J Ind Microbiol Biotechnol (2011) 38:1161–1169
DOI 10.1007/s10295-010-0891-6

ORIGINAL PAPER

Biotechnological process for obtaining new fermented products from cashew apple fruit by *Saccharomyces cerevisiae* strains

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Received: 31 May 2010 / Accepted: 21 October 2010 / Published online: 11 November 2010

Abstract In Brazil, the use of cashew apple (*Anacardium occidentale* L.) to obtain new products by biotechnological process represents an important alternative to avoid wastage of a large quantity of this fruit, which reaches about 85% of the annual production of 1 million tons. This work focuses on the development of an alcoholic product obtained by the fermentation of cashew apple juice. The inoculation with two different strains of yeast *Saccharomyces cerevisiae* viz. SCP and SCT, were standardized to a concentration of 10^7 cells ml^{-1}. Each inoculum was added to 1,500 ml of cashew must. Fermentation was performed at 28 ± 3°C and aliquots were withdrawn every 24 h to monitor soluble sugar concentrations, pH, and dry matter contents. The volatile compounds in fermented products were analyzed using the gas chromatography/mass spectrometry (GC/MS) system. After 6 days, the fermentation process was completed, cells removed by filtration and centrifugation, and the products were stabilized under refrigeration for a period of 20 days. The stabilized products were stored in glass bottles and pasteurized at 60 ± 5°C/30 min. Both fermented products contained ethanol concentration above 6% (v v^{-1}) while methanol was not detected and total acidity was below 90 mEq l^{-1}, representing a pH of 3.8–3.9. The volatile compounds were characterized by the presence of aldehyde (butyl aldehyde diethyl acetal, 2,4-dimethyl-hepta-2,4-dienal, and 2-methyl-2-pentenal) and ester (ethyl α-methylbutyrate) representing fruity aroma. The strain SCT was found to be better and efficient and this produced 10% more alcohol over that of strain SCP.

Keywords Cashew apple · Fermentation · Flavor compounds · GC/MS · *S. cerevisiae*

Introduction

In the northeastern region of Brazil there is a large cultivation of cashew apple fruit, which is generally consumed as fresh fruit or processed to obtain cashew apple juice. A large amount of this fruit, representing about 85% of its annual production and amounting to about 1 million tons, is wasted. It is therefore necessary to explore the potential of fermented products such as wine, which could be an alternative in utilization of the large volume of fruit produced in its peak season, whereby reducing its wastage.

During fermentation, the yeasts present in must metabolize glucose via the Embden-Meyerhof pathway, under anaerobic conditions for ATP production and primary products of fermentation such as ethanol and carbon dioxide. In addition to enzymes involved in alcoholic fermentation, yeast produces other enzymes involved in the formation of odorous compounds. These volatiles known as "secondary products of fermentation" belong to various functional groups, such as alcohols, esters, aldehydes, and acids.

The volatile compounds responsible for the wine aroma may have different origins, including the substances present

This article is based on a presentation at the 32nd Symposium on Biotechnology for Fuels and Chemicals.

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in fruit, which are not altered by fermentation, compounds resulting from biochemical transformations that occurred in compounds originally present in the raw material, new volatile compounds formed during fermentation, and some other compounds generated during the aging process [4].

The volatile constituents of fresh and commercially processed cashew apple juice from Brazil were analyzed by Maciel et al. [19] who identified 12 alcohols, eight terpenes, three ketones, two aldehydes, two acids, and three sulfur compounds. The principal compounds present in cashew apple juice were methyl-3-methyl pentanoate, *trans*-2-hexenal, ethyl-2-methyl-2-butenoate, and 2-methyl-2-pentenal [12].

Gil et al. [14] studied the importance of apiculate yeasts in the formation of the volatile compounds in wine. The authors concluded that yeasts *Hanseniaspora uvarum* and *Kloeckera apiculata*, which are commonly present in must as wild microorganisms, are retained from the grape, and these are important for the final wine quality, while the yeast mixture belonging to the genus *Saccharomyces* produces higher concentrations of ethyl isobutyrate, ethyl isovalerate, various other higher alcohols, and some acids.

