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# CASHEW WINE VINEGAR PRODUCTION: ALCOHOLIC AND ACETIC FERMENTATION

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**Abstract -** Cashew wine of demi-sec grade was produced in a stirred batch reactor. The kinetic parameters obtained for cashew wine fermentation were $Y_{X/S}$=0.061, $Y_{P/S}$=0.3 and $\mu_{max}$=0.16 $h^{-1}$. The yield and the productivity of cashew wine were 57.7% and 0.78 g/Lh respectively. A $2^2$ factorial experimental design was used for the cashew wine vinegar fermentation optimization study. The cashew wine vinegar process optimization ranges found for initial concentrations of ethanol and acetic acid as independent variables were 4.8 to 6.0% and 1.0 to 1.3% respectively.
*Keywords*: Cashew vinegar; Cashew wine; Fermentation kinetic parameters.

## INTRODUCTION

Brazil is one of the world's largest producers of tropical fruits such as oranges, pineapples, bananas, acerolas and persimmons amongst others. Particularly in the Northeast region, there is cashews are cultivated on a large scale. However, due to the higher commercial value of the cashew nut, only 15% of the juicy portion (*peduncle*) is utilized for the derived products. This is an unbearably great loss for the region, which is poor in food resources. Fermentation, however, can be a viable solution to avoid this loss. This includes alcoholic and acetic, as well as various other types of fermentation. Recently some research results on the fermentation of guava and banana (Bhatt et al., 1997), onion (Hirouchi et al., 2000), kiwi (Bertolini et al., 2001), orange (Corazza et al., 2001) and cajá (*spondias mombin* L.) (Dias et al., 2003) have been reported.

Cashew wine can be produced by alcoholic fermentation of peduncle juice. However, the peduncle has a high concentration of pectin which degrades into methanol when present in the juice. Methanol is a toxic substance that can cause death when ingested in excess. The maximum permitted concentration of methanol in wine is 35 mg/100 mL.

Acetic fermentation is the next step in alcoholic fermentation, where alcohol molecules are oxidized into acetic acid molecules by the action of *Acetobacter aceti* bacteria, giving it the characteristic vinegar taste (Hirouchi et al., 2000; Tesfaye et al., 2002).

The aim of the present work was to study alcoholic and acetic fermentation kinetics and also to verify the effects of the initial concentrations of ethanol and acetic acid as two independent variables on process yield and productivity. For process optimization the factorial design of experiments (Box et al., 1978) method was utilized.

## MATERIALS AND METHODS

### Microorganisms

Fleischmann ™ *Sacharomyces cerevisiea* bakers yeast was used for cashew wine fermentation. The inoculate utilized for cashew wine vinegar was a concentrated vinegar which was produced separately

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by acetic fermentation in the natural presence of *Acetobacter aceti* bacteria on sugarcane husk in a polyethylene reactor.

## Procedure of Cashew Wine Production

Mature cashews were selected by first eliminating all damaged and spoiled ones and especially those in which the process of fermentation had already started. The cashews were washed by submerging them in chlorine water for about 30 minutes in order to remove dirt and microorganisms and were finally washed with running water to remove the residual chlorine. Upon weighing the washed whole cashews the peduncle was separated and weighed. The peduncle juice was obtained by crushing the separated peduncle and filtering it through a cotton cloth. ºBrix of the integral peduncle juice was then determined. The ºBrix values were used to calculate sugar concentrations during alcoholic fermentation. To purify the juice, 3mL of gelatine (10%) per mL of juice were added so that the must pectin could be removed. Ammonium sulphate and potassium phosphate as nutrients and potassium metabisulfite as disinfectant were also added. In order to obtain a cashew wine with an alcohol concentration higher than 100 g/L, sugar was added to the peduncle juice **c**haptalization, to obtain an initial concentration of 120 g/L. Therefore, 30 g/L of sugar was added at the beginning of fermentation and thereafter 170 g/L, when the concentration of saccharose in the medium fell to 10 g/L. In this way the sucrose inhibition was reduced. Alcoholic fermentation was started by inoculation of the must through the addition of 20g/L of Fleischmann $^{TM}$ *Sacharomyces cerevisiea* general bakers yeast. Fermentation was interrupted when the substrate concentration level fell to less than 20g/L, which characterizes a dry wine and is appropriate for vinegar production. The concentrations in g/L of substrate (S), product (P), biomass (X) and total acid as well as pH were monitored for a period of 48 hours of alcoholic fermentation. A vacuum filtration was performed to remove microorganisms. The filtered wine was poured into pasteurized dark green glass bottles and was stocked for use in the production of cashew wine vinegar.

