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Fermentation of Cashew Apple (Anacardium Occidentale) Juice Into Wine by Different Saccharomyces Cerevisiae Strains: A Comparative Study

O. A. Apine Department of Biotechnology, Shivaji University Kolhapur, India 416004.

*** Prof. Jyoti P. Jadhav** Department of Biochemistry, Shivaji University Kolhapur, India 416004 *Corresponding author

ABSTRACT

Cashew nut production is the primary aim for the cashew tree cultivation. Large numbers of cashew apples were considered as a major agriculture waste. This work is focused on the development of alcoholic product by using cashew apple juice. Two different yeastSaccharomyces cerevisiae NCIM 3311 and NCIM 3282strains were used for fermentation with cell count 107 CFU/ml. Fermentation was performed by using 10% of inoculum at 24±2°C. After each 24 h sample aliquot was withdrawn from the flask to monitor soluble sugar concentrations, pH and dry matter contents. Among volatile compounds, major alcohols and volatile acids were analysed after every 24 h using the gas GC-MS and GC-FID system. After fermentationprocess the products were stabilized under refrigerationfor a period of 25 days. After the analysis of physicochemical properties and volatiles, strain NCIM 3311 was found to be better with 12 % alcohol after fermentation compared with the other NCIM 3282.

KEYWORDS Cashew apple, wine,Saccharomyces cerevisiae, GC-MS, GC-FID

Introduction

Most of the countries around the world are being actively engaged in the cultivation of cashew (*Anacardium occidentale* L.), with Brazil, India, Vietnam and Nigeria as the main cultivation centers. The primary aim of the cashew tree cultivation is for production of cashew nuts. Cashew apples were considered as a 'Gold Mine' of wasteland as it requires low inputs for production (Attri, 2009). In India harvesting of the cashew starts from February and lasts till the end of May or early June. Cashew tree bears cashew nut (true fruit) and cashew apple (pseudo fruit), accounting for 10 % and 90 % of the total fruit weight respectively (Talasila, 2012)

Cashew apples are elongated, round or pear-shaped fibrous fruits. The fully developed cashew apple would be firm, juicy, without any shade of green color and is easily detachable from the plant. The flavor, aroma and sugar concentrations are very high, and acidity and astringency are low at fully developed stage (de Figueiredo, 2002). 30 million metric tons of cashew apples are produced yearly in the world and 20 lakh tons in India (Mandal,1985; Michodjehoun-Mestres, 2009). About 10–15 tons of cashew apples are produced for every ton of cashew nut (Attri,2009). Cashew apple is a non-climacteric fruit found in three colors yellow, orange and red, with the same pale yellow pulp, weighs about 75– 80 g (Maciel, 1986). Cashew apple can be consumed raw and possesses good characteristics for utilization due to its fleshy pulp, soft peel, lack of seeds, high sugar content and strong exotic flavor (Garruti, 2003). Neither the cashew apples nor the juice extracted from the fruits is completely utilized in India, despite of having promising nutritional and medicinal properties; large amounts of cashew apples are being wasted in the field after nut separation.

Wine making would be better option to preserve nutraceuticals and more value to the cashew apples. It could provide a better alternative to process the large number of fruits during the peak harvest period whereby reducing its wastage due to rapid post-harvest deterioration.Wine from cashew apples is like a real gold mine from the waste if processed and promoted properly. In this study the attempt has been made to ferment fresh and ripe cashew apple juice to wine using two different *Saccharomyces cerevisiae*, denominated as NCIM 3282 and NCIM 3311. The products were evaluated for their physico-chemical composition and volatiles. Furthermore, certain prime parameters such as pH,soluble solids, and dry matter contents were also monitoreddaily during fermentation.