Dias [9], working with fermentation of cashew apple juice, reported that clarification and sulfitation pre-treatments before fermentation result in a positive influence on products aroma. These treatments also contribute to reduced concentration of ethanal, ethyl acetate, and propanol in fermented products obtained with *S. bayanus.* [9].

With an aim to expand the studies on utilization of cashew apple fruit, this work was planned to obtain two fermented beverages using fresh ripe cashew apple juice by utilization of two different strains of *Saccharomyces cerevisiae*, denominated as SCP and SCT and the products were evaluated for their physico-chemical and volatiles composition. Furthermore, certain prime parameters such as pH, soluble solids, and dry matter contents were also monitored daily during fermentation.

Materials and methods

Microorganisms

Two strains of *Saccharomyces cerevisiae* were used in this study. One strain, called SCP, which is used in baking processes, was obtained commercially in lyophilized form (Dr. Oetker Brazil Ltda, São Paulo, SP, Brazil), and another strain, SCT, which is commonly used in industrial processes (Taquari Plant Industrial, AL, Brazil) to produce ethanol. Both of these strains contained 70% moisture and were maintained at 40°C on standard agar based slants (Acumedia, Lansing, MI, USA).

Media and culture conditions

Cultures were aerobically maintained in a liquid medium containing cashew apple (*Anacardium occidentale* L.) juice, diluted at 1:1 (juice: H_2O). The ripe cashew apple fruits were purchased from Central Market in the city of Aracaju, Brazil, and selected according to color and physical conditions, and then cleaned, processed, and filtered to obtain clarified juice. The must was prepared by mixing cashew apple juice with sucrose so as to obtain a 30° Brix and other chemical solutions such as 1 g l^{-1} ammonium phosphate (Vetec Fine Chemical Ltda, Duque de Caxias, RJ, Brazil), 0.1 g l^{-1} of magnesium sulfate (Labsynth Ltda, Diadema, SP, Brazil), 0.1 g l^{-1} of sodium metabisulfite (Vetec Fine Chemical Ltda, Duque de Caxias, RJ, Brazil) and citric acid (Cromato Ltda, Diadema, SP, Brazil) solution 10% to adjust its pH to 4.0. The wort was then sterilized in an autoclave at 121°C at 1 atm pressure for 20 min. To prepare the pre-inoculum, the loop of the strain grown on standard agar based slant was added to 20 ml broth base medium (glucose 2%, peptone 1%, yeast extract 1%) in a 50-ml Erlenmeyer flask. The bottle was incubated for 24 h at 100 g and 30°C in an orbital shaker (BS-T Certomat B. Braun Biotech International). A volume of 18 ml of culture was transferred to a 250-ml Erlenmeyer flask containing 180 ml of juice, and it was incubated under identical conditions of pre-inoculum for 12 h, yielding a count of 10^7 cells ml^1.

Fermentation process

A volume of 1.5 l of cashew apple juice was placed in a 2-l Erlenmeyer flask to which 10% inoculum volume was added without agitation. The flasks were maintained at room temperature (30 ± 3°C) until its Brix stabilized, considering a maximum variation of 0.20. Following fermentation, the flasks were stored under refrigeration (4°C) for 24 h for the solid-phase separation. Next, the fermented liquid was transferred to another Erlenmeyer flask and the remaining mass was centrifuged at 3,000 × *g* for 5 min, discarding the solids. All liquid obtained in the process was filtered through cotton gravimetrically, retaining the container to cooling so to stabilize the fermentation process. Later the liquid was again filtered under the same conditions followed by its filling in sterilized wine bottles (750-ml capacity), which were sealed and pasteurized at 60 ± 5°C for 30 min, followed by cooling to 20°C for 10 min.

