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Vol. 16(20), pp. 1150-1158, 17 May, 2017
DOI: 10.5897/AJB2016.15613
Article Number: B05343C64272
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effect of different concentrations of bush passion fruit pulp and temperature in the production of beer

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Received 12 August, 2016; Accepted 28 April, 2017

Among many species of passion fruit, the bush passion fruit (*Passiflora cincinnata Mast.)* is a wild, edible fruit, of exotic flavor and has good acceptability for consumption. This study aimed to determine the physico-chemical characteristics of the bush passion fruit pulp, and to produce and characterize physico- chemically the ale beer made with bush passion fruit as a malt adjunct, using different percentages (10, 29, 39 and 49%) of this adjunct and temperatures (15 and 22°C) to evaluate its fermentative potential for production of beer and to verify the influence of the soluble solids content and the specific gravity decrease during the fermentation. The results indicated that the bush passion fruit is suitable to carry out the fermentative process and the bench test media containing 29% of malt adjunct obtained best results in fermentation at both 15 and 22°C, with alcohol yields of 7.61 and 8.29%(v/v), respectively.

Key words: Beer, adjunct, bush passion fruit, fermentative potential.

INTRODUCTION

Biochemically, the fermentation is an energy production process from organic compounds like carbohydrates by microorganisms towards alcohols, organic acids and related catabolites. The process of fermentation in the food production generates various benefits, such as food conservation and security by production of compounds that inhibit the proliferation of other microorganisms including pathogens; increase of the nutritional value in addition to the improvement of the sensory quality of the product thanks to flavoring substances (Bourdichon et al., 2012).

Nowadays, biotechnology includes a wide range of different processes, which can be applied in nutrition and agriculture sectors (Carvalho et al., 2009). There is need of new processes and development allowing the use of fruits which are characteristic for particular geographic places of the world. Passion fruit is a delicacy from tropical regions due this tasteful and sugary mucilage around the seeds although the major part corresponds to the soft underskin pulp.

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Fermented fruits are promising products due to the tendency of high consumer acceptance. Traditionally, grapes and apples are used for fermented beverages. Many countries, mainly European, can produce fruit wines by manufacturing similar to processes made with the grape. Most of them use apple, pear, blackcurrant, raspberry or cherry. In tropical countries, there is a wide variety of fruits, such as orange, guava, pineapple, passion fruit and mango, which are in economic terms, poorly exploited for the production of beverages from fruit. Brazil has a wide variety of fruits with great potential to be exploited economically for the production of beverages.

The adjuncts for brewing can be defined as any different carbohydrate source from the barley malt which contributes fermentable sugars to the wort (Stewart, 2000).

Adjuncts used in beer production allow the reduction of costs of raw materials, since the barley produced in Brazil does not meet the demand. Therefore, the use of domestic raw materials reduces costs in the production of beer extract, however, this practice should not interfere with the beer quality, giving inadequate characteristics to the product or not providing conditions for the development of the features that are expected.

It is possible to find in Brazil many types of fruit for most of the year due to a great extent and the varied climate that allows the cultivation of tropical fruits as much fruits of temperate or cold climate.

The passion fruit is classified as a tropical fruit whose aroma and taste are highly appreciated by the Brazilian consumer. Brazil occupies the first position as a producer and consumer of passion fruit (Monteiro et al., 2005). There are about 400 tropical and subtropical species of passion fruit (*Passiflora*), more than 150 are native to Brazil, of which about 60 produce fruit that can be eaten raw or in the form of juices, soft drinks, sweets and liqueurs. The juice of the passion fruit is a source of ascorbic acid, characteristic this combined with the aroma and distinctive taste, allows many placement opportunities in the international market (Sato et al., 1992).

In the wide biodiversity of passion fruit, the bush passion fruit (*Passiflora cincinnata* Mast.) from Passifloraceae family stands out due to its edible and wild fruit, of exotic flavor and good acceptability for consumption. The fruit *in natura* is very appreciated by populations from northern Brazil. This fruit has potential functional components, such as fibers, vitamins, carotenoids and inorganic compounds (calcium, iron, phosphorus) flavonoids, steroids and fatty acids.

In this present study, the bush passion fruit pulp was characterized physico-chemically and it was evaluated for the production of improved beers with bush passion fruit, as malt adjunct, using different percentages of this adjunct and fermentation temperatures to verify the influence of soluble solids and specific gravity decrease during fermentation.

Figure 1. Bush passion fruit.

