,00	38.35 ± 0.77A	10.7 ± 0.98D	18.65 ± 0.77C	23.2 ± 1.97B	25.1 ± 0.56A	20.3 ± 0.42D	16.9 ± 1.13C	18.45 ± 0.35BC
Fructose (g/L)	49 ± 0.4	58 ± 1.27A	8 ± 1.41C	48 ± 0.56B	48.5 ± 1.27B	54.20 ± 0.99A	46.65 ± 2.33C	40.95 ± 0.91D	49.40 ± 1.27B
Sucrose (g/L)	70 ± 0.00	1.7 ± 0.42C	67.07 ± 1.37A	1.8 ± 0.00C	6.3 ± 0.84B	1.4 ± 0.14B	1.3 ± 0.28B	5.95 ± 0.35A	1.6 ± 0.28B
Ethanol (%v/v)	ND	52.2 ± 0.84B	38.4 ± 0.14C	66.95 ± 1.34A	53.46 ± 1.99B	65.87 ± 1.23AB	62.05 ± 1.20C	63.81 ± 1.67BC	67.85 ± 0.63A
Acetic Acid (g/L)	ND	0.2 ± 0.07A	0.5 ± 0.07A	0.4 ± 0.00A	0.2 ± 0.07B	0.4 ± 0.03A	0.2 ± 0.00A	0.2 ± 0.04A	0.5 ± 0.02A

Results are the mean of three injections of each replicate (n = 3); the standard deviation values (±) are indicated. The same capital letters on the same line indicate significant differences between data within the same fermentation at $p < 0.05$.
ND: Not detected.

soluble solid concentrations (°Brix) were obtained at 48 h (14–9 °Brix) along with final sugars consumption (120–144 h) with 4–5 °Brix (Table S1), suggesting the capability of *S. cerevisiae* (CAY1007 and V249) with *T. delbruckii* (FRU5) and *H. opuntiae* (FRU10) for the fermentation process. This capability was confirmed by the fermentation kinetics obtained by HPLC until 48 h (Fig. 1) because, after this period, the concentrations of undesirable compounds were detected previously.

In simple fermentation (SF), the glucose consumption range was among 10–40 g/L, approximately, at 48 h (Fig. 1A). In this condition, FRU5 has a low glucose consumption, since significant concentrations of this sugar (38.35 ± 0.77 g/L) were detected (Table 1). Already in co-fermentation (CF), a range of glucose variations (18 g/L to 25 g/L) was observed (Fig. 1a) and, in this condition, FRU10 + V249 and FRU10 + CAY1007 showed higher glucose consumption.

With respect to fructose consumption, FRU5 in SF has low metabolism of this sugar showing a significant concentration (58 ± 1.27g/L) (Table 1). On the other hand, FRU10 in SF had a better utilization of fructose, while the other yeasts no show significant consumption of this sugar at 48 h (Fig. 1B). With respect to CF, all yeast co-cultured showed low fructose consumption capacity, especially FRU5 + CAY1007. (Fig. 1b).

Although there is no significant fructose consumption, FRU10 + V249 showed a better fructose utilization (40.95 g/L) at 48 h (Table 1). This result can favour the making of cashew wine, since fructosophilic yeast, *Hanseniaspora* ssp., positively interferes with the *S. cerevisiae* fermentation behaviour to enhance the flavour and yield desirable characteristics in beverages (Ciani & Fatichenti, 1999).

All fermentation process exhibited high sucrose consumption at 48 h except under the FRU10 SF condition (Fig. 1C), which may be related to low ethanol conversion levels while isolated at 48 h (Fig. 1D). Although FRU10 shows similar ethanol production values to FRU5, this ethanol production may be due to the glucose fermentation also present in cashew apple juice. Already, the sucrose consumption in CF has yielded an evident increase from 12 h in all of its yeast co-cultures (Fig. 1c), mainly in FRU5 + V249 and FRU5 + CAY1007; these displayed a higher sucrose consumption with 1.3 and 1.4 g/L, respectively, compared to the commercial *S. cerevisiae* CAY1007 and V249 in SF (Table 1).