## Optimization Cashew Wine Vinegar Production

The production of cashew wine vinegar was studied through a $2^2$ factorial design of experiments using seven 600 mL glass beakers. The $2^2$ factorial experimental design matrix, shown in Table 1, included three center points to provide a better estimate of the experimental error and also to indicate whether the best conditions for the production of the cashew wine vinegar, were within the range of the current experimental region. It represents all possible combinations of concentrations and their respective values for -1, 0 and +1 levels of ethanol (Cal) and inoculating acetic acid (Caa) concentrations along with the observed responses for productivity and yield. As shown in Table 1, the seven experiments on acetic fermentation were started by mixing 100 mL of cashew wine solution, 100 g of sugarcane husk and 100 mL of strong vinegar as inoculate, which had been drawn from the 50 L reactor. The concentration of the acetic acid formed was monitored for all seven experiments at five hour intervals during a period of 72 hours of total fermentation. At the end of the 72 hour period the solution was filtered under vacuum to remove sugarcane husk particles from the vinegar. After filtration the vinegar solution was poured into the white plastic bottles, capped, sterilized by pasteurization and stored.

## Analytical Methods

For cashew wine fermentation the parameters obtained were biomass yield ($Y_{x/s}$), product yield ($Y_{p/s}$) and cellular growth specific velocity ($\mu_{max}$) and were based on total sugars utilized. $Y_{x/s}$ and $\mu_{max}$ were estimated in the exponential growth phase of the initial sixteen hours of fermentation.

For analysis of sugar concentration, the property used was ºBrix. According to the method of the AOAC (2000), ºBrix is the total soluble solids. The concentration of sugars has a linear relationship with ºBrix (Torres Neto et al., 2006), as shown in Equation 1.

$$\text{Sugar (g/L)} = 10.13 * (\text{ºBrix}) + 1.445 \quad (1)$$

The alcohol concentration was determined by ebuliometer and Gay-Lussac alcoholmeter, the pH by pH meter and the total acid concentration by the volumetric neutralization method in accordance with the Analytical Norms of the Adolfo Lutz Institute (1985). The cell mass was determined gravimetrically (Torres Neto et al., 2006).

The concentration of methanol was determined with a gas chromatograph Varian CP-3380 equipped with a flame ionization detector, an electronic processor/integrator and a Hallcomid and Carbowax coloumn. The operational temperatures for the injector, detector and column were 130 ºC, 160 ºC and 86 ºC respectively. Ultra-pure nitrogen carrier gas with a flow velocity of 2.4 $dm^3/h$ and injected sample volumes of 5µL were utilized. The peaks of the chromatogram were identified using external standards having the following concentrations: 1-propanol: $6.03x10^{-2}$ $g/dm^3$; isobutanol: $4.42x10^{-2}$ $g/dm^3$; n-butanol: $3.10x10^{-2}$ $g/dm^3$; isoamyl alcohol: $3.20x10^{-2}$ $g/dm^3$ and methanol: $2.73x10^{-2}$ $g/dm^3$ (Torres Neto et al., 2006).

**Table 1: $2^2$ factorial experimental design matrix with the results for productivity and yield**

| Experiments | Ethanol concentration levels for cashew wine and (%) | Acetic acid concentration of vinegar inoculant levels and (%) | Productivity (g/Lh) | Yield (%) |
|---|---|---|---|---|
| 1 | - 1.0 (3.0) | - 1.0 (1.0) | 0.36 | 48.17 |
| 2 | + 1.0 (6.0) | - 1.0 (1.0) | 0.56 | 74.30 |
| 3 | - 1.0 (3.0) | + 1.0 (2.0) | 0.45 | 29.66 |
| 4 | + 1.0 (6.0) | + 1.0 (2.0) | 0.56 | 37.28 |
| 5 | 0.0 (4.5) | 0.0 (1.5) | 0.51 | 59.02 |
| 6 | 0.0 (4.5) | 0.0 (1.5) | 0.50 | 58.07 |
| 7 | 0.0 (4.5) | 0.0 (1.5) | 0.49 | 57.13 |