MATERIALS AND METHODS

Physico-chemical characterization of bush passion fruit pulp

The bush passion fruit (Figure 1) purchased at local fairs in the city of Feira de Santana, Bahia, Brazil, were selected, cleaned and processed to obtain the pulp by albedo, skin and seeds removing device (Macanuda brand, DBM-73 NR12 model). It is important to report that the pulp was made only from the mucilage. The pH was measured in digital apparatus, and soluble solids in a digital refractometer (Reichert brand, AR200 model).

Preparation of wort

The beer wort with original extract 12°P (approximately 12°Brix) and pH=5.0 was produced to obtain a clear *pilsen* beer in the pilot plant facilities, located at the Department of Technology of the Laboratory Fermentation of the State University of Feira de Santana, BA, Brazil. The bush passion fruit pulp with amount of soluble solids corrected was used as adjunct.

Sampling of the pulps

Pulp samples, previously filtered, were prepared to be fermented with yeast *Saccharomyces cerevisiae* Safale S-04, type *Ale* in different pulp percentages (10, 29, 39 and 49%), and at different temperatures (15 and 22°C) that were based on literature, in order to evaluate what percentage would produce higher amounts of ethanol in the same fermentation conditions and what would be the ideal temperature for fermentation. The samples were prepared with filtered fruit pulp. The amount of soluble solids content of the samples was corrected by sugaring process with commercial sucrose to the value of 12°Brix, value traditionally used in the industry in the production of conventional beer wort resulting in beer between 4 and 5% v ethanol/v. Following the same aim, the pH of the sample was adjusted to 5.0 using deacidification technique with $CaCO_3$ at a concentration in 100 mg/L. SO_2 was added in the form of potassium metabisulphite ($K_2S_2O_5$), as antimicrobial, since the pulp was not pasteurized. The techniques of sugaring, deacidification and microbial decontamination were performed according to Carvalho et al. (2016).

Propagation of yeast

Before starting the fermentation, the yeasts were hydrated and propagated in wort according to the specifications of the manufacturer Fermentis, Division of S. I. Lesaffre. After the hydration, the propagation of yeast was performed by adding hydrated yeast in an Erlenmeyer flask containing wort and it was incubated at 30°C for 24 h in a rotary shaker at 150 rpm, in Shaker ACB Labor.

Wort fermentation

In order to begin a fermentative process, a volume of 10% of the total volume to be fermented was removed from the propagation and added in the mixture of wort and adjunct previously prepared to ferment and with content of soluble solids and pH adjusted.

The fermentations with different percentages of adjunct (10, 29, 39 and 49%) added to the wort were conducted in Erlenmeyer flasks of 500 mL containing 250 mL of the mixture of wort, adjunct and propagation, placed in biochemical oxygen demand (BOD) incubator, Quimis Greenhouse Incubator brand, Q315M25 model, where the temperature was stabilized at 15 and 22°C. Every 12 h, samples were collected to carry out the analytical monitoring of the fermentation. The total time of fermentation was 120 h.

After the fermentation, the samples were kept for maturation, performed in a BOD incubator, stabilizing the temperature in 10° below the fermentation temperature (12°C for fermentation at 22 and 5°C for fermentation at 15°C), in order to clarify the beer. The total time of maturation was 360 h.

Determination of apparent extract, ethanol concentration and specific gravity

The values of specific gravity (g/mL), which correspond to relative density; apparent extract (°Plato), which is the extract measured during the fermentative process, and alcohol (% v/v) were determined through DDM 2911 bench densitometer from Rudolph Analytical Research. For transformation of the ethanol concentration of % v/v to g/L the following equation was used (EBC, 2005):

$$Ethanol\,(g/L) = Ethanol\,(\%\,v/v)\ x\ 0{,}789\ x\ \rho$$

For transformation of apparent extract content of °Plato to g / L, the following equation was used:

$$App.Extr.(g/L) = app.extr.(°P)\ x\ \rho\ x\ 10$$

Where: 0.789 g/cm^3 is the specific gravity of ethanol at 20°C and ρ is the wort specific gravity (g/mL).

Statistical analysis

The results obtained in the determinations in triplicate of soluble solid content, specific gravity, ethanol content and apparent extract of the last point of fermentation, were submitted to statistical analysis, being performed the analysis of variance by F test and comparisons between the means by the Tukey test at 5% level of probability.

Table 1. Physico-chemical analysis of the bush passion fruit pulp.

Physico-chemical analysis	Bush passion fruit
Soluble solids (°Brix), at 20ºC (TSS)	10.06 ± 0.15
pH	2.85 ± 0.11

TSS, Total soluble solids.

RESULTS AND DISCUSSION

Physico-chemical characterization of bush passion fruit pulp

The physico-chemical characteristics of bush passion fruit pulp were evaluated in order to analyze whether it is appropriate to start the procedures for the preparation of a beer using it as adjunct. The results of the physico-chemical analysis pulp are shown in Table 1.