Materials and Methods

Microorganisms and media

Two strains of Saccharomyces cerevisiae were used in this study. Freeze-dried yeast strains *Saccharomyces cerevisiae* NCIM 3282, and *Saccharomyces cerevisiae* NCIM 3311 was obtained from National Collection of Industrial Microorganisms (Pune, Maharashtra, India). The yeasts were propagated in a sterile nutrient broth composed of 2 % w/v glucose, 0.1% yeast extract, 0.2 % peptone, 0.5 % sodium chloride (pH 5.0) and routinely maintained on the agar based slants of same nutrient composition earlier mentioned.

Must and inoculum preparation

Fully ripe cashew apple yellow variety, Vengurla 7 officially developed by Regional Fruit Research Station Vengurle, Maharashtra, India. Cashew apples used for the study were collected from the Vaibhavwadi (16° 29' 47' N, 73° 44' 45'E) region and immediately processed. The fruits were washed and sanitized; any defective fruits were sorted out in the laboratory. During washing nuts attached to cashew apple were separated. Then cashew apples were steamed for 5 min, again washed with cool fresh water to bring the cashew apples to room temperature and then pressed to obtain the integral non-pulpy juice and filtered. The must was prepared by mixing cashew apple juice with sucrose so as to increase natural brix 16° to 28° additionally 0.1 g/l of diammonium phosphate added. The pH was adjusted to 4.0 with citric acid solution. The juice was sterilized overnight with 100 ppm of potassium metabisulphite under refrigeration (4°C). Pre-inoculum was prepared by inoculating loopful of respective yeast strains grown on the agar based slants was added to 150 ml of liquid medium containing cashew apple juice diluted at 1:1 (Juice: distilled water) along with broth based medium components earlier mentioned. The culture was maintained aerobically for 48 h yielding a count of 10^7 CFU/ml. The CFU was calculated by using neubauer chamber, this fermented Cashew juice was then used as inoculum.

Fermentation condition

Replicate fermentation was carried out in 5 l capacity Erlen-

meyer flask with a 3 l volume of cashew apple juice to which 10 % (v/v) inoculum was added. The flasks were maintained at room temperature for brix stabilization then maintained at 20° C throughout fermentation process. Fermentations were conducted for 7 days using a simple batch system under static conditions.

Physico-chemical composition

During the fermentation process, an aliquot was removed after every 24 h for the analysis. The total soluble solids (Brix), pH, dry matter and optical density (600 nm) were measured throughout the fermentation process with samples taken at the indicated time points by using a refractometer (ATAGO, Japan), pH meter (Metrolim, Switzerland) and spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan). Besides these determinations, other physico-chemical characteristics such as total acidity, alcohol content, and total reducing sugars were determined in final wine products, according to the methods described by Analysis of the Association of Official Analytical Chemists (AOAC 2000). All analyses were performed in triplicate in three distinct sets of fermentations.

GC-FID and GC-MS analysis

Volatile extraction by HS-SPME was carried out with 50/30µm layer of divinylbenzene–carboxen–polydimethylsiloxane (DVB-CAR-PDMS) (Supelco Co, Bellefonte, USA) fibre that was exposed to the sample headspace for a specified time.After extraction, the SPME assembly was removed from the headspace vial and inserted directly into the injection port of the GC. The SPME fibre was immediately thermally desorbed for 3 min at 250 °C in the SPME-specific liner of the injector port of either the GC-FID or GC–MS. The volatile flavour compounds of wine were initially identified using a GC–MS and only major alcohols and volatile acids were included in the study. Quantitative analysis of the volatile compounds performed by using a GC equipped with a flame ionization detector (FID). Sample preparation was based on the method of (Chen, 2006) with modifications. External individual standard solutions were prepared and diluted with 10% cashew apple juice-based aqueous solutions to obtain a range of concentrations, except that ethanol standard was diluted in 100% cashew apple juice. The selected volatiles included ethanol (1000- 15,000 ppm (v/v)), isoamyl alcohol (0.01- 0.17 ppm), isoamyl acetate and (0.004-0.1 ppm) and 2-phenylethyl alcohol (0.04-2.00 ppm). All calibration curves had R 2 values of at least 0.98. The samples were diluted with water prior to extraction and subjected to analysis. Triplicate analyses were performed for each sample

GC-FID

Agilent 7890 A gas chromatograph equipped with a FID and a capillary column stabilwax- DA (30m × 0.25mm × 0.25 µ). Helium was the carrier gas with a velocity of 1.5 ml/min and oven temperature was held 120 °C for 2 min and then raised at 5 °C with 15 min hold. The injector and detector temperature were 230 °C and 260 °C respectively.