The sucrose hydrolysis to free glucose and fructose is mediated by an invertase enzyme, which is synthesized by a yeast genus diversity like Candida, Hansenula, Saccharomyces, Kluyveromyces and Schwanniomyces (Nadeem et al., 2015). Therefore, the low rate of sucrose hydrolysis by *H. opuntiae* (FRU10) may be related to its lack of invertase, leading to low sucrose fermentation. (Čadež, Bellora, Ulloa, Hittinger, & Libkind, 2019). Hence, *H. opuntiae* (FRU10) will need to be used concurrently with other yeasts, such as *S. cerevisiae*, for fermented beverage production.

The difference in sugar utilization between different yeasts in the fermentative process (SF and CF) varied strongly. Thus, the high glucose and sucrose consumption during CF in all of the cultures show a potential for cashew wine production.

3.2. Ethanol and acetic acid production

In SF, the commercial *Saccharomyces* CAY1007 strain exhibited high ethanol production from 18 h to 48 h (Fig. 1D) as expected. The non-*Saccharomyces* FRU5 has similar ethanol production to the commercial *Saccharomyces* V249, 52.2 ± 0.84 and 53.46 ± 1.99, respectively (Table 1). *S. cerevisiae*'s comparable levels of ethanol production was also observed during the *T. delbrueckii* monoculture fermentation in making durian wine (Lu, Huang, Lee, & Liu, 2015): corroborating with the fermentative capability of *T. delbrueckii* (FRU5) observed during cashew must fermentation.

Fig. 1d shows an increase in ethanol production in all CF, probably due to high sucrose consumption during the fermentative process. The co-cultures with CAY1007 displayed high ethanol productions with

prominence in the FRU10 + CAY1007 co-culture (67.85 ± 0.63) (Table 1). However, this ethanol production capability of FRU10 + CAY1007 cannot be attributed to FRU10, since this isolate showed low ethanol production in SF (Fig. 1D). Probably, the FRU10 + CAY1007 high ethanol production could be due to an invertase produced mainly by various *Saccharomyces* species (Bhalla, Thakur, & Thakur, 2017).

With respect to acetic acid levels, ranges from 0.2 to 0.5 g/L were detected during the cashew must fermentative processes at 48 h (Table 1). This organic acid lower concentrations (0.2 g/L) were observed in both SF and CF with FRU5 (*T. delbrueckii*) and *Saccharomyces* V249. *T. delbrueckii* is the non-*Saccharomyces* yeast most used in making wine due to its low production of acetaldehyde, acetic acid, acetoin and ethyl acetate, which is required in the high purity fermentative process (Bely, Stoeckle, Masneuf-Pomarède, & Dubourdieu, 2008; Canonico, Agarbati, Comitini, & Ciani, 2016).

In grape wine, an acetic acid level above 0.8 g/L contributes to the bitter taste and 'vinegar-like' aroma, making a beverage unpleasant, whereas values ranging from 0.2 to 0.7 g/L are considered optimal acetic acid concentrations to improve the flavour profiles of the wine (Corison, Ough, Berg, & Nelson, 1979; Lambrechts & Pretorius, 2000; Swiegers, Bartowsky, Henschke, & Pretorius, 2005). The acetic acid levels detected in the fermentation with the cashew must exhibited an optimal concentration range (0.2–0.5 g/L) in both the SF and CF (Table 1).

3.3. Analysis of volatile compounds in the fermentation

The volatile composition of the wines was analysed by SPME and GC–MS technique. A total of 19 major volatile compounds were identified in the cashew must fermentations with FRU5 (*T. delbrueckii*), FRU10 (*H. opuntiae*) and *S. cerevisiae* CAY1007 and V249 (Table 2). The results indicate that alcohols, esters, aldehydes, and ketones were quantitatively the major group of volatile compounds produced throughout the cashew must fermentations. In the alcohol group, isoamyl alcohol exhibited high concentrations in all the fermentation tests, except in the SF of FRU10.

High significant concentrations of 3-methyl-1-pentanol, 1-dodecanol, benzoic acid and isoamyl alcohol in SF of FRU5 were produced. Volatile compounds of phenethyl alcohol, phenol 2, 4-bis (1, 1-dimethylethyl) and acetic acid were also highly produced by FRU10, while octanoic acid, ethyl ester had high production by means of CAY1007.