The results obtained for physico-chemical characterization of bush passion fruit pulp revealed the potential of this fruit for the brewing industry as a malt adjunct.

In relation to the chemical characteristics of the bush passion fruit, it was observed that the average value of pH was 2.85. The determination of the pH is very important because it is necessary to verify whether the microorganism used in fermentation resists the fed substrate pH.

The total soluble solids content presented an average of 10.06 °Brix in *P. cincinnata* Mast., fruit. The amount of soluble solids is used to indicate the degree of ripeness of the fruit, the lower this degree, the pulp or juice will be more acid, and less sweet, that is, the higher the degree, the higher amount of sugars in the fruit, what in this case is a positive factor for fermentation.

The found physico-chemical characteristics demonstrated passion fruit pulp potential for industrial use as a barley malt adjunct and for *in natura* consumption; it is an already explored alternative to the juice industry due to the exotic flavor and sour sweetness.

Analytical monitoring of the fermentation process

During fermentation, samples were taken periodically every 12 h for monitoring the fermentation process. The final values of the variables are shown in Tables 2 and 3. The results obtained in the determinations in triplicate of soluble solid content, specific gravity, ethanol content and apparent extract of the last point of fermentation, were submitted to statistical analysis, being performed the analysis of variance by F test and comparisons between the means by the Tukey test at 5% level of probability. It can be seen that the samples did not show statistically significant differences in values for the mean concentration of soluble solids, specific gravity, ethanol

Table 2. Values of soluble solids, specific gravity, alcohol content and apparent extract for fermentations conducted at 15ºC.

Adjunct (%)	TSS (°Brix)	Specific gravity (g/mL)	Álcohol (% v/v)	Álcohol (g/L)	Apparent extract (g/L)
10	2.6[a]±0.01	1.0101[a]±0.02	7.47	58.94[a]±0.01	26.26[a]±0.02
29	2.3[a]±0.02	1.0090[b]±0.01	7.61	60.04[b]±0.02	24.72[b]±0.02
39	2.4[a]±0.00	1.0094[b]±0.01	7.56	59.65[a]±0.01	25.23[b]±0.03
49	2.7[a]±0.01	1.0105[a]±0.00	7.52	59.33[a]±0.03	27.28[a]±0.01

***Media followed by same letter in a column did not differ statistically among themselves by the Tukey test at 5% probability. TSS, Total soluble solids.

Table 3. Values of soluble solids, specific gravity, alcohol content and apparent extract for fermentations conducted at 22°C.

Adjunct (%)	TSS (°Brix)	Specific gravity (g/mL)	Álcohol (% v/v)	Álcohol (g/L)	Apparent extract (g/L)
10	2.0[a]±0.02	1.0078c±0.00	7.79	61.46[b]±0.00	21.87[c]±0.01
29	1.2[b]±0.03	1.0047[d]±0.01	8.29	65.41[c]±0.02	14.07[d]±0.00
39	1.3[b]±0.01	1.0050[d]±0.02	8.25	65.09[c]±0.02	14.47[d]±0.02
49	1.3[b]±0.01	1.0050[d]±0.01	8.22	64.85[c]±0.02	15.08[d]±0.03

***Media followed by same letter in a column did not differ statistically among themselves by the Tukey Test at 5% probability.

content and apparent extract, after fermentation and maturation.

Tables 2 and 3 showed the concentration of soluble solids, specific gravity, alcohol content and apparent extract to different percentages of malt adjunct and temperatures of 15 and 22°C .It can be seen that there was higher consumption of the fermentation substrate when it was used 29% of bush passion fruit pulp under the same conditions of fermentations for others adjuncts percentages. The substrate consumption can be observed through the values of content of soluble solids, which initiated the fermentation in about 12°Brix and in the end of fermentation, it was 2.3°Brix in experiments at 15°C and 1.2°Brix in the experiments at 22°C. From these values it emphasizes the value of the alcohol content, 7.61 and 8.29% (v/v) for tests at 15 and 22°C, respectively.

In 120 h of fermentation, the yeasts produced 60.04 g/L of ethanol (7.61% v/v) in the test using 29% of adjunct to 22°C, consuming 14.07 g /L (92.7%) of the initial apparent extract while that the yeasts in the test using 49% adjunct at 15°C produced 59.33 g/L (7.52% v/v) alcohol, with a consumption of 27.28 g/L (85.8%) of initial apparent extract. The results showed that the increase of amount of adjunct does not favor the increase of ethanol production during fermentation. The initial sugar concentration of worts in relation to study in question may have contributed to the high production of ethanol, since it contained an original extract of 16.5°P (12 °Brix). The increase in ethanol production with a high initial substrate concentration in the beer wort was verified by Dragone et al. (2003). Most beers produced worldwide has an alcohol content of around 3 to 6% (v/v), while a "weaker" beer contains about 2 to 3% alcohol, an "average" beer is about 5% and a "strong" beer is between 6 to 12% v/v alcohol (Sohrabvandi et al., 2011). Thus, the bush passion fruit is able to produce beers with high alcohol content.