GC-MS analysis

Agilent 5975 C series GC-MS system was used for analysis. Separation of volatile compounds was achieved by using stablewax DA, with dimensions of 60 m × 250 µm × 0.25 µm. Helium was used as a carrier gas with a velocity of 1 ml/min. The oven temperature was initially at 60°C for 10 min then 10°C/min rise up to 230°C for 18 min then kept for 45 min. The mass spectrometer operated in the electron impact mode with a source temperature of 200 °C, an ionizing voltage of 70 eV, and a transfer line temperature of 250 °C. All the analysis was done in scan mode using MSD Chemstation software, the mass spectrometer scanned masses from 48 to 400 m/z at a rate of 3.41 scan/s. Peak identification was carried out by comparison of the volatile sample mass spectra with spectra in the NIST Mass Spectral Database.

Results and Discussion

Many tropical fruits such as mango, jackfruit, banana and cashew apple have been shown to be suitable for fermentation, mainly because of their appropriate taste, flavor, availability, high sugar and water content, and overall chemical composition (Ward and Ray, 2006). Some of them could enhance local or international markets by appropriate utilization processes, and fermentation remains as a technological attempt of such utilization (Onwuka and Awam, 2001; Muniz, 2006). Despite the high level of tannin content (causing astringency), cashew apples could be processed into beverage owing to its fleshy pulp, soft peel and lack of seeds, high sugar concentration and strong exotic flavor. However, it has been underutilized, and the development of a new product to minimize the apple waste includes fermentation of the juice, yielding an alcoholic beverage such as wine (Muniz, 2006).

Physico-chemical composition

Cashew apple juice (Filtered through 1-mm-meshsieve) and products during fermentation process were analyzed for their physico-chemical composition (Table1). The fermentation carried out with different strains (*Saccharomyces cerevisiae* NCIM 3282 and NCIM 3311) lasted for 168 h, and after this period, prior to pasteurization and maturation the physicochemical characteristics of cashew apple wine were determined (Table 2). 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. The consumption pattern of sugars during fermentation, performed with both strains (NCIM 3311 and NCIM 3282), can be observed in Fig.1a. There was a similarity in the reduction profile for both the strains. This result was in accord with soluble solids profile during fermentation process. The viable yeast cell populations of pure cultures of *S. cerevisiae* 3282 and *S. cerevisiae* 3311 showed comparable increase in the cell count up to day 120 h of the fermentation then declined towards the end of the fermentation Fig. 1b.

The pH reduction during the fermentation by with strain NCIM 3311 and NCIM 3282 were reduced by 0.4 and 0.6 respectively. Thus, both these products are within the value which guarantees the microbiological quality of wines (ToresNeto, 2006).The decrease in dry matter content especially fermentation carried out by NCIM 3311 strain was more than NCIM 3282 strain.The reduction of dry matter was mainly responsible for the dryness of the wine. The wine fermented with NCIM 3311 strain was comparatively drier like white grape wine. Wine fermented by NCIM 3282 strain was considered to be mild compared with white grape wine and the wine fermented with NCIM 3311.

