Preparation and physicochemical characterization of persimmon fermented beverage obtained by spontaneous and inoculated fermentation

Elaboração e caracterização física e química de fermentado de caqui obtido por fermentação espontânea e inoculada

Suelen da Silva GASPAR¹; Aline Manke NACHTIGALL²; Disney Ribeiro DIAS³; Brígida Monteiro VILAS BOAS⁴

¹ Mestranda em Microbiologia Agrícola, Universidade Federal de Lavras, suh_gaspar@hotmail.com
² Doutora em Ciência e Tecnologia de Alimentos, Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais – Campus Machado, aline.manke@ifsuldeminhas.edu.br
³ Doutor em Ciência dos Alimentos, Universidade Federal de Lavras, diasdr@dca.ufia.br
⁴ Autor para Correspondência - Doutora em Ciência dos Alimentos; Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais – Campus Machado, Núcleo de Alimentos, Rodovia Machado – Paraguacu km 3, Bairro Santo Antônio, Machado – MG, CEP: 37750-000; brigida.monteiro@ifsuldeminhas.edu.br

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Abstract

The aim of this work was to develop and evaluate physicochemical characteristics, their viability, and which is the most appropriate process to produce persimmon fermented beverage, obtained by three treatments, namely: spontaneous and inoculated fermentation with commercial Saccharomyces cerevisiae yeasts (Fleishmann baker yeast and CA-11). Persimmon pulp was diluted in water in a 1:1 ratio (v/v) and chaptalized to a soluble solids content of 24°Brix. Potassium metabisulfite was used as bacterial growth inhibitor and antioxidant. Bentonite was used as a fining agent to clarify the wine. All fermentation processes were conducted under the same conditions at 28°C, consisting of spontaneous fermentation and inoculated fermentation with Fleishmann yeast and CA-11. Must aliquots were collected daily to monitor cell population, soluble solids and pH. At the end of fermentation, beverages were transferred to clean containers, removing excess sediment. Afterwards, beverages were subjected to filtration and pasteurization. The following analyzes were performed: sugars (glucose, fructose and sucrose), alcohols (ethanol, methanol and glycerol), ethanol yield and total acidity. Yeast population was held adequate during the fermentation process. Fermentation processes conducted with commercial yeasts were more viable due to higher ethanol yields when compared to spontaneous fermentation. CA-11 yeast proved to be the most suitable for persimmon fermented beverage production due to lower residual reducing sugar contents.

Additional keywords: CA-11; CCMA 0200; Chemical composition; Diospyros kaki L.; yeast.

Resumo

O objetivo deste trabalho foi elaborar e avaliar as características físicas e químicas, sua viabilidade, e qual o processo mais indicado para produção de bebida fermentada de caqui obtido mediante 3 tratamentos, sendo estes: fermentação espontânea e inoculada com levedura Saccharomyces cerevisiae (fermento biológico Fleishmann e levedura CA-11). A polpa de caqui foi diluída em água na proporção 1:1 (v/v) e chaptalizada a um teor de sólidos solúveis de 24°Brix. Foi utilizado metabisulfito de potássio como agente inibidor do crescimento bacteriano e como antioxidante. Para clarificação do mosto foi realizada colagem. Todos os processos fermentativos foram conduzidos sob as mesmas condições a 28°C, sendo: fermentação espontânea e fermentação inoculada com fermento biológico e levedura CA-11. Aliquotas dos mostos foram coletadas, diariamente, para monitoramento de população de células, sólidos solúveis e pH. Ao término das fermentações, as bebidas foram transferidas para recipientes limpos, eliminando o excesso de borra. Em seguida, foram submetidas aos processos de filtração e pasteurização. As seguintes análises foram realizadas: açúcares (glicose, frutose e sacarose), álcoois (etanol, metanol e glicerol), produtividade em etanol e acidez total. A população de leveduras se manteve adequada durante o processo fermentativo. Os processos fermentativos conduzidos com fermento biológico e levedura CA-11 foram mais viáveis por proporcionar maior produtividade em etanol em relação a fermentação espontânea. A levedura CA-11 se mostrou a mais indicada para produção de bebida fermentada de caqui, devido aos menores teores de açúcar redutor residual.