The incomplete fermentation observed in the tests may result from lack of nutrients in the medium provided by the high concentration of adjunct. The majority of apparent initial extract may have been used by yeast to generate secondary fermentation products such as glycerol, organic acids, acetaldehyde, acetoin, butylene and others.

Reduction of specific gravity and cell growth

In the fermentations of worts with bush passion fruit pulp they were also studied the substrates reduction by decrease of the wort specific gravity and the cell concentration during fermentation. The specific gravity of the wort decreased with the production of ethanol, which has specific gravity of 0.789 g/L, by consumption of sugars such as glucose (specific gravity 1.54 g/L), fructose (specific gravity 1.69 g/L) maltose (specific gravity 1.54 g/L) and sucrose (specific gravity 1.59 g/L) for yeast and also by formation of volatile CO_2.

The graphical representations in Figures 2 to 5 showed that from the same specific gravity values, 1.0484 g/mL for all samples in the fermentations conducted at 15°C, at the end of fermentation (120 hours), there was a percent reduction of the specific gravity of test 2 (1.0090 g/mL), which was lower than of the other tests according to Figure 3 showing the in same conditions the yeast *Saccharomyces cerevisiae* Safale S-04 type *Ale* produced more ethanol with 29% of adjunct.

They can be observed the graphic representations of

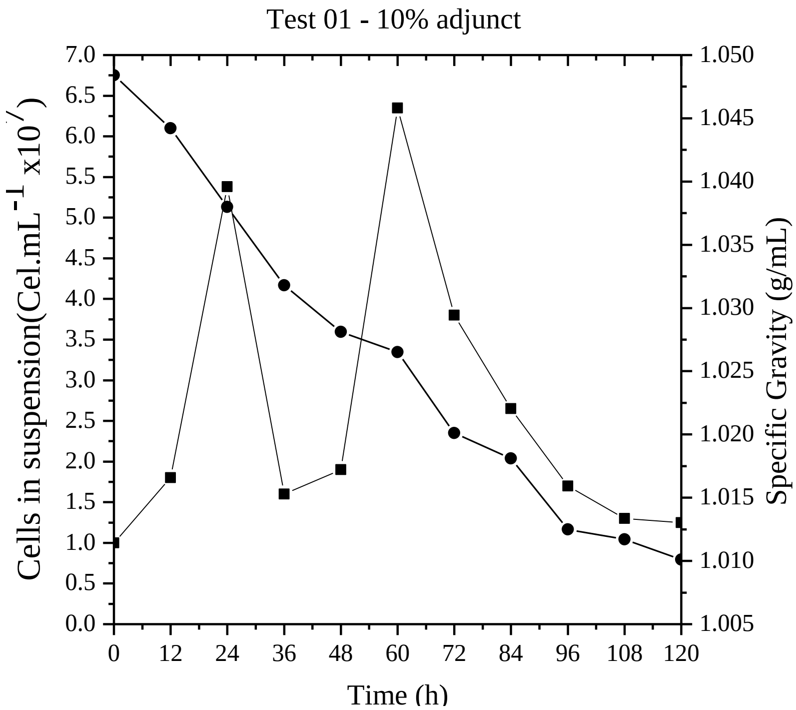

Figure 2. Graphical representation of the cell concentration in suspension (cel/mL) (▪) and specific gravity decrease (g/mL) (•) during the fermentation of the test with 10% of adjunct at 15°C.