The results of physico-chemical parameters of cashew apple juice and fermented wine from the same juicewas analysed with and without inoculation with the two strains (NCIM 3282 and NCIM 3311) of *S. cerevisiae*, after fermentation wine pasteurized and stored for a maturation period of 25 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 NCIM 3311 of *S. cerevisiae*. Lower values were observed (Table 2) for dry matter content in the wine fermented with NCIM 3311. Reducing sugars were lower by about 10 g l^{-1}, in the wine fermented with the NCIM 3311 strain when compared to wine fermented with the NCIM 3282 strain. Alcohol content in the fermented product with NCIM 3311 strain was 12 % against a concentration of 10% in the fermented wine prepared with NCIM 3282 strain, representing a gain in its efficiency of 10% in alcohol production by strain NCIM 3311 over that of strain NCIM 3282. This conclusion is drawn from the solids soluble data (Table 1) and total reducing sugars (Table 2). Earlier reported studies on the *Saccharomyces cerevisiae* strains SCP and SCThas alcohol content of 10 % and 11 % respectively (S. Arauja, 2011). Torres Neto, 2006 reported an alcohol content of 11.5% 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 by (Garruti, 2006) reported a lower alcohol content (8.5%) 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 168 h of fermentation (Table 1). The yield calculated from alcohol production following the procedure described by Hang et al. (Hang, 1981) was 92.30 % for the product inoculated with NCIM 3311 strain of *S. cerevisiae*, while in case of strain NCIM 3282 it was observed 76.92 %.

The fermented beverages produced with cashew apple juice inoculated with NCIM 3282 strain had a yield of about 76.30 % and its productivity rate was 0.47 g $l^{-1}h^{-1}$, which is comparatively lower than the beverages obtained by the usage of the NCIM 3311 strain.The titratable acidity of cashew apple wines was 76.8 and 71.65in the wines obtained byusing strain NCIM 3311 and NCIM 3282 of *S. cerevisiae*, respectively,which demonstrates that the fermented cashew apple winesobtained in this work had a medium total acidity whencompared to dry, red and white grape wine. This analysis is quite relevant since, in general, the lower concentrations of total acidity in red andwhite wines results in attributes of soft wines with morestructure and more complex aromas (Tsukatani, 2003).

Kinetic changes of selected volatiles during fermentation

During the fermentation, the yeasts along with presence of some non-saccharomyces yeast during fermentation released secondary products such as higher alcohols, esters, acids and carbonyl compounds in general mixed culture helps in producing a wider range and higher amounts of volatiles than the pure cultures. 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 (Rodriguez-Amaya, 2004). 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 mgl^{-1} they characterize for an unpleasant odor and may detract from the overall quality of the beverage (Rapp, 1979). Ethanol, isobutyl alcohol (2-methyl-1-propanol), isoamyl alcohol (3-methyl-1-butanol) and 2-phenylethanol were the major alcohols produced during the cashew apple juice fermentation. The kinetic changes of the alcohols were varied among the cultures, whereas the final amounts of alcohols at day 168h varied significantly with the *S. cerevisiae* 3311 constantly producing the higher amounts of each type of alcohols except for phenylethyl alcohol compared with *S. cerevisiae* 3282. While *S. cerevisiae* 3282 produced significantly greater amount of phenyl ethyl alcohol compared with NCIM 3311 (Fig. 2).

The profile of production and degradation of fatty acids of C2 to C8 was similar in both the fermentation except for hexanoic and heptanoic acids in *S. cerevisiae 3282*. Butyric and hexanoic acids present at relatively high concentrations in the juice were utilized during fermentation to trace levels by both cultures. For the yeast cultures, acid formation and utilization trends are similar *S. cerevisiae* 3311while *S. cerevisiae* 3282 showed better production of heptanoic acid (Enanthic acid) which is also known as wine oil. Esters developed from the acid during aging process add to the varital aroma of the wine. Acetic acid was constantly produced throughout the fermentation with *S. cerevisiae* 3311 *and S. cerevisiae* 3300highest amount of acetic acid (Fig. 3). Non-*Saccharomyces* yeasts have been associated with high acetic acid production, thus, are considered as spoilage yeasts (Pretorius, 2000). They are also important as precursors for esters formation during aging (Amerine, 1983). Cashew apple fruit is rich in amino acids such as alanine, serine, leucine, pH, phenylalanine, proline, glutamic acid, tyrosine, and aspartic acid. The aromatic phenyl ethyl alcohol 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 (Garruti, 2006).