Palavras-chave adicionais: CA-11; CCMA 0200; composição química; Diospyros kaki L.; levedura.
Introduction

Brazil is the world’s third largest fruit producer, with production of 43,892,946 tons (FAO, 2014). Persimmon is among fruits that are highlighted in the domestic market, as it has great importance for the fruit sector (Fachinello et al., 2011). The most produced cultivars in Brazil are ‘Rama Forte’, ‘Giombo’ and ‘Fuyu’ (Edagi & Kluge, 2009). Persimmon plants of Rama Forte cultivar are vigorous and very productive. Its fruits are medium-sized (130 g), flattened, with soft pulp, often tannic, with a quite pleasant and very consistent flavor, even after astringency (Pio et al., 2003). Persimmon tree (Diospyros kaki L.) cultivation has some limiting factors, as follows: short harvest period, due to its maturation is concentrated in a short time period; large postharvest losses due to persimmon short conservation time; improper management and postharvest techniques (Sommer, 1992); injuries by climatic or phytopathological factors (Paganini et al., 2004); and little knowledge of how excess production could be used.

Thus, adoption of techniques that reduce postharvest losses is an effective practice that may create new jobs and technologies (Dias et al., 2003). Fruit processing in beverage production is a viable technique for reducing losses, because even if the quality of a fruit was not maintained, post-processing would ensure better use of production.

Several fruits can be used to produce fermented beverages with typical characteristics, provided that must and nutrient corrections are made, and that the existing laws for this product are accomplished (Brasil, 2012). Fermentation biotechnology can be effectively used for fermented fruit preparation (Dias et al., 2010). According to Dias et al. (2010), fermented fruit nutritional composition depends on the fruit, which widely contributes to beverage characteristics, on must composition, inoculum type, fermentation conditions and post-fermentation procedures. In Brazil, studies with different fruits, such as watermelon (Fontan, 2011), pineapple (Oliveira et al., 2012), raspberry (Guimarães, 2012) and acerola (Seglowick & Brunelli, 2013) were carried out.

In technical regulations for fermented fruit identity and quality standards, proposed in Portaria N. 64 of 23 April 2008, the basic ingredients used in fermented fruit production are healthy, fresh and ripe fruit must, while sugar and water are optional ingredients. The yeast strain used for fermented fruit beverage production is a determining factor to ensure complete fermentation, as well as to improve final characteristics.

Thus, this study aimed to evaluate the different processes of persimmon fermented alcoholic beverage from spontaneous and inoculated fermentation (Fleishmann yeast and CA-11 yeast), assessing their physicochemical characteristics, their viability, and which is the most suitable production process.

Material and methods

Persimmon pulp

Persimmons, Rama Forte cultivar, were harvested from a commercial orchard. Ripe fruits were transported to an experimental orchard, where rotten fruit were discarded.

Persimmons were washed in running water with neutral detergent and were sanitized in a 200 mg L\textsuperscript{-1} sodium hypochlorite solution for 15 minutes. The pulp was extracted using an electric device. Persimmon pulps were placed in plastic bags, 500 g each, and stored in a freezer at -18°C until fermented persimmon preparation.

Statistical design

The experiment was conducted in a completely randomized design with four repetitions, consisting of 3 treatments (spontaneous fermentation, Fleishmann yeast and CA-11 yeast). The experimental plot consisted of 300 mL of must.

Fermented persimmon preparation

For must preparation, pulp was defrosted for 24 hours in the refrigerator (~ 8°C) and diluted with distilled water in the 1:1 ratio (v/v).