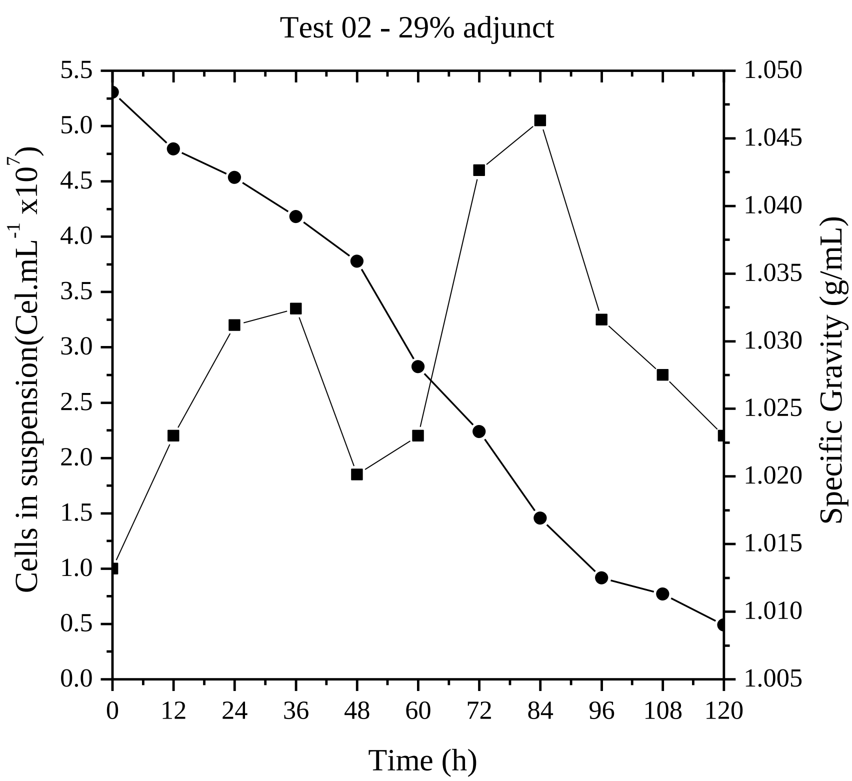

Figure 3. Graphical representation of the cell concentration in suspension (cel/mL) (▪) and specific gravity decrease (g/mL) (•) during the fermentation of the test with 29% of adjunct at 15°C.

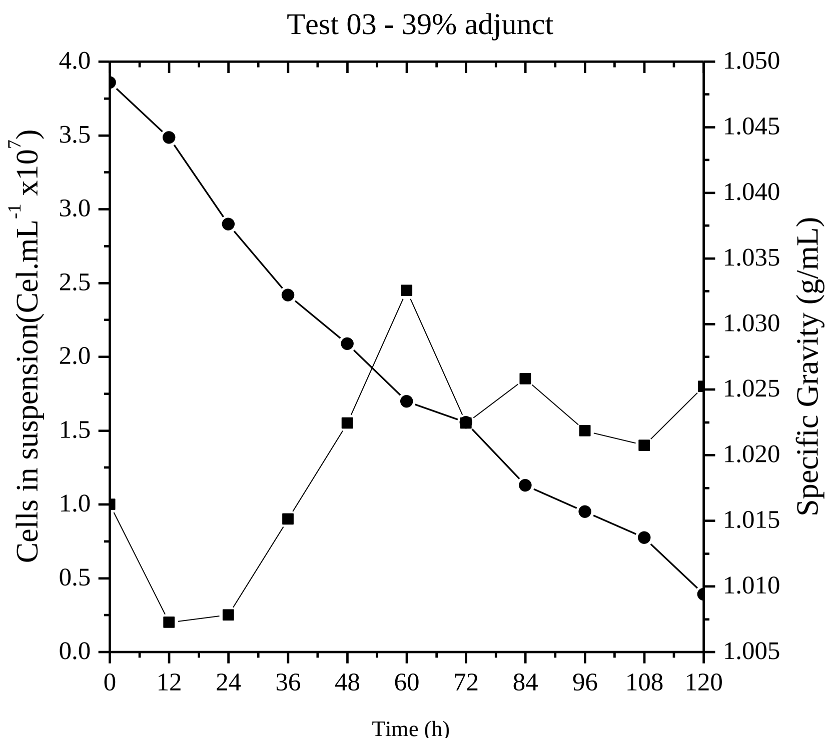

Figure 4. Graphical representation of the cell concentration in suspension (cel/mL) (▪) and specific gravity decrease (g/mL) (•) during the fermentation of the test with 39% of adjunct at 15°C.

