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Murcott mandarin ferment beverage: technological process and control parameters

Josinaldo Florêncio da SILVA FILHO[1*], Eduardo Ramirez ASQUIERI[2], Elaine Meire de Assis Ramirez ASQUIERI[3], Aline Gomes de Moura e SILVA[2]

Abstract

This study aimed to develop an alcoholic fermented beverage from Murcott mandarin juice (*Citrus reticulata* × *Clonorchis sinensis*), focusing on its physicochemical properties and control parameters. Mandarins were selected, sanitized, and juiced, with pulp and seeds subsequently separated by sieving. Chaptalization raised the initial 11 °Brix to 28 °Brix by adding sucrose, followed by sulfitation and inoculation with *Saccharomyces cerevisiae*. Fermentation lasted 168 h, followed by 3 months of slow fermentation, including decantation and filtration before bottling. Physicochemical analyses included moisture, proteins, lipids, carbohydrates, ash content, pH, total acidity, reducing sugars, sucrose, alcohol content, and tannins. Initially, the juice had 10.46 °Brix and a pH of 3.8. During fermentation, soluble solids dropped from 25.4 °Brix to 9.2 °Brix, and reducing sugars decreased to less than 1% by the fifth day. The final product reached 13 °GL alcohol content, stabilized at pH 3.54, and had a total acidity of 135 meq/L. This study demonstrates the technological viability of Murcott mandarin as a raw material for fermented beverages, offering alternative options in beverage technology and diversifying the market.

Keywords: alcoholic fermented beverage; *Saccharomyces cerevisiae*; fermentation process; physicochemical characterization.

Practical Application: Murcott tangerine wine — technological feasibility, valorization, and market.

1 INTRODUCTION

In recent years, the fruit market has grown steadily. According to data from the Food and Agriculture Organization of the United Nations (FAO, 2024), world fruit production will increase by approximately 20.52% between 2012 and 2022, demonstrating a significant advance in agricultural activity.

Brazilian production is 41.6 million tons. In view of this, several studies have been carried out on fruit-fermented products, such as the preparation and characterization of non-grape fermented products. A notable example is the study on fermented orange (Barreto et al., 2023), jackfruit (Asquieri et al., 2008), fig (Moisescu & Antonce, 2022), açaí (Ferreira et al., 2021), passion fruit from the caatinga (Santos et al., 2021), Baru (Silva et al., 2021), blueberry (Feitosa et al., 2023), melon (Salas-Millán et al., 2022), karonda (Arora et al., 2023), pitaya (Sales et al., 2021), jaboticaba (Santos et al., 2024), banana and watermelon (Yeshaneh, 2024), among others, demonstrating the feasibility of using different tropical fruits as a substrate in the production of fermented alcoholic beverages.

The Murcott mandarin represents an opportunity for the development of new products, being a fruit with technological potential that has yet to be explored. It is rich in fiber, carotenes, and antioxidant compounds (Rico et al., 2019; Wang et al., 2019).

In view of the above, the aim of this study was to produce an alcoholic fermented drink from mandarin juice, as well as to characterize its physicochemical properties and process parameters.

1.1 Relevance of the work

This study developed an alcoholic fermented mandarin beverage that has significant scientific and technological value, as it exploits the potential of a fruit that is little used in the industry. The research contributes to the utilization of raw materials, helping to channel excess production, adding value to the mandarin production chain, and stimulating the diversification of artisanal drinks. The study also makes it possible to evaluate fermentation parameters, stability, and all product characterization, promoting innovation, sustainability, and new markets.

2 MATERIAL AND METHODS

For the preparation of the tangerine ferment, we used Murcott tangerine fruit, purchased from the Goiás Central Supply Station (CEASA-GO) and transported to the Food Chemistry and Biochemistry Research Laboratory at the Federal University of Goiás (UFG).

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[1]*Universidade Federal de Goiás, School of Agronomy, Goiânia, Goiás, Brazil.*
[2]*Universidade Federal de Goiás, Faculty of Pharmacy, Goiânia, Goiás, Brazil.*
[3]*Universidade Federal de Goiás, Faculty of Nutrition, Goiânia, Goiás, Brazil.*
*Corresponding author: josinaldo@discente.ufg.br
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2.1 Physico-chemical analysis

The physicochemical analyses of the mandarin juice and fermented juice were carried out in triplicate and included determination of moisture (AOAC 925.09), proteins (AOAC 920.152), lipids (AOAC 983.23), carbohydrates (by difference), and ash (AOAC 942.05).