Persimmon pulp soluble solids content was 18.7 °Brix (Table 1), determined using a digital refractometer with automatic temperature compensation (25°C) (AOAC, 2005). Ideally, must soluble solids content should be 24°Brix to obtain a beverage with alcohol content 12% (v/v). Therefore, must chaptalization was conducted, that is, correction with sucrose. Commercial sucrose (granulated sugar) was used for solution preparation. According to Cataluña (1988), each 25 g of sucrose added to a liter volume raise the must °Brix in about two units.

<table>
<thead>
<tr>
<th>Table 1 - Sucrose use for persimmon wine chaptalization.</th>
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<tbody>
<tr>
<td>Pulp °Brix</td>
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</tr>
<tr>
<td>18.7</td>
</tr>
</tbody>
</table>

SO\textsubscript{2} was added to the must in potassium metabisulfite (K\textsubscript{2}S\textsubscript{2}O\textsubscript{5}) form. Maximum SO\textsubscript{2} concentration in wine is 350 mg L\textsuperscript{-1}, according to the Ministry of Agriculture (Brazil, 2012). Must concentration was of 200 mg of K\textsubscript{2}S\textsubscript{2}O\textsubscript{5} per liter of must. Metabisulfite mass was diluted in 200 mL must and added at once to the total volume.

Bentonite was added to the must at a 1 g L\textsuperscript{-1} concentration, according to Vogt et al. (1986). The volume of the compound to be added was calculated in relation to must total volume, and 3.6 g of bentonite were added to the total volume. The compound was
added to the must at the beginning of fermentation and remained throughout the fermentation process.

For Fleischmann, 100 g was inoculated in 250 mL of YEFG medium (Yeast Extract Glucose Agar), Himedia (Mumbai, India). For CA-11 yeast reactivation, 5 g of yeast was inoculated in 50 mL of YEFG medium. Subsequently, yeasts were incubated in B.O.D. (Biochemical Oxygen Demand) at 28°C for 24 hours. An aliquot of each inoculum was transferred to a Neubauer chamber after being diluted 3 times, and methylene blue was added for determination of viable cells in suspension, in accordance with BIO-RAD (1992). Inoculum population was of 10^7 mL^-1 for both commercial yeasts. Then, the inoculum was transferred to centrifuge tubes and taken to centrifuge for cell washing and nutrient removal from the medium. Centrifugation took place at 4,000 rpm and 22°C for 3 times, 10 minutes each. Afterwards, inocula were added to the must to start fermentation. In the case of spontaneous fermentation, no yeast was inoculated and the must was left to ferment at the same conditions of inoculated musts.

After preparation, corrections, and inoculation, Erlenmeyer flasks were incubated in a B.O.D. (Biochemical Oxygen Demand) incubator at 28°C. At each day of fermentation, a 2 mL sample was collected from each Erlenmeyer flask for cell counting, according to BIO-RAD technique (1992), soluble solids and pH determination (AOAC, 2005). The end of fermentation was determined by soluble solids content stabilization (*Brix) (Ough, 1996), after three days with the same reading. Fermentation time was calculated in hours for each treatment.

After fermentation finished, fermented solutions were taken to the refrigerator (~ 8°C) for 72 hours, in order to facilitate solid material sedimentation. After decantation, the solution underwent vacuum filtration in a Büchner flask, 1000 mL, where a Büchner funnel was attached and cellulose filter was used (Dias et al. 2003; Dias et al., 2007).

After filtration, the beverage was packaged in glass vials and subjected to pasteurization in thermostatic bath. Fermented beverage temperature was raised to 65°C for 30 minutes. Afterwards, vials were cooled and stored in the refrigerator.