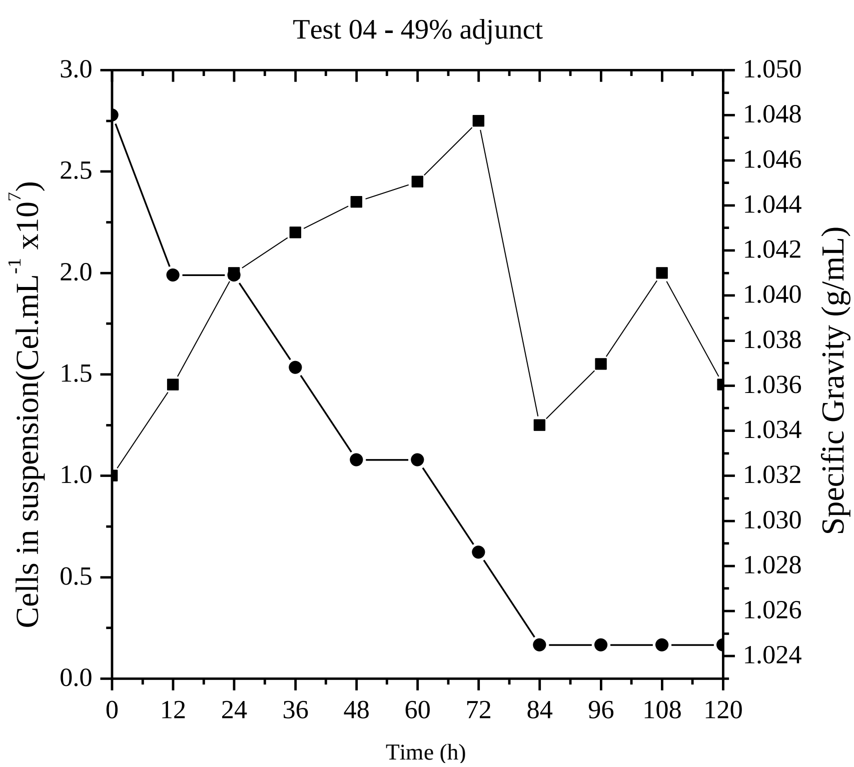

Figure 5. Representation of the cell concentration in suspension (cel/mL) (▪) and specific gravity decrease (g/mL) (•) during the fermentation of the test with 49% of adjunct at 15°C.

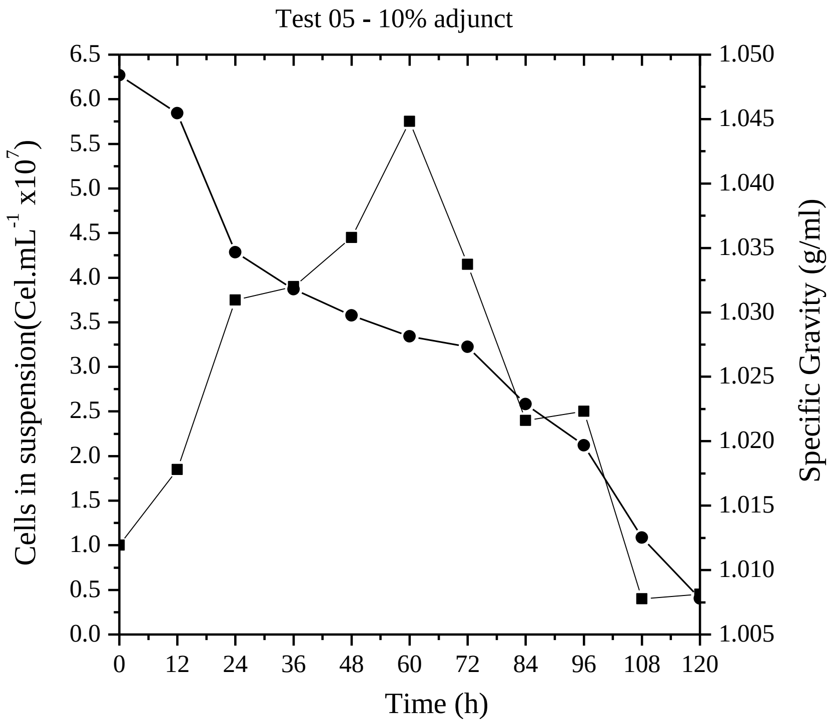

Figure 6. Graphical representation of the cell concentration in suspension (cel/mL) (▪) e specific gravity decrease (g/mL) (•) during the fermentation of the test with 10% of adjunct at 22°C.

the cell concentration obtained by cell count in Neubauer chamber for different adjuncts percentages (10, 29, 39 and 49%) and at temperatures of 15 and 22°C. In the experiments conducted at 15°C it is observed that the yeasts reached maximum cell concentration after 60 h of fermentation, obtaining 6.35×10^{7} (cells/mL) by method of cell viability in the test using 10% of bush passion fruit pulp (Figure 2). In the test using 29% pulp, it is observed that yeasts reached a maximum cell concentration after 84 h of fermentation, obtaining 5.05×10^{7} (cells/mL), as shown in Figure 3. In the test using 39% of pulp, the yeasts reached a maximum cell concentration after 60 h of fermentation, obtaining 2.45×10^{7} (cell /mL), according to Figure 4. And in the test using 49% of pulp, the yeasts reached a maximum cell concentration after 72 h fermentation, obtaining 2.75×10^{7}(cells/mL) (Figure 5). It is also possible to notice that from these times the yeasts began to enter in the impracticability phase (or the death phase).