Conclusion

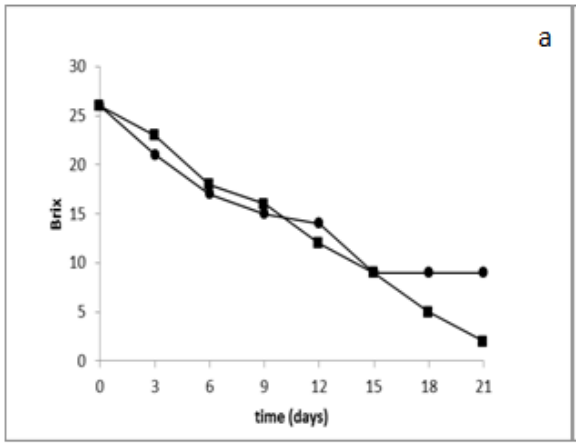
Apart from its nutritional value cashew apples in India were considered as a major waste, lack of processing technologies and market campaigning is the most definite reason for the wastage. Except fenny and pectin those are the main cashew apple products produced in India. Wine from cashew apple juice would be a better option to develop as value added product. In present study the cashew apple wines obtained with both strains of NCIM 3282 and NCIM 3311 are characterized as mild, less structured, and of adequate flavor. Among the two strains NCIM 3282 and NCIM 3311 of *S. cerevisiae* tried in this work for the preparation of cashew apple wines, the strain NCIM 3311 was found to be better and more efficient for alcohol production than the wine fermented by NCIM 3282 strain. The kinetic study of principal volatile compounds such as major alcohols and volatile acids present in cashew apple wine marks promising flavour and aroma profile of wine. Cashew wine, prepared by fermentation of cashew apple juice using *S. cerevisiae* NCIM 3311, would be another good prospect for the alcoholic beverage industry. However, more research may be conducted to find out the method forreduction of tannin concentration in cashew apple must and, consequently, inwine to minimize the astringent flavor

Table 1.Physicochemical characters of a cashew apple juice fermented with two strains (NCIM 3282 and NCIM 3311) of *S. cerevisiae*

Fermentation (h)	Wine from strain 3282			Wine from strain 3311		
	Soluble solids (Brix)	pH	Dry extract ($g\ l^{-1}$)	Soluble solids (Brix)	pH	Dry extract ($g\ l^{-1}$)
0.0	26.0	4.01	25.29	26.0	4.01	30.13
24.0	23.0	3.70	21.33	22.0	3.80	25.33
48.0	20.0	3.70	19.28	18.0	3.80	19.06
72.0	17.0	3.60	18.84	12.0	3.60	16.75
96.0	14.0	3.60	18.15	9.0	3.60	10.18
120.0	9.0	3.60	17.33	5.0	3.60	10.09
144.0	9.0	3.50	16.31	5.0	3.60	08.59
168.0	9.0	3.50	15.27	3.0	3.60	05.50

Table 2Physico chemicalt parameters of cashew apple juice and wine elaborated with *S. cerevisiae* **strains (NCIM3282 and NCIM 3311).**

Characteristics	Must without inoculum	*S. cerevisiae* strain	
		NCIM 3311	NCIM 3282
pH	4.2±0.00	3.6±0.00	3.5±0.00
Soluble solids	26.00± 0.00	3.2±0.00	5.2±0.00
Dry Matter	29.68±0.23	6.52±0.02	16.51±0.12
Total reducing sugar	119.21± 0.00	6.05±0.25	14.87±0.50
Protein	0.43±0.00	NR	0.02±0.00
Nitrogen	0.007±0.05	NR	NR
Total acidity	3.61±0.00	76.8±0.00	71.65±2.5
Alcohol	NR	12.00±0.00	10.00± 0.00