Physicochemical analysis

During the fermentation process, an aliquot was removed every 24 h to determine soluble solids content (°Brix), pH, and dry matter. Besides these determinations, other physicol-chemical characteristics such as total acidity, alcohol content, and total reducing sugars were determined in final wine products, according to the methods described by Instituto Adolfo Lutz [15]. All analyses were performed in duplicate in three distinct sets of fermentations.

Extraction and characterization of volatile constituents

The volatile compounds present in cashew apple juice and fermented wines (samples withdrawn daily) were extracted by using solvent extraction methodology described by Mamede [22]. The extracts were analyzed for the presence of volatile constituents in a gas chromatograph (GC3800, Varian) system coupled with a mass spectrometer (MS4000, Varian). A volume of 1 μl of sample diluted in ethyl ether (Dinâmica Ltda, Diadema, SP, Brazil) was injected in an injector operating in split mode (1:10). The separation of the compounds occurred in a capillary column (Varian VF-5MS, 30 m × 0.25 mm i.d. × 0.25 μm film thickness), programmed at 30°C (5 min) followed by a ramp, 2°C/min to 195°C, where it was maintained for a further period of 20 min. The total ion chromatograms were analyzed using the workstation MS Data Review (Varian) and compounds were identified based on the mass spectrum of the analyte compared to the compound database from the NIST library along with the linear retention index value of each compound. Furthermore, an analysis was performed to compare the peak intensities of the analytes with spectra recorded in the literature [1].

Results and discussion

Physico-chemical composition

Both cashew apple juice (filtered through 1-mm-mesh sieve) and products during fermentation process were analyzed for their physico-chemical composition (Table 1).

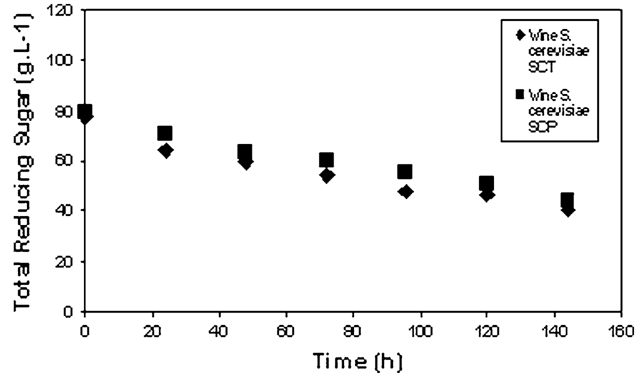

Fig. 1 Sugar consumption during fermentation of cashew apple wine obtained by the two strains (SCT and SCP) of *S. cerevisiae*

The fermentation carried out with different strains (SCT and SCP) lasted for 144 h, and after this period, physico-chemical characteristics of cashew apple wine were determined prior to pasteurization and maturation, and results of these parameters are presented in Table 1.

From the data presented in Table 1 it could be observed that there was a continued decrease in soluble solid content during the fermentation process. However, at the end of fermentation, sugar consumption was higher (43.4%) in wine elaborated with strain SCT when compared to the product obtained with strain SCP (37.4%). The consumption pattern of sugars during fermentation, performed with both strains (SCP and SCT), can be observed in Fig. 1. There was a similarity in the reduct in profile for both the strains. This result was in accord with soluble solids profile during fermentation process. The pH reduction was of 0.2 unit in the fermentation performed with strain SCP against a reduction of only 0.1 unit in the product obtained with SCT strain. Thus, both these products are within the value which guarantees the microbiological quality of wines [34]. The decrease in dry matter content in both fermentations indicated that the production of cashew apple wine was considered to be mild when compared to dry white grape wines [32].