Physicochemical analysis

Chromatographic analyzes were performed 1 day after the end of fermentation. Samples were removed from the refrigerator at 8°C and brought to room temperature for approximately 1 hour prior to HPLC analysis. Afterwards, samples were centrifuged under 1000 rpm at 4°C and filtered through 0.20 µm porosity ultrafilter membrane (cellulose nitrate). A 300 µL sample aliquot was used for chromatographic run, automatically injected. Glucose, fructose, sucrose, ethanol, methanol, and glycerol chromatographic analyses were conducted in a liquid chromatograph (HPLC). Values were determined using the methodology modified from Schwan et al. (2001) and Shimadzu (1998). Shimadzu liquid chromatograph, LC-10AI model (Shimadzu Corp., Japan), equipped with refractive (RID-10A model) and ultraviolet (SPD-10AI model) index detectors was used. Shimpack SCR-101Hmodel (Shimadzu, 1998) column was used. Sugars and alcohols were detected by refractive index detector and column at room temperature. In the mobile phase, perchloric acid was used at 100 mM and flow rate of 0.8 mL min^-1. Quantification was performed based on comparison with calibration curves, determined using standards. HPLC analytical standards from Sigma-Aldrich were used.

Ethanol yield (g L^-1 h^-1) was calculated by the ratio between ethanol concentration (g L^-1 h^-1) and fermentation time, in hours.

Total acid values were determined by titration using 0.1 mol L^-1 sodium hydroxide solution and phenolphthalein basic indicator, according to Adolfo Lutz Institute (1985). Results were expressed in malic acid meq L^-1.

Statistical analysis

Statistical analysis of response variables were conducted using the Sisvar software (Ferreira, 2008). Analysis of variance and mean comparison were performed through Scott-Knott’s 5% test.

Results and discussions

During the fermentation process (Figure 1), cell concentration was maintained between 10^7 and 10^8 cells mL^-1, showing good adaptation to the fermentation medium. In a kinetic study with fermented apple (Souza et al., 2011), populations affected by the CA-11 yeast at the end of the fermentation process were considered high (10^6 cells mL^-1), showing CA-11 yeast efficiency to achieve high viable cell populations in the fermentation process, which was also observed in this study. Initial value was close to 10^7 cells mL^-1, which is the optimal concentration to initiate fruit must fermentation (Dias et al., 2003).

In the fermentation period, bacteria was not observed during yeast counting in a Neubauer chamber. It is believed that must sulfitation and fining have contributed to this fact, as Saccharomyces cerevisiae strains were able to reach higher populations due to natural microbiota reduction (Wiecheteck et al., 2005; Nogueira et al., 2008; Dierings, 2008).

According to Figure 2, must sugar use occurred faster for inoculated yeasts from the beginning of fermentation until soluble solids stabilization (*Brix). Fermentation time was on average 18 ± 0.81 days for spontaneous fermentation, 9 ± 1.70 days for Fleischmann yeast fermentation, and 11 ± 0.57 days for fermentation with CA-11 yeast, taking into account soluble solids stabilization.
Cell concentration (Log cells mL$^{-1}$) values during fermentation for obtaining persimmon fermented beverages by spontaneous fermentation (Spontaneous), fermentation inoculated with Fleishmann yeast (Fleishmann), and CA-11 yeast (CA-11).

Figure 2 - Soluble solids consumption (°Brix) values during fermentation for obtaining persimmon fermented beverages by spontaneous fermentation (Spontaneous), fermentation inoculated with Fleishmann yeast (Fleishmann), and CA-11 yeast (CA-11).

The beverage produced by spontaneous fermentation showed the highest soluble solids content (14°Brix) in relation to the other ones produced by Fleishmann yeast (11°Brix) and CA-11 yeast fermentation (8°Brix) (Figure 2).

The process conducted with spontaneous fermentation had a low fermentation rate, which may be attributed to the presence of various non-Saccharomyces yeasts at the beginning of fermentation, contributing to a lower substrate consumption rate (Alves, 2009). These yeasts are characterized by low fermentative efficiency. The lowest substrate consumption rate in the spontaneous process may be related to yeast adaptive difficulties related to nutrition conditions, pH, acidity and temperature at which fermentation was conducted (Lebeau et al., 1998).