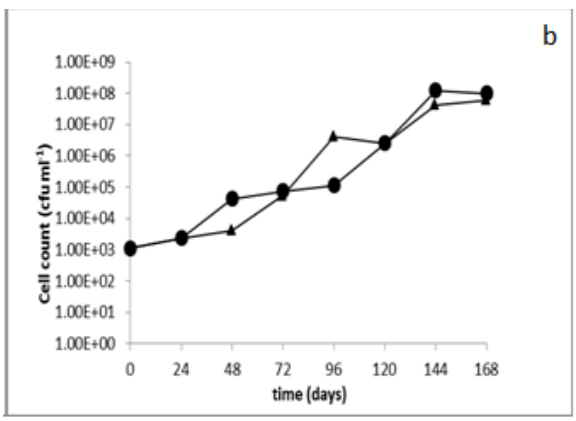
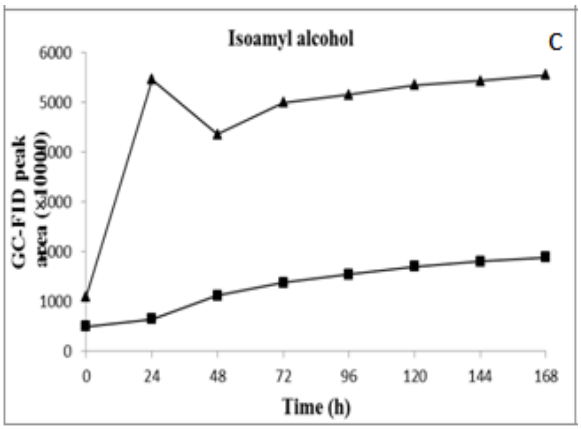


Fig.1 a) Evolution of yeast as a viable cell count and b) Brix changes in cashew wine during fermentation with *S. cerevisiae* **3282 (+), S. cerevisiae 3311 (+)**



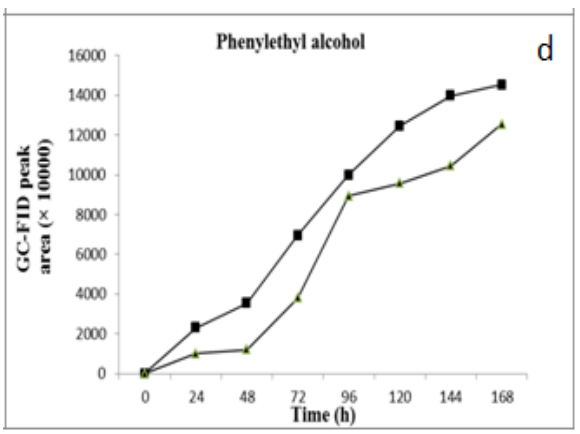
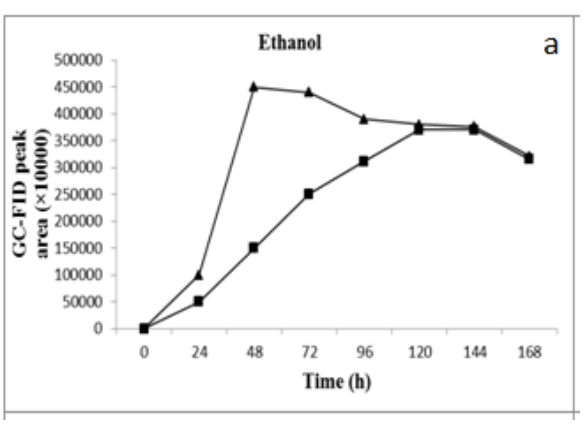
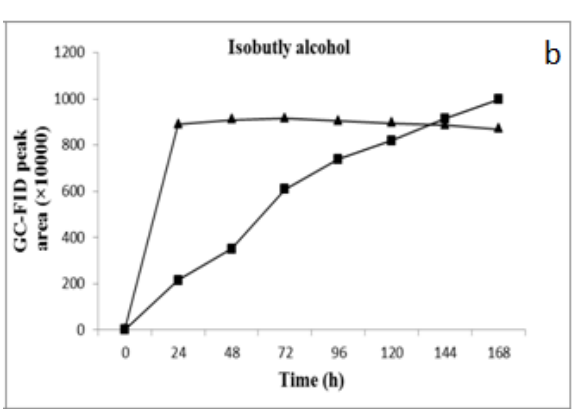
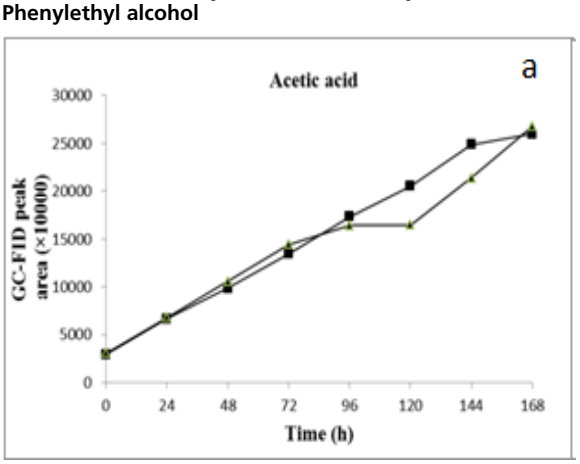
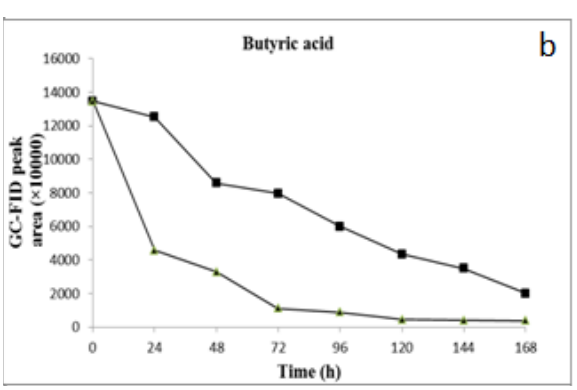