Table 1 Physico-chemical characteristics of cashew apple products obtained with the usage of two strains (SCT and SCP) of *S. cerevisiae* during fermentation

Fermentation (h)	Wine form strain SCP			Wine form strain SCP		
	Soluble solids (°Brix)	pH	Dry extract (g l^{-1})	Soluble solids (°Brix)	pH	Dry extract (g l^{-1})
0.0	30.0	4.00	29.59	30.0	4.00	30.13
24.0	25.6	3.60	24.33	26.4	3.60	25.33
48.0	21.8	3.60	20.25	21.6	3.60	19.06
72.0	19.8	3.70	17.54	19.2	3.70	16.75
96.0	19.2	3.80	15.75	18.0	3.80	14.18
120.0	18.8	3.80	15.32	17.2	3.90	13.09
144.0	18.8	3.80	16.31	17.0	3.90	13.59

Table 2 Physico-chemical characteristics of cashew apple juice and wines elaborated with two strains (SCT and SCP) of *S. cerevisiae*

Characteristics	Must without inoculum	Wine elaborated with *S cerevisiae* with strain	
		SCP	SCT
pH	4.1 ± 0.00	3.9 ± 0.00	4.00 ± 0.00
Soluble solids (°Brix)	31.00 ± 0.00	19.20 ± 0.00	17.40 ± 0.00
Dry matter (%)	31.06 ± 0.25	15.02 ± 0.11	12.915 ± 0.99
Total reducing sugar (g l^{-1})	101.219 ± 0.00	43.94 ± 0.10	33.09 ± 2.23
Protein (%)	0.33 ± 0.00	NR	NR
Nitrogen (%)	0.05 ± 0.05	NR	NR
Total acidity (mEq l^{-1})	2.61 ± 0.11	89.55 ± 2.69	83.83 ± 0.00
Ash (%)	0.11 ± 0.00	0.12 ± 0.04	0.11 ± 0.00
Alcohol (% v v^{-1})	NR	10.0 ± 0.00	11.0 ± 0.00

The results of physico-chemical analysis of cashew apple juice used for the production of wines with and without inoculation with the two strains (SCP and SCT) of *S. cerevisiae,* pasteurized and stored for a maturation period of 20 days are presented in Table 2. The data obtained on the analyzed variables of soluble solids and dry matter contents of the uninoculated medium was higher as compared to fermented wines prepared by inoculation with the strain of SCT of *S. cerevisiae*.

Lower values (13%) were observed (Table 2) for dry matter content in the wine fermented with SCT. Reducing sugars were lower by about 10 g l^{-1}, in the wine fermented with the SCT strain when compared to wine fermented with the SCP strain. Alcohol content in the fermented product with SCT strain was 11°GL (Gay-Lussac degree) against a concentration of 10°GL in the fermented beverage prepared with SCP strain, representing a gain in its efficiency of 10% in alcohol production by strain SCT over that of strain SCP. This conclusion is drawn from the solids soluble data (Table 1) and total reducing sugars (Table 2). Torres Neto et al. [34] reported an alcohol content of 11.5°GL in the fermented cashew apple wine inoculated with *S. cerevisiae*, used in bread making, with two stages of must enrichment, while in another study conducted, Garruti et al. [13] reported a lower alcohol content (8.5°GL) in cashew apple wine obtained with the *S. bayannus* strain.

In addition to the above data, it was observed that during the maturation and stabilization period of cashew apple wine, the parameters such as dry matter content, soluble solids, and pH suffered minor variations between the two products at the end of 144 h of fermentation (Table 1).

The yield calculated from alcohol production following the procedure described by Hang et al. [15] was 97.8% for the product inoculated with SCT strain of *S. cerevisiae*, having productivity of 0.6 g l^{-1} h^{-1}, which is about 34% lower than the yield reported by Torres Neto et al. [34]. This difference can be explained in the form of enrichment, which was done in two stages during the fermentation. Silva et al. [33] cite that the enrichment done in two steps is a common practice performed to minimize the microbial inhibition by the substrate. The fermented beverages produced with cashew apple juice inoculated with SCP strain had a yield of about 89%, and its productivity rate was 0.55 g l^{-1} h^{-1}, which is 8.9% lower than the beverages obtained by the usage of the SCT strain.

The total acidity of cashew apple wines was 89.55 (mEq l^{-1}) and 83.83 (mEq $l^{-1)}$ in the beverages obtained by using strain SCP and SCT of *S. cerevisiae*, respectively, which demonstrates that the fermented cashew apple wines obtained in this work had a medium total acidity when compared to dry, red and white grape wines, whose acidity should not exceed 130 (mEq l^{-1}) according to Brazilian legislation [7]. This analysis is quite relevant since, in general, the lower concentrations of total acidity in red and white wines results in attributes of soft wines with more structure and more complex aromas [36].