Throughout the fermentation process, a small pH decrease was observed (Figure 3), which is part of organic acids production, such as acetic, and succinic acid (Borzani et al., 1983). These pH values were similar to those of several fermented fruit. Lopes & Silva (2006) obtained a final pH of 3.5 for fermented barbary fig. Final pH values of persimmon beverages obtained by spontaneous fermentation, Fleishmann yeast, and CA-11 yeast were 3.2, 3.5 and 3.5, respectively (Figure 3).
Figure 3 - pH values during fermentation for obtaining persimmon fermented beverages by spontaneous fermentation (Spontaneous), fermentation inoculated with Fleishmann yeast (Fleishmann), and CA-11 yeast (CA-11).

Low pH of fermented fruit is an important factor to inhibit bacterial contamination (Chiarelli et al., 2005; Torres Neto et al., 2006), besides promoting yeast growth, which are microorganisms (Muniz et al., 2002) that have optimum growth in acidic pH. Final pH of fermented alcoholic beverages lies typically between 2.0 and 4.0. Values above 4.0 may cause microbiological and staining changes (Araújo et al., 2009).

In Table 2, sugar final concentrations (sucrose, glucose and fructose) are shown. In fermented persimmon, there was no significant difference between sucrose final content in processes conducted with spontaneous fermentation and CA-11 yeast.

Table 2 - Physicochemical characteristics obtained at the end of the fermentation process for obtaining persimmon fermented beverages by spontaneous fermentation (Spontaneous), fermentation inoculated with Fleishmann yeast (Fleishmann), and CA-11 yeast (CA-11).

<table>
<thead>
<tr>
<th>Physicochemical analysis</th>
<th>Fermentation processes</th>
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<tbody>
<tr>
<td></td>
<td>Spontaneous</td>
<td>Fleishmann</td>
<td>CA-11</td>
</tr>
<tr>
<td>Sucrose (g L⁻¹)</td>
<td>0.89 a</td>
<td>0.61 b</td>
<td>0.77 a</td>
</tr>
<tr>
<td>Glucose (g L⁻¹)</td>
<td>30.86 a</td>
<td>2.46 b</td>
<td>2.51 b</td>
</tr>
<tr>
<td>Fructose (g L⁻¹)</td>
<td>43.79 a</td>
<td>33.19 b</td>
<td>8.69 c</td>
</tr>
<tr>
<td>Glycerol (g L⁻¹)</td>
<td>0.63 a</td>
<td>0.43 c</td>
<td>0.58 b</td>
</tr>
<tr>
<td>Methanol (g L⁻¹)</td>
<td>0.16 c</td>
<td>0.24 a</td>
<td>0.20 b</td>
</tr>
<tr>
<td>Ethanol (%)</td>
<td>6.80 c</td>
<td>9.80 b</td>
<td>12.19 a</td>
</tr>
<tr>
<td>Ethanol yield (g L⁻¹ h⁻¹)</td>
<td>0.158 b</td>
<td>0.436 a</td>
<td>0.445 a</td>
</tr>
<tr>
<td>Total acidity (meq L⁻¹)</td>
<td>130.0 a</td>
<td>93.0 b</td>
<td>75.0 c</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the line do not differ statistically by Scott-Knott’s test at 5%

Glucose values at the end of the fermentation process did not differ statistically between the beverage fermented with Fleishmann yeast and CA-11 yeast. It was observed that, at the end of fermentation, residual glucose levels in fermentation processes conducted with Fleishmann yeast and CA-11 yeast were close to zero, indicating total substrate consumption and good wine quality (Pato, 1982). However, this observation does not apply to fructose content in the fermentative processes analyzed.

It was observed that glucose is preferentially consumed in detriment of fructose. This effect is called diauxie, in which the yeast favors one substrate over another (Schlegel et al., 1990). The process conducted with spontaneous fermentation left a larger glucose and fructose remaining content (Table 2). What probably happened is that yeasts in spontaneous fermentation were not capable of fermenting glucose and fructose quickly, resulting in lower alcohol content and higher residual sugar amount in the final beverage.