Fig. 2 Changes in alcohol in cashew wine during fermentationwith *S. cerevisiae* **3282 (+),and** *S. cerevisiae* **3311(+) a) Ethanol, b) Isobutyl alcohol, c) Isoamyl alcohol and d) Phenylethyl alcohol**









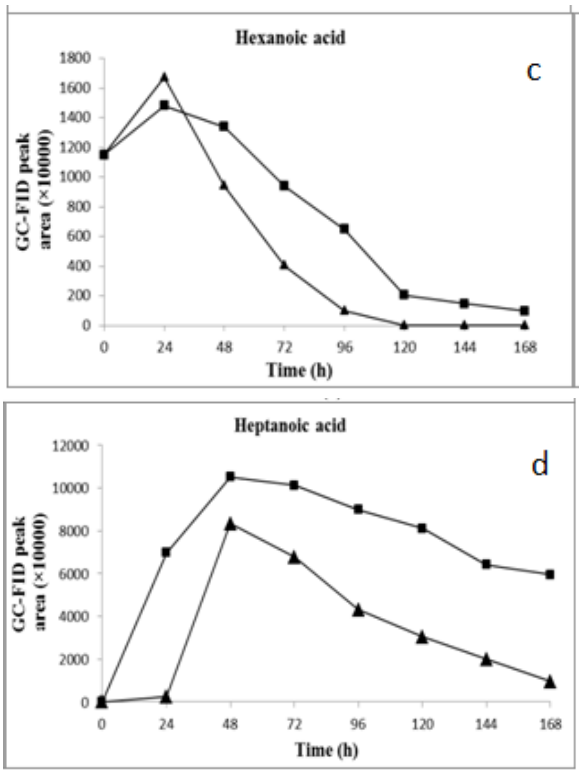


Fig. 3 Changes in volatile acids in cashew wine during fermentationwith *S. cerevisiae* 3282 (+), and *S. cerevisiae* 3311 (+) a) Acetic acid, b) Butyric acid, c) Hexanoic acid and d) Heptanoic acid

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