During the 6-day fermentation period, samples were collected for extraction and analysis of volatile constituents. The gas chromatographic analysis with mass spectrometric detection of volatile fractions of fermented cashew apple juice obtained with the strain SCP and SCT detected a total of 47 and 72 compounds, respectively, and 44 of these compounds were positively identified. Figure 2 shows the total ion chromatogram obtained on the analysis of the volatile extracts of cashew apple wines prepared with the use of two strains (SCP and SCT) of *S. cerevisiae.* Identification of 16 alcohols, seven ketones, four aldehydes, two ethers, four esters, five acids, four terpenes, and others aromatic and aliphatic hydrocarbons was achieved. Of the total compounds identified, 25 were found only in the volatile extract obtained from the fermented cashew apple wine elaborated with the strain SCT of *S. cerevisiae*. In this context, the present work is the first of its kind to evaluate two strains of *S. cerevisiae* and then compare the product profile, proving the potential of strain SCT as the one producing a large number of compounds that are known to possess fruity or floral aroma.

Table 3 presents the data on compounds detected in the volatile extract obtained from cashew apple wine inoculated with the strains SCP and SCT of *S. cerevisiae*. All compounds identified in this study have also been previously reported in literature as constituents of fermentation processes in various matrices. Furthermore, a database (Flavor Base Pro-2007; Leffingwell, USA) that includes the presence of volatile compounds in different products was also consulted.