2.1.1 pH

Determined using the methods described in AOAC 981.12.

2.1.2 Total acidity (fixed and volatile)

Total acidity was determined by titration with 0.1N NaOH. Total acidity was determined by titration with 0.1 N NaOH to pH 7.0, using phenolphthalein as an indicator. Volatile acidity was obtained by water vapor drag and then titration with 0.1N NaOH (Instituto Adolf Lutz [IAL], 2008) and fixed acidity, the difference between total and volatile acidity (Amerine & Ough, 1976)

2.1.3 Reducing sugars

By the 3,5-dinitrosalicylic acid (DNS) method according to Miller (1959). When DNS comes into contact with boiling water, it reacts with the sugars present in the sample, forming 3-amino-5-nitrosalicylic acid with a dark brown color, allowing it to be read using a spectrophotometer.

2.1.4 Sucrose

According to the method of Miller (1959), with modifications by Silva et al. (2003).

2.1.5 Alcoholic strength

Determined by the density of the distillate using a Gay--Lussac alcoholmeter placed directly on 250 mL of distillate at 20°C (IAL, 2008).

2.1.6 Tannins

Determined by spectrophotometry using the Folin-Denis reagent, as described by (Conecchio Filho (1972).

2.2 Technological process for making mandarin fermented fruit beverage

The fermented fruit beverage was made from 25 kg of mandarins. Following the technological processes in Figures 1 and 2, the fruit was selected by hand based on visual criteria, ensuring that it had a shiny appearance and was orange to slightly green. Damaged, unripe, or spoiled fruit was discarded. Only fruit with a diameter of between 7 and 9 cm and free from damage was selected, washed, and then sanitized in a 200 ppm chlorine solution. Equipment and utensils were also sanitized by immersion in active chlorine.

The juice extraction process consisted of opening the fruit in half, followed by complete extraction of the pulp using an