According to Walker (1998), some Saccharomyces species use fructose slower than glucose, leading to fructose accumulation at the end of fermentation. Excess fructose at the end of fermentation, mainly by fermentation with commercial yeasts, may be related to differences in fructose transport and
assimilation/oxidation mechanisms by yeast. In our experiments, it was observed that Fleishmann yeast was not a good fructose fermenter and that CA-11 yeast is a better fructose fermenter in persimmon must, as it consumed large amounts of this sugar, leaving a lower remaining sugars content and producing more ethanol in the final beverage.

There was a significant difference between the three fermented beverages in relation to final glycerol (g L⁻¹), methanol (g L⁻¹), and ethanol (%) values (Table 2). According to Balli et al. (2003), in a fermented fruit, glycerol concentration varies from 1 to 10 g L⁻¹, depending on the yeast. In relation to methanol, the Brazilian legislation for fermented fruit established that the maximum methanol concentration allowed is 0.35 g L⁻¹ (Brasil, 1988).

Values obtained for glycerol in all beverages are not within the concentration range, suggesting deviation in the metabolic pathway for the formation of this compound (Alves, 2009). At the end of fermentation, a higher glycerol concentration was observed in the beverage fermented spontaneously.

Methanol is naturally present in alcoholic beverages, but in smaller amounts in relation to other components. It is highly toxic for humans. It was observed that in all fermentation processes, values obtained for methanol are well below the maximum concentration established by legislation, which is below the concentration considered to be toxic, indicating that the methodology used for the fermentation was suitable.

There was a significant difference between the three fermented beverages in relation to ethanol content. It was observed that fermentation conducted with CA-11 yeast showed the highest final ethanol content, with alcohol content of 12.19º, followed by fermentation with Fleishmann yeast, with alcohol content of 9.8º, and spontaneous fermentation, with alcohol content of 6.8º (Table 2). It can be observed that the three beverages, obtained either by inoculated fermentation as by spontaneous fermentation, obtained values within the standards required by law, which established that the alcohol content for fermented fruit must be between 4% to 14% (v/v) at 20°C (Brasil, 2012).

Comparing the results for ethanol concentration produced during persimmon must fermentation, it was observed that fermentation conducted with Fleishmann yeast and the process conducted with CA-11 yeast showed the best fermentation performance, evidenced by higher ethanol rates. Spontaneous fermentation showed lower ethanol concentration in the last day of fermentation. Lower substrate consumption rate may be related to fermentation agent difficulties to adjust to the medium (Lebeau et al., 1998), and to lower yeast resistance to high ethanol concentrations, formed in the last days of fermentation (Alves, 2009).

It was observed that ethanol yield (Table 2) did not differ statistically between fermentations conducted with Fleishmann yeast and CA-11 yeast, which had higher ethanol yield values in a lower fermentation time compared to spontaneous fermentation. However, ethanol concentration and yield for beverages made with CA-11 yeast were the largest.

It was observed that total acidity values obtained for the three fermented persimmon beverages were statistically different from each other (Table 2), but were within the limits established by Brazilian law, which determine minimum of 50 meq L⁻¹ and maximum of 130 meq L⁻¹ (Brasil, 2012).

Oliveira (2010), while preparing a cagaita fermented alcoholic beverage using CA-11 yeast, observed a total acidity content of 55.59 meq L⁻¹, which is also within the limits specified by law (Brasil, 2012).

Alcarde et al. (2012), after comparing the chemical composition of sugarcane spirits fermented by different S. cerevisiae commercial strains, concluded that spirits obtained through fermentations with CA-11, BG-1, and CAT-1 yeasts showed chemical composition within the limits established under Brazilian law. In addition, they also found that the CA-11 strain was the one that produced spirits with the best chemical composition related to sensory quality.

Conclusions

Fermentation processes conducted with Fleishmann yeast and CA-11 yeast are more viable for higher ethanol yields compared to spontaneous fermentation. CA-11 yeast proved to be the most suitable for fermented persimmon beverage production due to higher ethanol content and lower residual reducing sugar contents.

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