During simple fermentation, V249 also displayed high significant concentrations of isoamyl alcohol, ethyl 9-decenoate and decanoic acid (Table 2). Isoamyl alcohol is one of the flavour compounds produced in the simple fermentation of *S. cerevisiae* in cashew juice, as described in a previous work (Apine & Jadhav, 2015).

Concerning the CF, the volatile compounds with statistically different values were decanoic acid and dodecanoic acid in the FRU5 + CAY1007 co-culture. Beta-farnesene (6E), 2-propenoic acid, 3-phenyl-, ethyl ester and octanoic acid exhibited high contents in the FRU10 + CAY1007 co-culture, while phenyl acetate, dodecanoic acid, ethyl ester had statistically different values in the FRU10 + V249 fermentation. These detected compounds may contribute to the organoleptic properties, as flavour and aroma do in the beverage (Chen & Liu, 2016).

Properties of floral, fruity, sweet and honey aromas are attributed to phenethyl alcohol, ethyl 9-decanoate and acetic acid, while laurel leaf aromas, associated with decanoic acid, are considered essential for a fine wine flavour (Zhang, Luan, Duan, & Yan, 2018). Phenethyl alcohol was produced by FRU5 and FRU10 in SF and CF, while decanoic acid was produced by V249, indicating the aroma and flavour contributions by these isolates in the cashew wine.

Non-*Saccharomyces* yeasts have a positive contribution to fruit wine quality, mainly regarding flavour complexity unlike to *S. cerevisiae* monoculture that limits the flavour variety during the fermentative process, which is a disadvantage in the beverage production. (Canonico et al., 2016; Jolly, Varela, & Pretorius, 2014). Among the non-*Saccharomyces* yeast species, *T. delbrueckiii* is the main example of these yeasts used in making wine due to its low acetaldehyde, acetic acid, acetoin and ethyl acetate production (Liu et al., 2018).

A desirable volatile compound, 3-methyl-1-pentanol, used as flavouring and fragrance agent due to its mushroom-like odours and whiskey notes, was highly produced by *T. delbrueckii* (FRU5) in the SF (Table 2). Regarding CF, the 3-methyl-1-pentanol production was observed only in the *S. cerevisiae* and FRU5 co-cultures, showing the organoleptic potential of *T. delbrueckii* to prepare a cashew wine. However, in the CF of *T. delbrueckii* or *H. opuntiae* with *S. cerevisiae*, it was observed the production of the 2-propenoic acid, 3-phenyl-, ethyl ester compound that has a balsamic odour and sweet flavour.

In addition to *T. delbrueckii*, other non-*Saccharomyces* yeasts, such as *H. opuntiae*, contribute toward wine aroma quality (Luan, Zhang, Duan, & Yan, 2018), which was also observed in our results with respect to phenylethanol (phenylethyl alcohol) production. This volatile compound has intense floral and sweet notes, and its high production was observed in SF by *H. opuntiae* (FRU10) (20.17 ± 0.96 mg/L) and *T. delbrueckii* (FRU5) (17.49 ± 2.04 mg/L) (Table 2). In CF, higher values of phenylethyl alcohol were observed in the fermentation of FRU5 and FRU10 with *S. cerevisiae* V249.

Non-*Saccharomyces* yeasts were described as producing phenylethyl alcohol in both SF and sequential fermentation with *S. cerevisiae* in the grape must fermentation (Azzolini, Tosi, Lorenzini, Finato, & Zapparoli, 2014; Luan et al., 2018). Recently, Gamero et al. (2019) also detected phenylethyl alcohol during the simple fermentation of *S. cerevisiae* and *Hanseniaspora guilliermondii* in cashew apple juice. Therefore, this study and the volatile compound analysis shows the potential of the non-*Saccharomyces* to modulate the aroma profiles in cashew wine.

In addition to yeast species and strains, the cell viability and the condition of the fermentation influence on the formation of volatiles. Although the presence and yeast viability has not been evaluated in this study, previous work indicates that during fermentation until to 48 h both, *S. cerevisiae* and the non-*Saccharomyces* species used in this work are still viable (González-Royo et al., 2014; Harlé et al., 2018), contributing to the profile of volatiles and modulating the aroma profiles in cashew wine.