## RESULTS AND DISCUSSION

### Kinetic Parameters of Alcoholic Fermentation of Cashew Wine

Figure 1 shows the course of degradation of substrate (S), variation in biomass (X) and production of ethanol (P) as functions of fermentation time. It can be observed that in 14 hours of fermentation the concentration of substrate decreased due to its consumption by microorganisms, falling from 150 g/L to about 10 g/L. In order to increase the sucrose concentration, after 15 hours sucrose was added a second time (170 g/L). This is a practice which avoids inhibiting the fermentation by substrate, as reported by Bortolini et al. (2001). The concentration of sucrose again started decreasing with fermentation time, but at a slower rate than that in the first step. This observation can be explained on the basis of the existence of increased product (alcohol) concentration in the medium. This reduction in production velocity can also be verified after 10 hours of fermentation in the first step. However, the concentration of alcohol could reach to 100 g/L at the end of 48 hours, resulting in a desired alcoholic grade of cashew wine. The fermentation process took 132 hours (not shown in the Figure 1) and was monitored to obtain demi-sec wine. From the biomass concentration curve it can be seen that in the first 15 hours a small increase in the biomass occurred, remaining at this level until 28 hours and thereafter decreasing slowly to the initial value of 20 g/L in 48 hours. Therefore, the use of a yeast concentration of 20 g/L (*Saccharomyces cerevisiae*) in the present work was adequate for producing a wine of alcoholic grade within the standard limits fixed by Brazilian legislation. Silva et al. (1999) also reported the ideal yeast concentration for alcohol production to be about 20 g/ L, where cell growth could be kept at its minimum level.

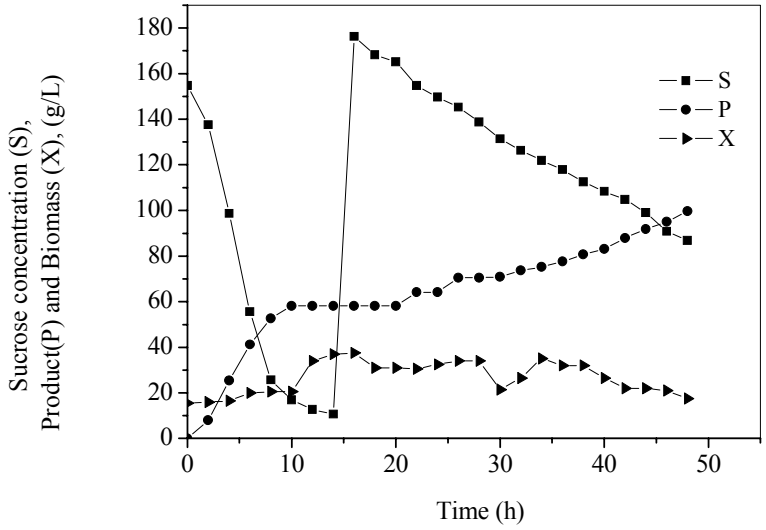


**Figure 1:** Fermentation kinetics of cashew wine production: concentration of substrate (S), concentration of ethanol (P) and concentration of biomass (X) (expressed in g/L).

It should be emphasized that methanol is highly toxic and its concentration in alcoholic drinks should not exceed the 35 mg/100 mL limit. The chemical component analysis of the cashew wine produced showed a very low level of methanol (1.39 mg/100 mL), which indicates that the removal of pectin from the must was very efficient.

The experimental and literature values for the concentration of alcohol and saccharose, yield and productivity of the cashew wine along with the kinetic parameters Yx/s, Yp/s and $\mu_{max}$ are given in Table 2. The alcoholic grade (102.9 g/L) and the saccharose concentration (7.12 g/L) indicate that the cashew wine produced is of the demi-sec grade. The yield (55.7%) and the productivity (1.43 g/Lh) are higher than those reported by Bortolini et al. (2001), who studied the production of kiwi wine. The kinetic parameter values (Yx/s, Yp/s and $\mu_{max}$) are similar to those obtained by Andrietta and Maugeri (1994), while the Yx/s value in the present work is two times higher. However, the literature value for Yp/s is higher which can be due to the yeast used in the work of Andrietta and Maugeri (1994), a selected efficient yeast, while the one used in the present work was of the general bakers' quality. Andrietta and Maugeri (1994) obtained the kinetic parameters of the process of alcoholic fermentation for the production of alcohol from sugarcane.

The kinetic parameters determined in the present work have great importance for the future modeling and simulation studies for the scale-up of cashew wine production.