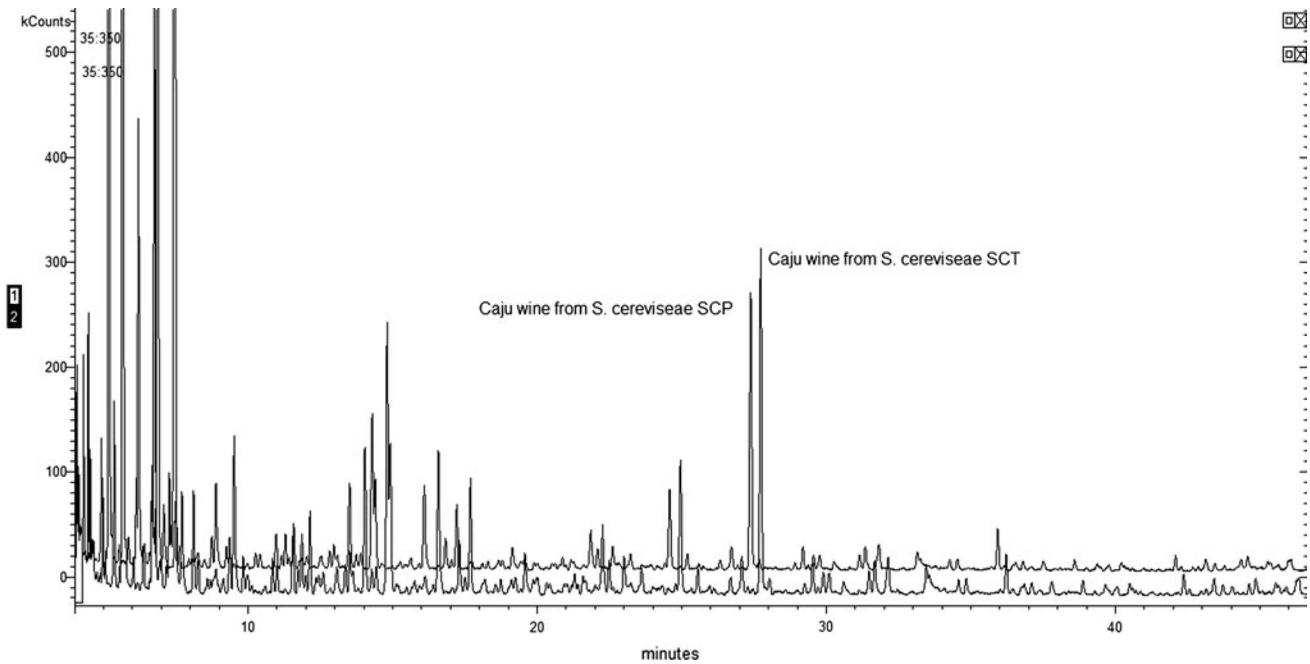

Fig. 2 Gas chromatographic profile of volatile constituents present in cashew apple wine obtained by fermentation with the strains SCP and SCT of *S. cerevisiae*

Among the principal chemical classes representing aroma of fermented products, alcohols was the group that contributed to a larger number of constituents. The so-called "higher alcohols" are formed from the metabolism of amino acids present in wine or resulting from degradation of protein from yeast cells [30]. When these are present in wine, they contribute positively to the aroma characterization of the drink, but when they are present at levels greater than 400 mg.l^{-1} they characterize for an unpleasant odor and may detract from the overall quality of the beverage [29]. They are also important as precursors for esters formation during aging [3]. Cashew apple fruit is rich in amino acids such as alanine, serine, leucine, pH, phenylalanine, proline, glutamic acid, tyrosine, and aspartic acid [17]. The aromatic phenyl-ethyl alcohol (16.8%) detected in this study could be derived from the amino acid phenylalanine. The presence of this compound is important as it is known to impart herbaceous aroma in the fermented beverages [13].

The compound isoamyl alcohol (3-methyl-1-butanol) represented only 1% of the total area of all constituents and it is also considered to be one of the most important characteristics of higher alcohols in wine production, and it is formed from the amino acid leucine [13].

The contribution of aldehydes and ketones present in wines is not well known, and very little information is available on the role of these compounds in wine flavor [6, 24]. In this study, one of the ketones identified was 3-methyl-2-pentanone, which is commonly characterized for beer aroma [26], while among the aldehydes identified was the presence of 2,3,4-trimethyl-3-hexenal which is known to contribute green notes of aroma. The presence of ester, ethyl isovalerate (1.75%) has been reported in earlier studies of fermented cashew apple wines and it is responsible, along with other esters such as ethyl 2-methylbutyrate (0.6%), for the fruity aromas.

The major compound of the volatile fraction was 3-methoxy-3-methyl-butanol which is usually not desired as it rapidly transformer into aldehydes, in the presence of oxygen. This degradation is accompanied by reactions that promote mercaptan formation (from sulfur amino acids), which possess extremely unpleasant odors and these may consequently be non-acceptable in the beverage obtained from cashew apple [20].

Among the two wines obtained by the usage of strains SCP and SCT, a larger number of constituents was detected in fermented cashew apple prepared with the usage of SCT. The compounds 3-methyl-hexane, 5-methyl-1-hexane, 3,3-dimethyl-butanone, diethyl acetaldehyde, ethyl isopropyl ketone, 4-ethyl-2-methyl-hexane, 2-propyl-1-heptanol, limonene, 2,3-epoxycarene, *o*-cymene were detected only in the fermented beverages obtained with the SCT strain. Berger [4] emphasized the strain- and species-dependent formation of volatile compounds during fermentation. When must of the Spanish grape cultivar Monastrell was fermented with different strains of *S. cerevisiae*, major

Table 3 Volatile constituents identified in cashew apple wines produced with two strains *S. cerevisiae* (SCP and SCT)