3.4. PCA of volatile compounds during the fermentative process

In order to understand the influence of the fermentation conditions of the eight groups (FRU5, FRU10, FRU5 + CAY1017, FRU5 + V249, FRU10 + CAY1017, FRU10 + V249, CAY1017 and V249) on the volatile releases in cashew wine, a PCA was carried out using the volatile quantitative data. Fig. 2A and B shows the two principal components in the function of volatiles (A) and variables (B), representing 84 and 93.5% of the variation explained, respectively.

The PCA results, as shown in Fig. 2A, depicted a close association between the 19 volatile compounds, whereby the first and second principal components (PC) explained the variance in 89.88 and 3.65%. Compounds such, isoamyl alcohol (1), phenylethyl alcohol (2), ethyl 9-decenoato (3) and octanoic acid ethyl ester (5) on PC 1 showed positive values, while the volatiles decanoic acid (7), 3-methyl-1-pentanol (9), 2-propenoic acid, 3-phenyl-, ethyl ester (12), octanoic acid (13), pentanoic acid 2-hydroxy-4-methyl-, ethyl ester (16) and acetophenone (17) were positively loaded on PC 2. The volatiles 1, 2 and 16 were highly positioned on the positive sides of both PC 1 and PC 2.

The projection of volatile compounds shows the correlation between the eight fermentative processes that were studied (Fig. 2B). Simple fermentation conditions (FRU5, FRU10, CAY 1007 and V249) are located at the positive and negative extremes of PC 2, while co-fermentation conditions are located in the middle, suggesting the sample discrimination depends on fermentative conditions. The correlation

Table 2
Principal volatile concentrations (mg/L) in the cashew must fermented by *S. cerevisiae* (CAY1007 and V249) and non-*Saccharomyces* (FRU5 and FRU10): quantified by gas chromatography and mass spectrometer (GC-MS).

