



CIDER PRODUCTION FROM APPLE OF VARIETY EVA AND FOUR STRAINS OF ALCOHOLIC YEASTS

Luciana Trevisan Brunelli¹, Vitor Massami Imaizumi², Marcelo Henrique Breda³ & Waldemar Gastoni Venturini Filho⁴

SUMMARY: The aim of this study was to produce Eva apple ciders using four strains of alcoholic yeast and to analyze their physicochemical and sensory characteristics. The yeast used in the fermentation process were baker's yeast; top fermentation beer yeast; white wine yeast, and red wine yeast. It was produced dry cider (<20 g L⁻¹) and sweet ciders (50 g L⁻¹ and 100 g L⁻¹). The experimental design was composed by twelve treatments. The tests were conducted with two replicates. The fruits were washed, crushed and pressed to extract juice which was inoculated with four yeast strains. Fermentation process was held in PVC fermenters (20 L), at 18 °C until the limit attenuation. After this phase, beverages were bottled to 4.5 liters bottles, with removal of atmospheric air. Maturation process was held at 10 °C for 30 days. The matured beverage had its sugar content adjusted according to the treatment and then put in 660 ml glass bottles. All ciders were as chemically analyzed for alcohol content, total acidity, volatile acidity, fix acidity, turbidity, and color. Ciders were sensory evaluated by 9-point hedonic scale test. All four types of yeasts interfered with the physicochemical parameters of dry and sweet ciders. Beverages produced with white wine yeast had lower turbidity and higher total acidity. Beverages produced with white wine yeast presented higher sensory quality in all parameters, such as appearance, aroma, flavor, overall assessment. All ciders produced in this study were within the standards set by Brazilian law.

KEYWORDS: fermentation, alcoholic beverage, physicochemical analysis, sensory analysis.

PRODUÇÃO DE SIDRA A PARTIR DE MAÇÃ DA VARIEDADE EVA E QUATRO CEPAS DE LEVEDURAS ALCOÓLICAS

RESUMO: O objetivo deste trabalho foi produzir sidras a partir de maçã da variedade Eva, usando quatro cepas de levedura alcoólica, e analisar as suas características físico-químicas e sensoriais. As leveduras usadas no processo de fermentação foram a de panificação, cervejeira de alta fermentação, de vinho branco e de vinho tinto. Foram produzidas sidras seca (<20 g L⁻¹) e doces (50 g L⁻¹ e 100 g L⁻¹). O experimento contou com 12 tratamentos e 2 repetições, totalizando 24 parcelas. As maçãs foram lavadas, trituradas e prensadas para extração do suco, o qual foi inoculado com as quatro cepas de leveduras. A fermentação transcorreu em dorna de PVC (20 L), à 18 °C, até a atenuação limite. Em seguida, as sidras foram trasfegadas para garrações (5,5 L), sob atesto, e mantidas a 10 °C por 30 dias, para a sua maturação. A bebida maturada teve seu teor de açúcar ajustado de acordo com os tratamentos e então foi acondicionada em garrafas de vidro de 660 mL. As sidras foram físico-quimicamente analisadas para teor alcoólico, acidez total, acidez volátil, acidez fixa, turbidez e cor. As bebidas foram analisadas sensorialmente por teste de escala hedônica estruturada de nove pontos. As cepas de levedura interferiram na composição físico-química das sidras. As bebidas produzidas com levedura de vinho branco apresentaram menor turbidez, maior acidez total e melhor qualidade sensorial para todos os parâmetros avaliados (aparência, odor, sabor e avaliação global). Todas as sidras produzidas neste estudo estavam em concordância com as os padrões da legislação brasileira.

PALAVRAS-CHAVE: fermentação, bebida alcoólica, análise físico-química e análise sensorial

1 INTRODUCTION

The Brazilian law (BRASIL, 2009) defines cider as "alcoholic beverage of four to eight percent of alcohol by volume, at twenty degrees Celsius, obtained by the alcoholic fermentation of apple must of fresh, healthy

and mature apple, concentrated apple juice or both, with or without the addition of water" (BRASIL, 2009).

Around 90% of the Brazilian apple crop is composed by Gala and Fuji varieties. The varieties Eva, Anna and Condessa, due to low demands of cold weather, are recommended for warmer climate regions (SEBRAE, 2017).

The Eva variety, developed by the Agronomic Institute of Paraná (IAPAR), is better adapted to the weather conditions of Sao Paulo state. In terms of market

^{1 2 e 4} Faculdade de Ciências Agrônomicas – Unesp. E-mails: lutbrunelli@gmail.com ; vtr_massami@hotmail.com ; venturini@fca.unesp.br
³ Cervejaria da cuesta. E-mail: breda@cervejariadacuesta.com.br

strategy, its cultivation becomes economically interesting, in addition to the fruits presenting good characteristics, the variety shows precocity (FAGUNDES et al., 2017).