Volatile compounds in both wines from strains SCP and SCT	LRI	Volatile compounds present only in the flavor of wine from strain SCT	LRI
		Cis-1,2-dimethylcyclopentane	739
		Methoxy-1,3-dioxalane	740
		3-Ethyl-hexane	741
1-Ethoxy-butane	742		
		Fatty ester	744
		3,3-Dimethyl-2-butanone	745
		Alcohol	746
		Methyl-cyclohexane	749
		NI	751
		Diethyl-acetal	752
3-Methoxy-3-Methylbutanol	753		
		NI	756
2-Terc-buthyl-3-ethyl-oxirane	760		
		Ethylisopropyl-cetone	762
3-Methyl-2-pentanone	763		
		NI	767
		4-Ethyl-2-methyl-hexane	768
		NI	768
2-Ethyl-1-hexanol	769		
HC	770		
Fatty ester	771		
2-Ethyl-1-pentanol	774		
Alcohol	778		
Fatty acid	781		
3-Hexanone	784		
Fatty acid	786		
6-Methyl-2-heptanone	787		
NI	789		
2,2-Dimethyl-3-pentanone	792		
Isoamylic alcohol	793		
		2,3-Buthanediol	803
3,3-Dimethyl-2-pentanone	805		
2-Methyl-2-pentenal	807		
		2-Propyl-1-heptanal	812
Dihydrocitronellol	814		
		Ethyl-cyclohexane	816
		Cyclogeraniolane	819
2,4-Dimethyl-2,4-heptadienal	824		
NI	826		
NI	828		
NI	834		
4-(2-Methyl-3-oxocyclohexyl)butanal	837		
Ethyl á-methylbuthyrate	839		
		HC	844
NI	846	NI	846
3-Hydroxi-decanoic acid	849		
		HC	854
		HC	855

Table 3 continued

Volatile compounds in both wines from strains SCP and SCT	LRI	Volatile compounds present only in the flavor of wine from strain SCT	LRI
NI	856		
3-phenyl-2-butanol	858		
Allyl 2-ethyl butyrate	891		
Butyraldehyde diethyl acetal	893		
		4-Methyl-2-propyl-1-pentanol	914
HC	917		
Dimethylacethylcetone	920		
Hydroxi alcohol	921		
ac *z,z*-9,12-octadecadienoico (grape seed oil)	933		
NI	934		
2,3,4-Trimethyl-3-hexenal	938		
		HC	947
1,2,4-Trimethilene-cyclohexane	952		
NI	961		
L-limonene	1,015		
Ethyl isovalerate	1,038		
L-carveol	1,062		
		o-cimene	1,034
Phenylethyl alcohol	1,110		
a,a-dimethyl-*p*-isopropyl-benzyl alcohol	1,242		

LRI Linear retention Index

differences in the ester content of the wines were observed, and these are not derived from the same metabolic pathway [23].

Studies suggest that much of the sensory expression of the wine bouquet is due to the presence of terpenoid compounds [8]. Moreover, the relationship between the amounts of each terpene in a wine can serve as a clue to discover the variety of fruit used. About 50 monoterpenes are known to be present in wine. Terpenes belong to the secondary constituents of plants, and their biosynthesis begins with acetyl co-enzyme A (SCoA). Since volatile compounds can be used as fingerprints in flavor identification of wines, these consequently can lead to the identification of fruit used in wine production.

In this work, an important finding in the formation of volatile compounds in fermented beverages obtained with both strains (SCP and SCT) is the absence of acetaldehyde, a constituent when present in high concentrations in products, is responsible for an oxidized odor note [5]. Even though the musts were inoculated with different strains of *S. cerevisiae*, the methanol contents were below the quantification level of this compound (5 mg l^{-1}) in both fermentations. The Brazilian legislation [7] establishes a maximum limit of 0.35 g l^{-1} of methanol presence in wines. This observation is much more relevant since musts used in this study were not subjected to clarification, which is known to remove pectin, which consequently results in a reduction of methanol production during the process [14, 18, 21, 28, 31, 35].

The overall profile of volatile constituents during fermentation can be observed in the chromatograms present in Fig. 3. In terms of composition and concentration of volatile constituents during the fermentation process, it was observed that the sec-isoamyl alcohol, phenylethyl alcohol, and dimethylacetyl acetone were formed and their concentrations increased during fermentation.