**Table 2: The experimental and literature parameter values for the production of cashew wine**

| Parameter | Experimental | Literature |
|---|---|---|
| Alcoholic concentration of wine (g/L) | 102.9 | 126.3 -176.8 * |
| Saccharose in wine (g/L ) | 7.12 | Between 5.1 and 20 (demi-sec)* |
| Yield (%) | 55.7 | 38.7 - 47.2 ** |
| Productivity (g/Lh) | 1.43 | 0.74 - 2.0 ** |
| Yx/s | 0.061 | 0.033 *** |
| Yp/s | 0.300 | 0.445 *** |
| $\mu_{max}$ | 0.16 $h^{-1}$ | 0.19 $h^{-1}$**** |

Reference: *Brasil (1997); **Bortolini et al. (2001); ***Andrietta & Maugeri (1994); ****Silva et al. (1999).

## Optimization of Acetic Fermentation of Cashew Wine Vinegar

Figure 2 depicts acetic acid production kinetics for 72 hours of fermentation. It can be observed that there is good reproducibility of the three replicate kinetic curves at the central point (Table 1: experiments 5, 6 and 7), where the initial concentrations of alcohol (Cal) and acetic acid (Caa) were 4.5% and 1.5% respectively. The profiles of these three kinetic curves show that the maximum concentration of acetic acid produced was reached at about 50 hours of fermentation. After this time, the production of vinegar started decreasing. This could be due to the oxidation of acetic acid by oxygen present in the fermentation medium when the concentration of alcohol was very low.

The empirical nonlinear regression coded models for the productivity and yield of the cashew wine vinegar for 41 hours of fermentation are given by Equations 2 and 3. The coefficient values are statistically significant at confidence levels 95% and 90% respectively

$$\text{Productivity} = 0.491 + 0.078\text{Cal} + \\ +0.215\text{Caa} - 0.021\text{Cal.Caa} \quad (2)$$

$$\text{Yield} = 51.95 + 8.44\text{Cal} - \\ -13.88\text{Caa} - 4.63\text{Cal.Caa} \quad (3)$$

Table 3 shows the variance analysis parameters: regression coefficient $R^2$ and F-test.

The response surfaces for the 41 hour period of fermentation were analyzed because in these cases the linear empirical coded models (Equations 2 and 3) showed better values for the correlation coefficient (R) and higher F-test ratio. It can be observed that the F-value/F listed value ratio is greater than 1. According to Box et al. (1978), the model is statistically significant at the determined confidence level when the F-value is greater than the

F listed value.

Figure 3 illustrates the effect of initial concentrations of ethanol and acetic acid on the productivity of vinegar. It can be verified that at a 4.5 % initial concentration of ethanol and for any concentration of acetic acid, a productivity of 0.55 g/Lh is obtained. It can also be observed that productivity increases when the initial concentration of ethanol is changed from the (-1) to the (+1) level.

In Figure 4 the response surface for the process yield at 41 hours of acetic fermentation is shown. For an ethanol concentration above 4.8% and an acetic acid concentration below 1.3% a yield of 70% can be observed.

Values of the initial concentration of ethanol and acetic acid for optimization of the process of cashew wine production can be obtained on the basis of the response surface results. Thus, maximum values of 0.55 g/Lh for productivity and 75% for yield were achieved when the corresponding initial concentration of ethanol was between 4.8 and 6.0% and that of acetic acid was between 1.0 and 1.3%. Operating under these conditions the cashew wine vinegar produced had an acetic acid concentration 4% higher than the minimum required by the Brazilian legislation.

**Table 3: Variance analysis parameters for productivity and yield for 41 hours of fermentation**

| Source of variation | Sum of squares Productivity Yield | Mean square ProductivityYield | Degree of squares | F-value Productivity Yield |
|---|---|---|---|---|
| Regression | 0.0281 1141.32 | 9.37E-3 380.44 | 3 | 38.72 5.74 |
| Residues (error) | 7.25E-4 198.82 | 2.42E-4 66.27 | 3 | - |
| Total | 0.0288 1340.14 | - | 6 | - |
| Correlation coefficient (R) | 0.9880 0.9223 | - | - | - |
| F listed value | - | - | - | 9.28* 5.39** |