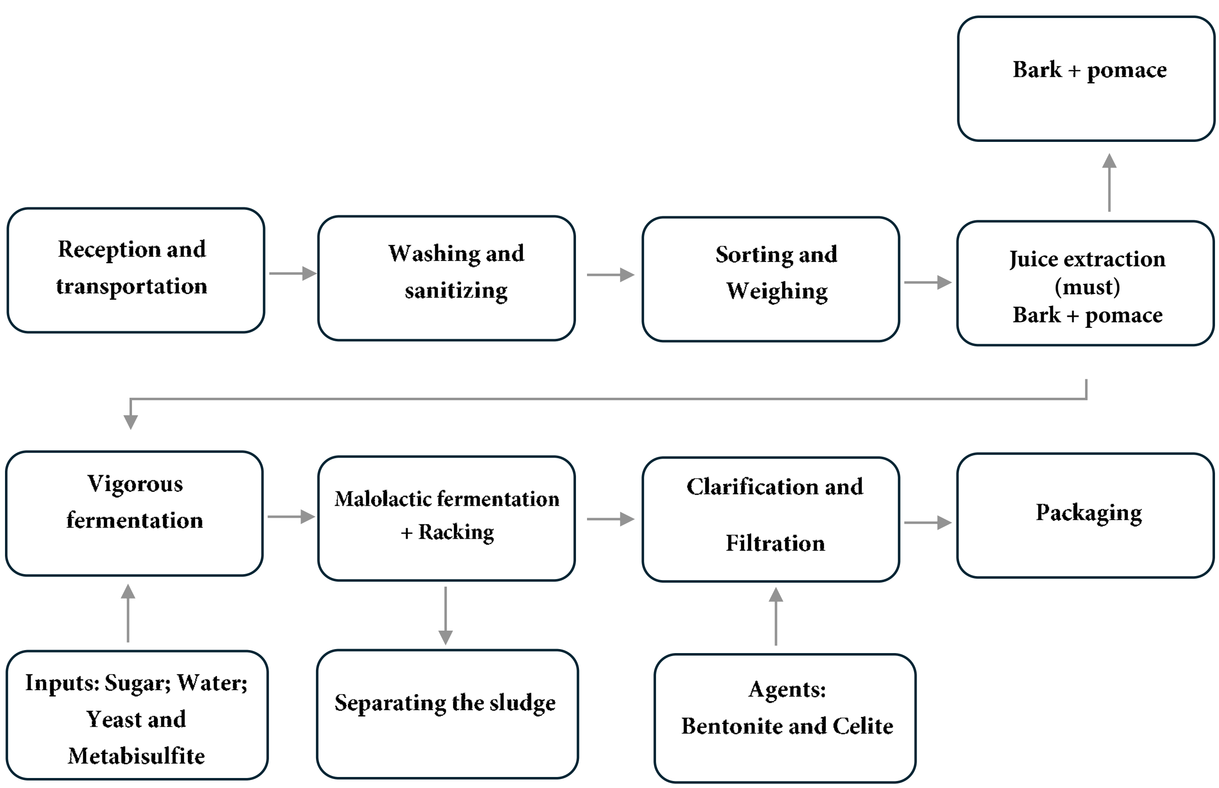

Figure 1. Flowchart of the technological process to obtain the fermented product.

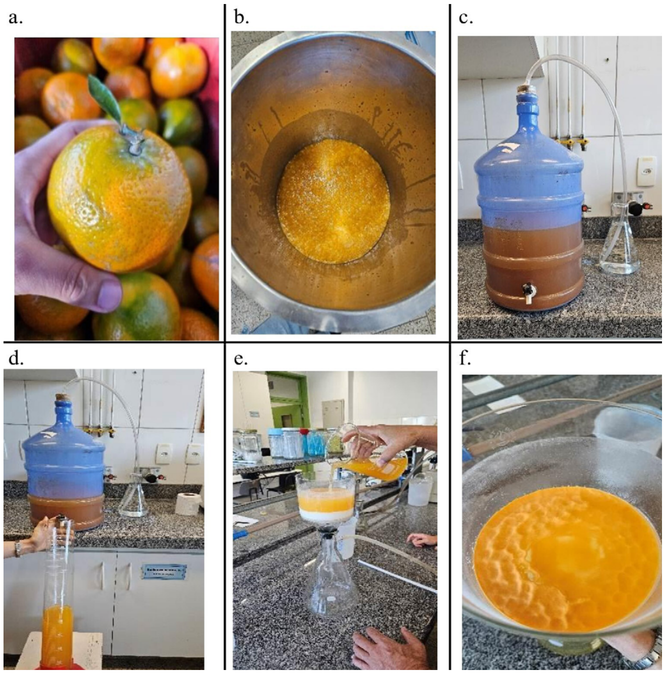

Figure 2. Processes for obtaining the fermented product. **(a)** Murcott mandarin; **(b)** Must during vigorous fermentation; **(c)** Fermentation barrel; **(d)** Decanting and transferring the fermentate; **(e)** Filtering the fermentate before bottling; and **(f)** Dregs retained after filtering.

electric orange squeezer. The liquid obtained was transferred to a graduated cylinder with the aid of a sieve in order to retain the pomace and seeds, preventing these residues from being sent along with the juice to the bioreactor.

2.2.1 Chaptalization

Chaptalization is a technique used in oenology with the aim of adjusting the sugar (sucrose) content of the must, knowing that 1.2 g of sucrose in 100 mL of must makes it possible to increase the °Brix by one degree, allowing fermentation to reach the desired alcohol content in the final drink (Asquieri et al., 2004).

Asquieri et al. (2004) stated that theoretically, yeasts convert 1% sugar into 0.57% alcohol, but this 2:1 ratio is not easily observed in practice. This is due to the difficulties yeasts face in developing in environments with a high alcohol content of 12 °GL. For this reason, a greater amount of sugar is needed to raise the alcohol level to the desired value.