The efficiency of fermentation and the profile of the end product quality are closely linked to the amount and health of the used yeast (Briggs et al., 2004). So, evaluating and predicting the viability of the cell, and the yeast performance during fermentation phase is an important condition (Carvalho et al., 2009).

It can be observed that during the first hours of fermentation in all tests, the number of viable cells increases and then decreases quickly. This behavior can be explained by the flocculation, a reversible process, it makes some yeast cells adhere to each other forming aggregates (Guido, 2003). According Dequim (2001), flocculation, in beer production, is important to obtain a clear product and good aroma.

In the same way of the experiments performed at 15°C, from the experiments conducted to 22°C it was observed that for fermentation with 10% of adjunct, the yeasts reached a maximum cell concentration after 60 h, obtaining 5.75×10^{7} (cel/mL) (Figure 6). In the fermentation using 29% adjunct, after 72 h, the yeasts reached a maximum cell concentration corresponded to 13.5×10^{7} (cells/mL), according to Figure 7. In the test using 39% adjunct, the yeasts reached a maximum cell concentration after 84 h, obtaining 20.0×10^{7} (cells/mL), as shown in Figure 8. In the test using 49% of adjunct, the yeasts reached a maximum concentration after 72 h of fermentation, obtaining 16.5×10^{7} (cells/mL) (Figure 9). It can also be noticed in the graphs that from these times, the yeasts begin to enter in the impracticability phase.

As well as the tests performed at15°C, the graphs in Figures 5 to 8 showed that, starting from specific gravity values equal to 1.0484 g/mL in fermentations conducted at 22°C, at the end of fermentation (120 h) there was a percentage reduction in the specific gravity of the test 6 (1.0047 g/mL) (Figure 6), which was lower than that of test 5 (1.0078 g/mL) (Figure 5), and of tests 7 and 8 (1.0050 g/mL) (Figure 7 and 8, respectively), showing

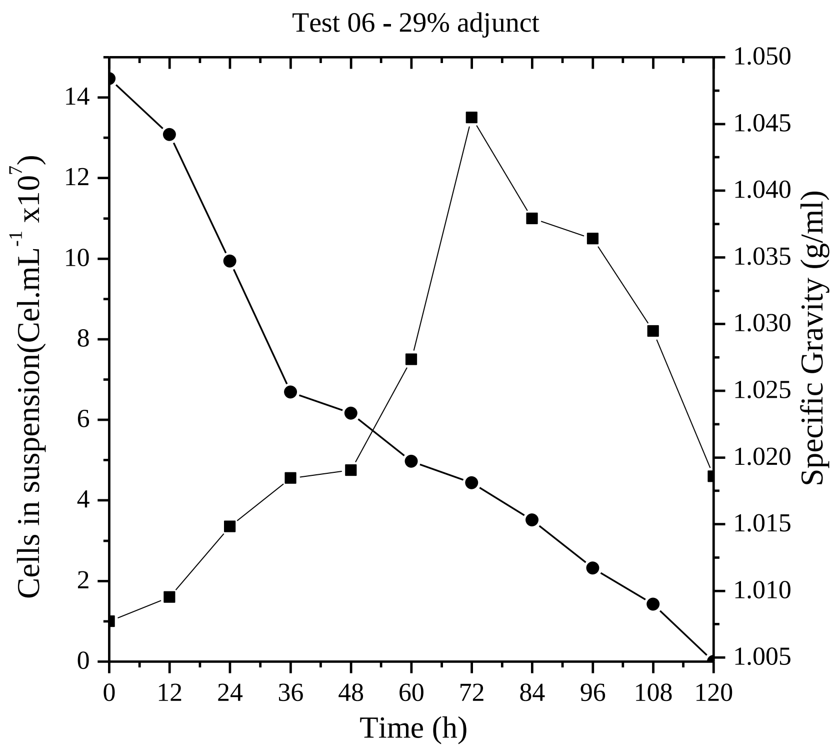

Figure 7. Graphical representation of the cell concentration in suspension (cel/mL) (▪) and specific gravity decrease (g/mL) (•) during the fermentation of the test with 29% of adjunct at 22°C.