According to Fagundes et al. (2017), apple cultivation in the city of Botucatu-SP is currently declining, according to the producers, due to the great difficulty of dealing with the production technology, resulting in fruits with reduced size. Another fact is that most part of apple producers in Botucatu use an organic production system, which has a rate of fruit waste higher than the conventional system, due to the problem of consumer standard requirements.

Discarded fruits (around 30%), despite the lack of standard, can be used in several products manufacture, such as cider (SEBRAE, 2017).

The use of regional varieties of apples for cider production increases the profit of small farmer and reduces losses, once the surplus of non-commercialized fresh fruits may be used to make beverage (PAGANINI et al. 2004).

The alcoholic fermentation of apple juice corresponds to the conversion of the sugar from apple must into alcohol, performed by yeasts (LAZZAROTTO, 2012).

According to Ribéreau-Gayon (2006), grapevine yeast strains influence the amount and nature of secondary products formed from alcoholic fermentation and the aromatic characteristics of the wine.

The objective of this work was to evaluate the effect of different strains of yeast on physicochemical and sensory characteristics of ciders with different sugar concentrations.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL PLANNING

The experimental design consisted on the combination of four types of alcoholic yeast (baker's, beer, red wine and white wine) with three sugar concentrations in the final beverage ($<20 \text{ g L}^{-1}$; 50 g L^{-1} ; 100 g L^{-1}), totaling 12 treatments. The tests were performed with two replicates. The experiment was completely randomized.

2.2 PROCESSING

Fresh apples were washed with water from public network and crushed in a hammer mill Mecamau model 037. The crushed mass was pressed (100 bar) in a hydraulic press AGM, using a synthetic fabric (voil) in order to hold insoluble solids. The juice was divided into four lots and inoculated with four types of yeast. The amount of yeast used to inoculate was 3.0 g of yeast per liter of must. Before the inoculation of the batch of each treatment (14 liters), yeast was added to one liter of must and the suspension was kept in fermentation for 30 minutes. The fermentation process was performed in 20

liters PVC fermenters, in freezer with digital temperature controller, at 18 °C.

At the end of the primary fermentation, the liquid was transferred to 4.5 liter bottles using a peristaltic pump Masterflex 77800-60. The maturation stage was performed at 10 °C for 30 days. After this period, each sample was divided into three equal parts in which sugar was added in different concentrations. The beverages also received potassium metabisulfite, at an equivalent concentration of 30 mg L^{-1} of SO_2 . Finally, the beverages were bottled in champagne-type bottles and stored at 10 °C until analysis.

2.3 PHYSICOCHEMICAL ANALYSIS

The apple juice was analyzed regarding the following parameters: soluble solids content, total acidity, ratio, and pH (BRASIL, 2005). As ciders were analyzed for alcohol content, total acidity, volatile acidity, fix acidity, pH and turbidity, according to BRASIL (2005) and color, according to Tanner and Brunner (1985).

2.4 SENSORY ANALYSIS

Sensory analysis was carried out under white light, where samples were codified with randomized numbers. Cider samples (50 mL) were tasted by a panel of 50 members in glass cups at 5 ± 1 °C. The test was performed using 9-point hedonic scale test, where tasters gave scores from 1 (like extremely) to 9 (dislike extremely).

This research was registered in Plataforma Brasil, Ministério da Saúde (CAAE 22931013.8.0000.5411), and has been approved by the Committee of Ethics in Research from Faculdade de Medicina de Botucatu, number 476.653, in 02/12/2013.

2.5 STATISTICAL ANALYSIS

The results of physicochemical and sensory analyzes were submitted to analysis of variance and means were compared by the Tukey test at 5% of probability. Tests were performed using software Assistat (SILVA; AZEVEDO, 2016).

3 RESULTS AND DISCUSSION

3.1 PHYSICOCHEMICAL ANALYSIS OF APPLE JUICE

The pH (3.94) is within the range mentioned by Nogueira and Wosiacki (2016) for apple juice produced in Brazil, which comprises the values from 2.9 to 4.3.