*Confidence level of 95%
**Confidence level of 90%

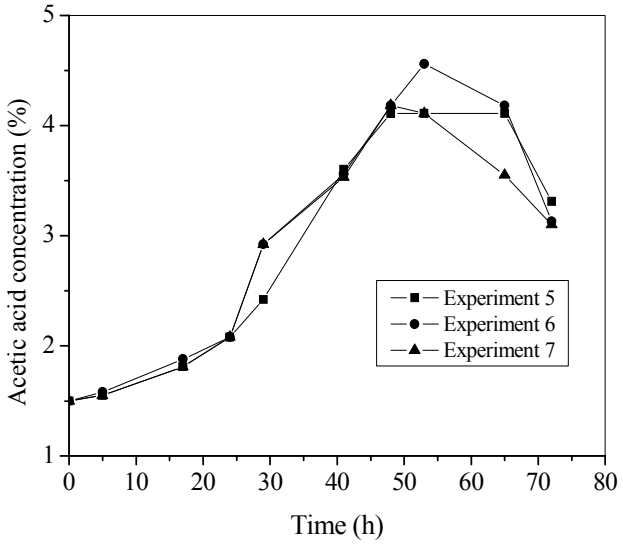


**Figure 2:** Vinegar production kinetics for acetic fermentation of cashew wine. Experiments 5, 6 and 7 at central point having initial concentrations of alcohol and acetic acid of 4.5% and 1.5% respectively

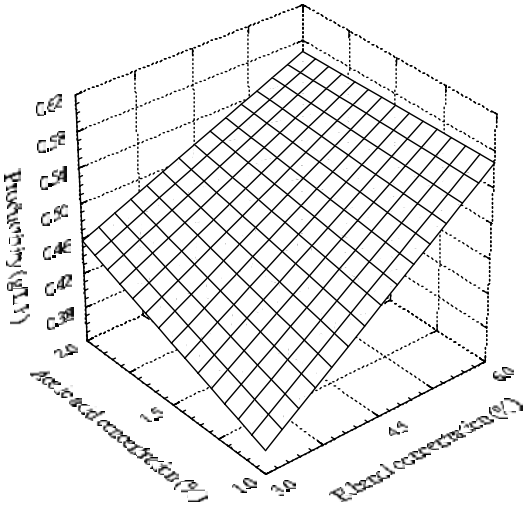

**Figure 3:** Response Surface: Variation in vinegar productivity (g/L $h^{-1}$) as a function of initial concentrations of ethanol and acetic acid for 41h of fermentation.

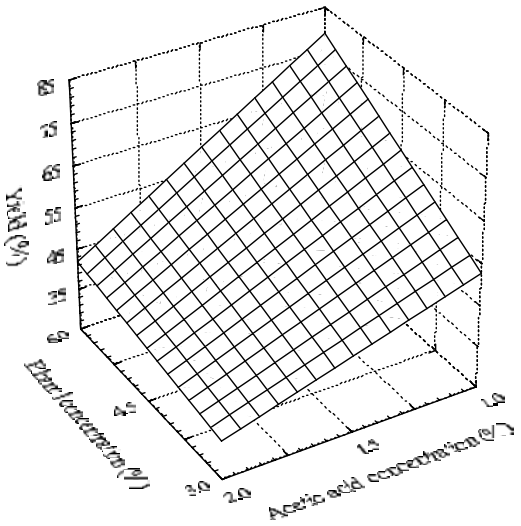

**Figure 4:** Response Surface: Variation in vinegar yield (%) as a function of initial concentrations of ethanol and acetic acid for 48h of fermentation.

## CONCLUSIONS

Cashew wine of demi-sec grade having an alcohol concentration of 102.9 g/L and a saccharose concentration of 7.12 g/L was produced in a period of about 48 hours of fermentation. This grade was adequate for obtaining the desired factor levels of the experimental design for studying their effects on the productivity and yield of the cashew wine vinegar fermentation process. The methanol concentration of the c6ashew wine produced, was about 1.39 mg/100 mL, which is quite a bit lower than the maximum permitted by Brazilian legislation on fermented alcoholic beverages. The yield and the productivity of cashew wine were 57.7% and 0.78 g/Lh respectively. The kinetic parameter values for the cashew wine process (Yx/s, Yp/s and $\mu_{max}$), were similar to those obtained in the literature.

The cashew wine vinegar process was optimized when the fermentation was carried out with an initial concentration of ethanol between 4.8% and 6.0% and of acetic acid between 1.0 and 1.3%. The maximum productivity achieved was 0.55g/L.h and that of the yield, above 75%. Under these conditions the concentration of acetic acid in the cashew wine vinegar produced was higher than 4%, as required by Brazilian legislation.

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