The volatile compounds 2-ethyl-1-hexanol, limonene [2], 3-methyl-2-butanol [2, 13], 3-hexanone [16], 2,3-butanediol, ethyl α-methylbutyrate [12], L-carveol [11], and *o*-cimene [21], which characterize the flavor of cashew apple juice, were also found in the wines elaborated in this study.

Conclusions

The cashew apple wines obtained with both strains of SCP and SCT are characterized as semi-hard, less structured, and of adequate flavor. Among the two strains SCP and SCT of *S. cerevisiae* tried in this work for the preparation of cashew apple wines, the strain SCT was found to be better and more efficient as alcohol production in wine obtained with this strain was 10% higher than in the wine obtained by the usage of strain SCP. The principal volatile compounds present in cashew apple wine elaborated with the usage of the strain of SCT of *S. cerevisiae* and which could be responsible for its char-

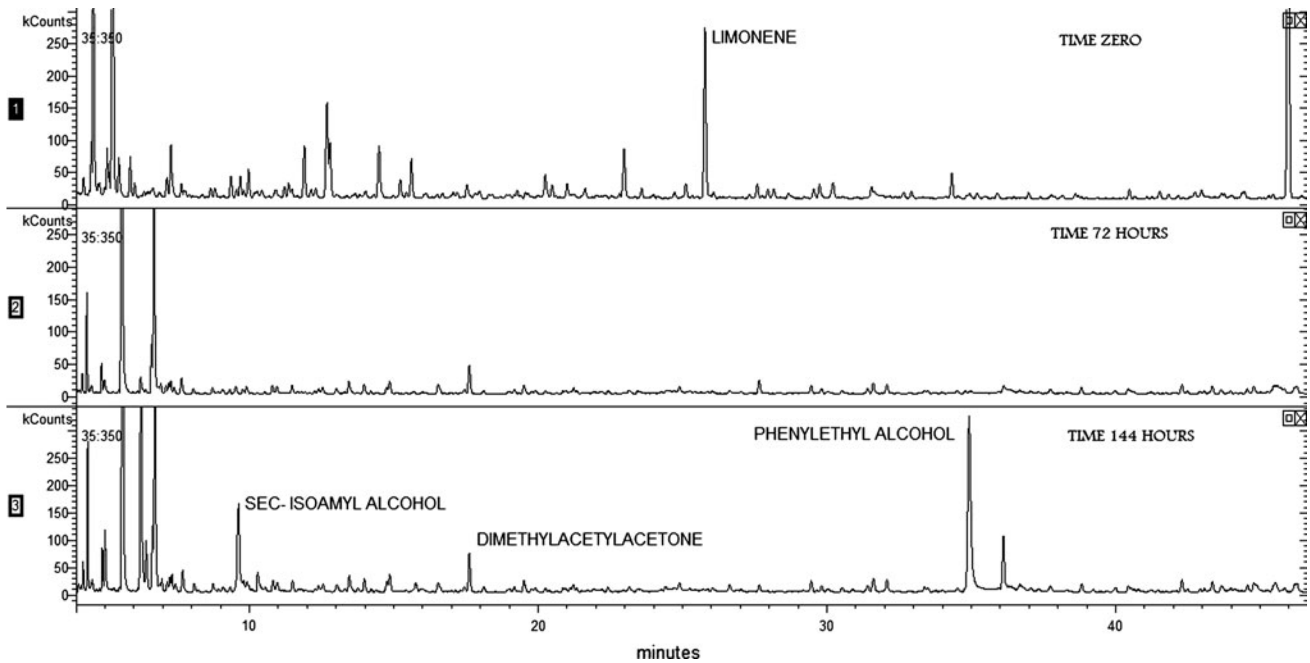

Fig. 3 Gas chromatographic profile of volatile constituents of cashew apple juice fermentation at time zero, after 72 h of fermentation, and after 144 h of fermentation

acteristic aroma, are 2,3,4-trimethyl-3-hexenal and phenyl-ethyl-alcohol.

Acknowledgments We appreciate the financial support given by CNPq (National Council for the Development of Science and Technology, Brazil) and to CAPES for fellowship offered to the author, Suzane M. Araújo.

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