The total acidity content (0.31%) was lower than that published by Paganini et al. (2004) for apple juice of Eva variety (0.41%). According to the English classification of apples for cider making; Total acid contents above 0.45% comprise apples called "Sharp", while lower contents comprise sweet apples "Sweet" (BEECH, 1972; LEA, 1995). The composition of apple juice is a

consequence of natural factors where orchards are located (soil, climate), agronomic factors of fruit production (cultivars, cultivation, fertilization, phytosanitary treatments, harvesting times) and technology of maintenance (JANZANTTI, FRANCO, WOSIACKI, 2003). Kempka et al. (2013) studied the clarification process in apple juices produced from Gala and Fuji cultivars. In this article, the authors obtained total acidity values of 1.09 ± 0.03 g of malic acid.100ml⁻¹ from Gala juice and 0.93 ± 0.02 g of malic acid.100ml⁻¹ from Fuji juice. The acids content in an apple is an important parameter for industrialization. Currently, 95% of apples used by industry are Gala and Fuji varieties.

The soluble solids content (13.03 °Brix) is close to those found by Rizzon et al. (2005), which analyzed juice of Gala, Golden Delicious and Fuji, obtaining means of 12.4, 12.9 and 14.0 °Brix, respectively. According to these authors, the difference in the concentration of soluble solids is not only associated with the genetic aspect, but also with other factors, mainly those related to photosynthesis, such as heat intensity, solar radiation and soil moisture. Thus, regions that present warm climate during the maturation stage, favor sugar increase (RIZZON et al., 2005).

The relationship between soluble solids content and total acidity (ratio) helps to classify and separate fruits between industrial use and market sale. Apple juices with less than 20 ratio have higher industrial aptitude due to the higher acidity content (CZELUSNIAK et al., 2003; NOGUEIRA, 2003). Ratio value of apple juice found in this research (42.45) indicates that this variety is suitable to be consumed as fruit (without processing). However, the waste from organic apple is greater than in conventional system, what favors its processing to produce beverages and other foods as an alternative to improve profits.

PH values (below 4) and soluble solids content (around 13 °Brix) make Eva apple juice suitable for alcoholic fermentation. With this concentration of solids soluble in the must, the cider is expected to have an alcoholic content between 6.5 and 7.5% v/v, according to Brazilian legislation (BRASIL, 2009).

Table 1 - Physicochemical parameters of apple juice

Parameter	Mean
pH	3.94
Soluble Solids (°Brix)	13.03
Total Acidity (g malic acid .100 mL ⁻¹)	0.31
Ratio	42.45

3.2 PHYSICO-CHEMICAL ANALYSIS OF CIDER

Ciders (<20 g L⁻¹, 50 g L⁻¹ and 100 g L⁻¹) manufactured using white wine yeast presented the highest total and fix acidity rates (Table 2). This behavior may be related to the metabolism of white wine yeasts that produce greater amounts of organic acids related to others. The values of volatile acidity, regardless cider type (dry and sweet) and yeast, were close to the concentration of 5 meq L⁻¹, demonstrating that the total acidity values are directly related to the fix acidity contents. The total and volatile acid content are below the maximum limits allowed by the legislation, 130 meq L⁻¹ and 30 meq L⁻¹ respectively. The low levels of volatile acidity indicate that beverages did not present microbiological changes (HASHIZUME, 2001).

Dry cider (<20 g L⁻¹) alcohol content was around 6.8% v/v. In this case, the alcoholic fermentation was exclusively derived from apple juice sugars. Alcoholic contents produced from all four yeast treatments matches with the soluble solids content of the raw material (apple juice) that was around 13 °Brix (Table 1). Brazilian legislation does not allow the addition of sugar during cider making (BRASIL, 2012). Sweet ciders (50 g L⁻¹ and 100 g L⁻¹), made using red wine yeast presented higher alcohol content. This behavior indicates that as cider was bottled without filtration, yeasts remained in the beverage, which probably fermented a part of the sugars added before bottling stage, increasing alcohol content., Regardless sugar concentration and type of yeast, all ciders presented alcohol content within the limit (4 to 8% v / v) established by Brazilian legislation (BRASIL, 2012).

The different types of yeast influenced color intensity. According to De Clerk (1958), yeasts influence the color intensity in beer, because during the fermentation process there is coloring matter in the foam elimination, and oxidized tannins reduction. Probably, these same phenomena occur during cider fermentation. Wiecheteck et al. (2005) observed a change in color intensity in apple must of Fuji cultivar before fermentation (0.999) and after fermentation (0.206). The color intensity found in ciders was within the values (0.101-0.552) cited by Nogueira et al. (2003) who evaluated 10 brands of Brazilian cider.

3.3 CIDER SENSORY ANALYSIS

The best ciders were fermented using white wine yeast (Table 3).This yeast produced lowest turbidity beverages.