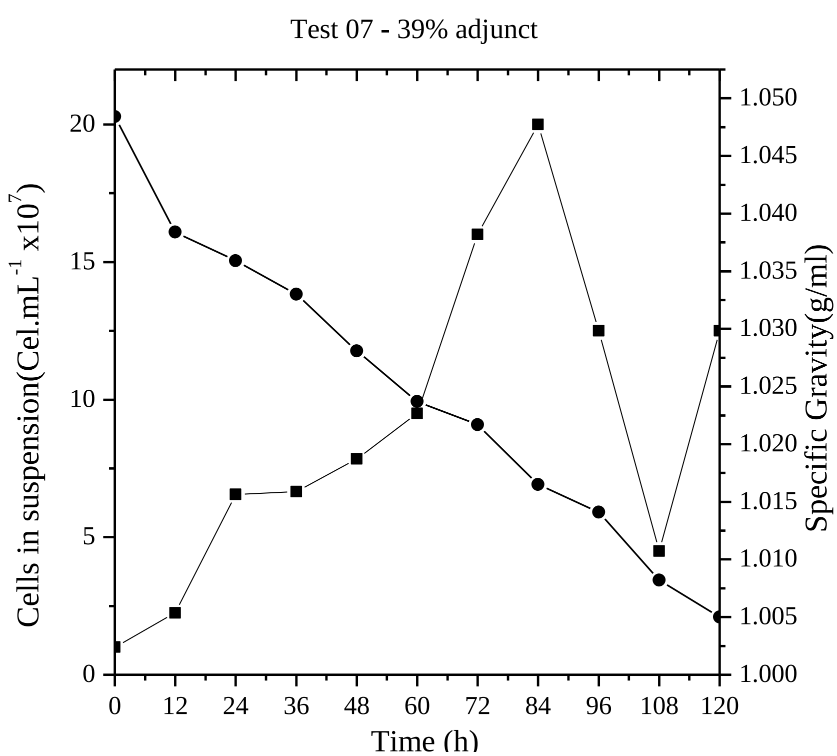

Figure 8. Graphical representation of the cell concentration in suspension (cel/mL) (▪) and specific gravity decrease (g/mL) (•) during the fermentation of the test with 39% of adjunct at 22°C.

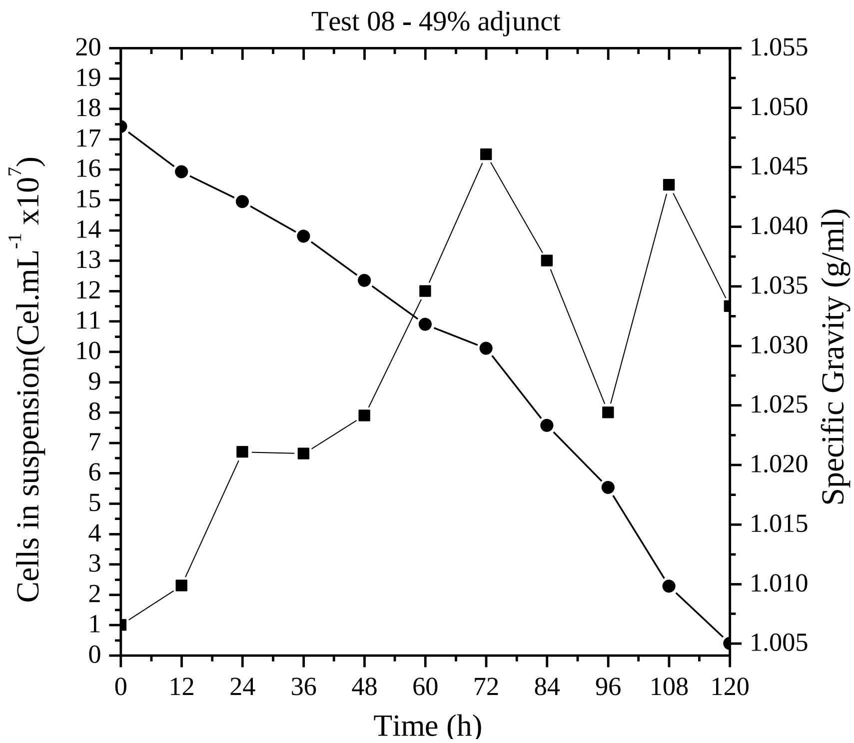

Figure 9. Graphical representation of the cell concentration in suspension (cel/mL) (▪) and specific gravity decrease (g/mL) (•) during the fermentation of the test with 49% of adjunct at 22°C.

once again that in the same conditions, the yeast *S. cerevisiae* Safale S-04 type *Ale* produced more ethanol with 29% adjunct.

This work demonstrates that the bush passion fruit has favorable characteristics that can be used in the fermentation of the beer. The increase of adjunct amount (39 and 49%) did not favor the fermentation of the wort, between different temperatures and adjunct percentages used, the wort containing 29% of bush passion fruit pulp conducted at 22°C showed better values of ethanol production (8.29% (v/v)), consuming 14.07 g/L (92.7%) of the apparent initial extract, and to have better cell growth and reduced specific gravity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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