N°	Compound (mg/L)	Cashew must	FRU5	FRU10	CAY1007	V249	FRU5+ CAY1007	FRU5+ V249	FRU10+ V249	FRU10+ CAY1007
1	Isoamyl alcohol	1.60 ± 0.09G	19.95 ± 1.2A	8.28 ± 0.02F	14.25 ± 0.02D	18.24 ± 0.24B	15.61 ± 0.53C	15.97 ± 0.06C	17.46 ± 0.13B	12.98 ± 0.4E
2	Phenylethyl alcohol	1.76 ± 0.14G	17.49 ± 2.04B	20.17 ± 0.96A	8.68 ± 0.04F	11.75 ± 0,5E	11.79 ± 0.69E	14.07 ± 0.53D	16.24 ± 0.28C	8.57 ± 0.31F
3	Ethyl 9-decenoate	ND	ND	ND	8.73 ± 0.09A	7.60 ± 0.02B	2.36 ± 0.01D	4.98 ± 0.16C	7.18 ± 0,37B	1.60 ± 0.55E
4	Decanoic acid, ethyl ester	ND	ND	ND	3.16 ± 0.02B	7.79 ± 0.07A	ND	ND	ND	ND
5	Octanoic acid, ethyl ester	ND	ND	0.42 ± 0.0D	6.88 ± 0.14A	5.26 ± 0.07BC	6.77 ± 0.31A	4.94 ± 0.13BC	5.74 ± 0.6B	4.79 ± 0.18C
6	beta-Farnesene, (6E)-	ND	ND	0.22 ± 0.05C	ND	0.23 ± 0.0C	ND	0.12 ± 0.0C	1.13 ± 0.0B	6.57 ± 0.14A
7	Decanoic acid	2.66 ± 0.27B	0.12 ± 0.01D	0.59 ± 0.0D	1.55 ± 0.07C	ND	5.81 ± 0.76A	ND	ND	0.74 ± 0.86D
8	2-phenyl acetate	ND	2.31 ± 0.09ED	5.49 ± 0.4A	2.12 ± 0.04E	2.45 ± 0.04CDE	3.26 ± 0.19BC	3.07 ± 0.11BC	3.65 ± 0.23B	2.02 ± 0.06E
9	3-methyl-1-pentanol	ND	5.06 ± 0.07A	0.31 ± 0.01B	ND	0.15 ± 0.0B	0.30 ± 0.0B	0.30 ± 0.0B	ND	ND
10	1-dodecanol	ND	4.54 ± 0.24A	4.16 ± 0.4A	ND	1.92 ± 0.04C	ND	2.78 ± 0.26B	ND	0.29 ± 0.01D
11	Phenol, 2,4-bis (1,1-dimethylethyl)	3.58 ± 0.0A	3.66 ± 0.47A	4.41 ± 0.55A	2.67 ± 0.11B	2.09 ± 0.07BC	1.35 ± 0.28CD	1.40 ± 0.25CD	1.05 ± 0.08D	1.10 ± 0.05D
12	2-propenoic acid, 3-phenyl-, ethyl ester	5.82 ± 0.13A	ND	ND	ND	ND	ND	0.60 ± 0.14C	0.57 ± 0.07C	4.54 ± 0.77B
13	Octanoic acid	8.17 ± 0.02A	0.23 ± 0.04E	1.36 ± 0.02DE	1.69 ± 0.71C	0.81 ± 0.15CDE	3.92 ± 0.33B	0.75 ± 0.07DE	1.02 ± 0.02CDE	4.17 ± 0.13B
14	Dodecanoic acid, ethyl ester	ND	0.35 ± 0.02C	ND	1.87 ± 0.12B	1.20 ± 0.14B	0.27 ± 0.08C	0.24 ± 0.04C	3.19 ± 0.16A	0.21 ± 0.04C
15	Dodecanoic acid	0.67 ± 0.00B	ND	ND	ND	ND	1,63 ± 0,18A	ND	ND	ND
16	Pentanoic acid, 2-hydroxy-4-methyl-, ethyl ester	12.80 ± 0.21A	1.09 ± 0.16C	2.06 ± 0.05B	0.51 ± 0.03C	0.50 ± 0.0C	0.47 ± 0.08C	0.46 ± 0.09C	0.57 ± 0.0C	1.20 ± 1.08BC
17	Acetophenone	2.60 ± 0.37A	0.46 ± 0.01B	0.81 ± 0.03B	0.19 ± 0.0B	0.21 ± 0.0B	0.60 ± 0.02B	ND	0.24 ± 0.0B	0.22 ± 0.03B
18	Benzoic acid, ethyl ester	0.20 ± 0.0B	2.06 ± 0.07A	ND	ND	ND	ND	ND	ND	ND
19	Acetic acid, 2-phenylethyl ester	ND	2.31 ± 0.09DE	5.49 ± 0.40A	2.12 ± 0.04E	2.45 ± 0.04CDE	3.26 ± 0.19BC	3.07 ± 0.11BCD	3.65 ± 0.23B	2.02 ± 0.06F

All values were the means of triplicate determinations ± SD. The same capital letters on the same line denote significant differences at $p < 0.05$.
ND: Not detected.