White wine yeast was also responsible for producing best aromas. This occurred in the treatments where the sugar concentration was <20 g L⁻¹ (dry) and 100 g L⁻¹ (sweet). Aroma compounds come from four sources: raw material, fermentation, maturation and aging in wood. According to Ribéreau-Gayon (2006), grapevine yeast strains influence the quantity and nature of secondary products formed from alcoholic fermentation and the aroma character of the wine; the same might occur

during cider fermentation. As with the aroma attribute, white wine yeasts were also responsible for the production of ciders with the best flavors. This makes sense since the flavor can be defined from the interaction between drinks aromas and tastes (sweet, salty, sour, bitter and umami). In addition to the preference for cider fermented using white wine yeast, tasters preferred sweet drinks, as score increased from dry to sweet.

4 CONCLUSIONS

- Variety Eva apple must chemical characteristics were suitable for cider production;
- The four yeast types (baker's, beer, red wine and white wine) interfered on physicochemical parameters of dry ($<20 \text{ g L}^{-1}$) and sweet (50 g L^{-1} and 100 g L^{-1}) ciders;
- Dry cider showed that all yeasts similarly converted sugars into ethanol, resulting in beverages statistically equal on alcohol content;
- Cider from white wine yeast showed lower turbidity and higher total acid content;
- Cider made with white wine yeast showed superior in all attributes studied: appearance, aroma, flavor and overall evaluation, presenting better sensorial quality.
- All ciders elaborated in the present study were within the standards established by Brazilian legislation.

Table 2 - Ciders physicochemical parameter.

Yeast	Baker's	Beer	White wine	Red wine
Sugar Content		<20g L⁻¹ (dry cider)		
Total acidity (meq L ⁻¹)	64.00b	51.50c	78.25a	64.75b
Volatile acidity (meq L ⁻¹)	5.00a	5.37a	5.38a	5.00a
Fix acidity (meq L ⁻¹)	59.00b	46.13c	72.88a	59.75b
pH	3.72b	3.90a	3.66b	3.72b
Alcohol content (% v/v)	6.65a	6.85a	6.67a	6.84a
Color (A _{440 nm} +A _{520 nm})	0.217b	0.412a	0.112b	0.115b
Turbidity (NTU)	13.33a	15.00a	8.09b	12.48a
Sugar Content		50g L⁻¹ (sweet cider)		
Total acidity (meq L ⁻¹)	46.80c	46.63c	96.88a	82.00b
Volatile acidity (meq L ⁻¹)	5.75a	5.00a	5.00a	5.62a
Fix acidity (meq L ⁻¹)	41.05c	41.63c	91.88a	76.38b
pH	3.68b	3.88a	3.63b	3.73b
Alcohol content (% v/v)	6.68b	6.74b	6.72b	6.96a
Color (A _{440 nm} +A _{520 nm})	0.167ab	0.187ab	0.130b	0.238a
Turbidity (NTU)	13.95a	13.45a	8.67b	11.52a
Sugar Content		100 g L⁻¹ (sweet cider)		
Total acidity (meq L ⁻¹)	46.30b	51.63b	92.25a	83.00a
Volatile acidity (meq L ⁻¹)	5.50a	4.63a	4.65a	5.63a
Fix acidity (meq L ⁻¹)	40.80b	47.00b	87.63a	77.38a
pH	3.63b	3.89a	3.66b	3.82a
Alcohol content (% v/v)	6.48b	6.49b	6.64b	7.10a
Color (A _{440 nm} +A _{520 nm})	0.233b	0.225b	0.335a	0.210b
Turbidity (NTU)	13.75a	14.00a	7.84b	14.86a

Means followed by equal letters do not differ by the Tukey test ($p < 0.05$).

Table 3 - Scores of ciders sensory evaluation.

<20g L⁻¹				
Yeast	Baker's	Beer	White wine	Red wine
Aparency	6.60b	6.64b	7.24a	6.00b
Aroma	5.36c	6.36b	6.94a	6.22b
Flavor	5.80b	5.36b	6.76a	5.52b
Overall assessment	5.88b	5.80b	6.46a	6.16ab
50g L⁻¹				
Aparency	7.24b	7.04a	7.76a	6.48c
Aroma	6.40a	6.28a	6.40a	6.32a
Flavor	6.56b	6.32b	7.08a	6.48b
Overall assessment	6.96b	6.20c	7.24a	6.16c
100g L⁻¹				
Aparency	7.12b	7.04c	8.16a	7.54b
Aroma	6.52c	6.28c	7.56a	7.20b
Flavor	6.52c	6.32c	8.04a	7.00b
Overall assessment	6.04c	6.20c	8.20a	7.32b

Means followed by equal letters do not differ by the Tukey test ($p < 0,05$).

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