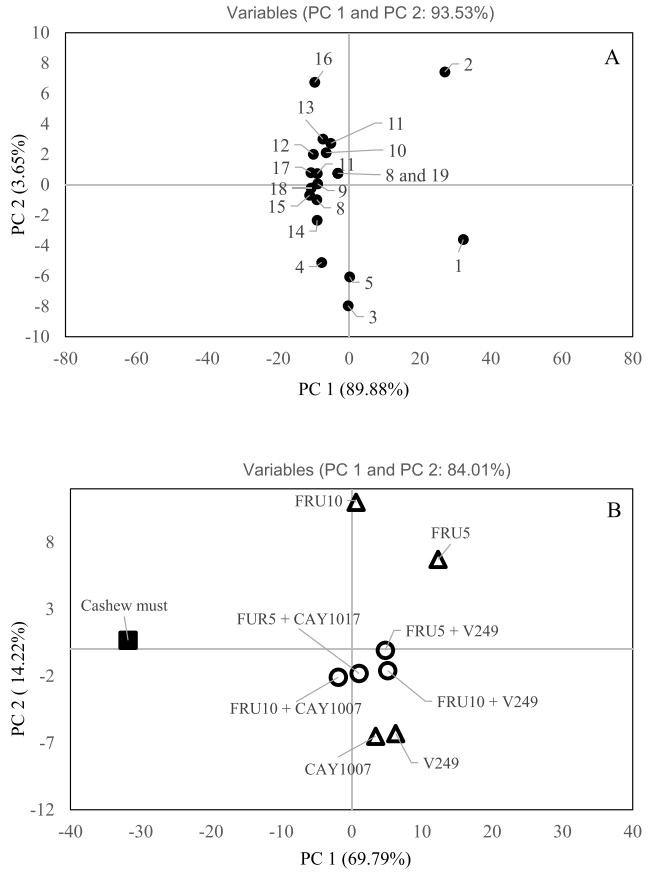

Fig. 2. PCA plots illustrating sample volatiles (A) and variables (B), representing 93.5% and 84% of variance, respectively. The variables are cashew must and yeasts, S. *cerevisiae* (CAY1007 and V249) and non-*Saccharomyces* (FRU5 and FRU10), used in fermentation. The number of volatile compounds is presented in Table 2.

between fermentation and the volatile compounds showed that most volatile compounds are located in the middle region, in Fig. 2A, displaying the relation of the co-fermentation process with *S. cerevisiae* and non-*Saccharomyces*. Desirable volatile compounds, phenylethyl alcohol (2) and 3-methyl-1-pentanol (9), are associated with pleasant rose and mushroom-like odours, respectively (Zhang et al., 2018); these were primarily correlated with the co-fermentation between the FRU5 and commercial yeasts. Moreover, 2-propenoic acid, 3-phenyl-, ethyl ester (12) and fruity and balsamic odour characteristics (Hasegawa, Hashimoto, Fujihara & Yamada, 2016) were correlated with the FRU10 + CAY1007 fermentation, indicating that co-fermentation processes contributed to desirable cashew wine flavours.

4. Conclusion

The results of this study revealed that non-*Saccharomyces* isolated from tropical fruits and co-cultured with *S. cerevisiae* displays a high ethanol production from the consumption of glucose, sucrose and fructose from cashew must. The cashew must co-fermentative process, using the non-*Saccharomyces T. delbrueckii* (FRU5) and *H. opuntiae* (FRU10) with *S. cerevisiae* CAY1007 and V249, also allowed the high desirable production of volatile compounds in fruit wines. All fermentative processes exhibited a desirable concentration of acetic acid for cashew wine production. The volatile compounds, phenethyl alcohol and 2-phenethyl acetate, can intensify floral and sweet characteristics during the co-fermentation process. In addition, the production of 3-methyl-1-pentanol with mushroom-like odours and whiskey notes was observed only in the co-cultures of *S. cerevisiae* with *T. delbrueckii*, inviting new perspectives for making exotic fruit wine using these yeasts.

Author contribution section

Conceptualization: Francine Ferreira Padilha and Maria Lucila Hernández- Macedo.

Methodology: Ester Seixas Bullé Rêgo, Ana Luiza Freire, Ana Maria de Resende Machado and Fátima de Cássia Oliveira Gomes.

Validation: Francine Ferreira Padilha, Maria Lucila Hernández-Macedo and Carlos Augusto Rosa.

Formal analysis: Ayslan Santos Pereira da Costa and Marcelo da Costa Mendonça.

Investigation: Francine Ferreira Padilha, Maria Lucila Hernández-Macedo and Carlos Augusto Rosa.

Resources: Maria Lucila Hernández- Macedo, Carlos Augusto Rosa and Marcelo da Costa Mendonça.

Writing - Original Draft: Maria Lucila Hernández- Macedo and Ester Seixas Bullé Rêgo.

Writing - Review & Editing: Maria Lucila Hernández- Macedo and Ester Seixas Bullé Rêgo.

Visualization: Ester Seixas Bullé Rêgo, Ana Luiza Freire, Ana Maria de Resende Machado and Fátima de Cássia Oliveira Gomes.

Supervision: Francine Ferreira Padilha, Maria Lucila Hernández-Macedo and Carlos Augusto Rosa.

Project administration: Francine Ferreira Padilha, Maria Lucila Hernández- Macedo and Carlos Augusto Rosa.

Funding acquisition: Maria Lucila Hernández- Macedo and Marcelo da Costa Mendonça.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.109291.

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