The initial value of total soluble solids in the mandarin juice was 11 °Brix. In order to obtain an alcoholic fermentate with approximately 12 °GL at the end of fermentation, the soluble solids content (°Brix) was adjusted to 28 °Brix, as indicated in Equation 1. The must, with a volume of 11.8 L and an initial content of 11 °Brix, was supplemented with 2.4 kg of sucrose to achieve the desired value. It was observed that, after adding the sugar, the final soluble solids content was 28 °Brix, a value compatible with that reported by Asquieri et al. (2004).

$$g\ sucralose = \frac{12g\ sucralose\ x\ liters\ of\ must(L)}{1000ml}(for\ 1°Brix) \quad (1)$$

To correct the °Brix of the 11.8 L volume, which currently stands at 11 °Brix, 17 °Brix would be missing from the required 28 °Brix. Therefore, the grams of sucrose found in equation 1 should be multiplied by the amount of °Brix missing, which in this case would be the amount to be added to the must to reach 28 °Brix.

2.2.2 Sulfitation

To determine the amount of sodium bisulfite needed for 11.8 L of must, we used the following parameters: sodium bisulfite contains 65% of its weight in SO_2, and, as permitted by Brazilian legislation, the maximum addition of SO_2 is 350 mg per liter of must, so an initial addition of 100 mg of SO_2 per liter was made. To calculate the exact amount (Equation 2), the following operations are performed (Asquieri et al., 2004).

$$g\ the\ SO_2 = \frac{0{,}1g\ SO_2 x\ litersofmust}{1} \quad (2)$$

2.2.3 Concentration correction

As metabisulfite ($Na_2S_2O_5$) is 65% SO_2, an inverse proportion calculation of three must be used to correct the concentration (Equation 3).

$$g\ de\ SO_2\ 100\% = \frac{g\ de\ SO_2\ x\ 100}{65\%} \quad (3)$$

2.2.4 Yeast addition

The dry yeast was calculated according to the formula for the volume of must (Equation 4) (Ough, 1996).

$$1g\ dry\ yeast = \frac{mL\ of\ must\ x\ 2\ x\ 10^6\ cells/mL}{10^{10}\ cells/ml} \quad (4)$$

To calculate the active yeast (Equation 5), it was necessary to correct for the humidity of the fresh yeast using an inverse rule of three (Asquieri et al., 2004).

$$g\ of\ active\ yeast\ (fresh) = \frac{g\ dry\ yeast\ x\ 100}{32\%\ humidity} \quad (5)$$

After calculating the necessary amount of yeast to be added to the must to obtain the desired alcohol content, the fresh yeast was weighed on an analytical balance with four decimal places of precision and then diluted with the help of a small fraction of the must in a water bath (40 °C for 60 min).

2.2.5 Tumultuous fermentation

Tumultuous fermentation was monitored until the sugar values stabilized, and the effects of fermentation in the bioreactor were observed in the first 24 h (Figure 2).

2.2.6 Fermentation kinetics

Fermentation was monitored at 24-h intervals until the end of vigorous fermentation, which lasted 168 h, by analyzing total titratable acidity, temperature, and total soluble solids (°Brix), according to the methodologies described above.

The bioprocess was carried out until the concentration of total soluble solids in the must stabilized at 9 °Brix, indicating that ethanol was no longer being produced.

After vigorous fermentation ended, the must was transferred from the bioreactor to a controlled environment, where it remained in slow fermentation for another 3 months. During this period, the must was transferred, decanted, and underwent monthly sulfitation (Figure 2).

2.2.7 Decanting, racking, and filtration

The sludge accumulated during the fermentation process was discarded monthly through racking. At the end of the decanting and racking stages, before bottling, the fermented product underwent a vacuum filtration process using bentonite and filter paper, with the aim of removing solid residues and ensuring that no solid fermentation by-products were transferred to the final bottled product (Figures 3D, 3E, 3F).

2.2.8 Bottling

After the end of the slow fermentation period. The alcoholic fermentate was stored in transparent glass bottles that had previously been sterilized in boiling water. The bottles were kept in a horizontal position under refrigeration for the following months.

3 RESULTS AND DISCUSSION

The results obtained for the different physicochemical characterization and proximal composition analyses carried out on the mandarin juice are shown in Table 1. The values for lipids, proteins, and carbohydrates were lower than those found in the studies by Belo et al. (2018), El-Otmani et al. (2011) and Pacheco et al. (2017), with values of 0.3–0.9% for lipids; 4–5%

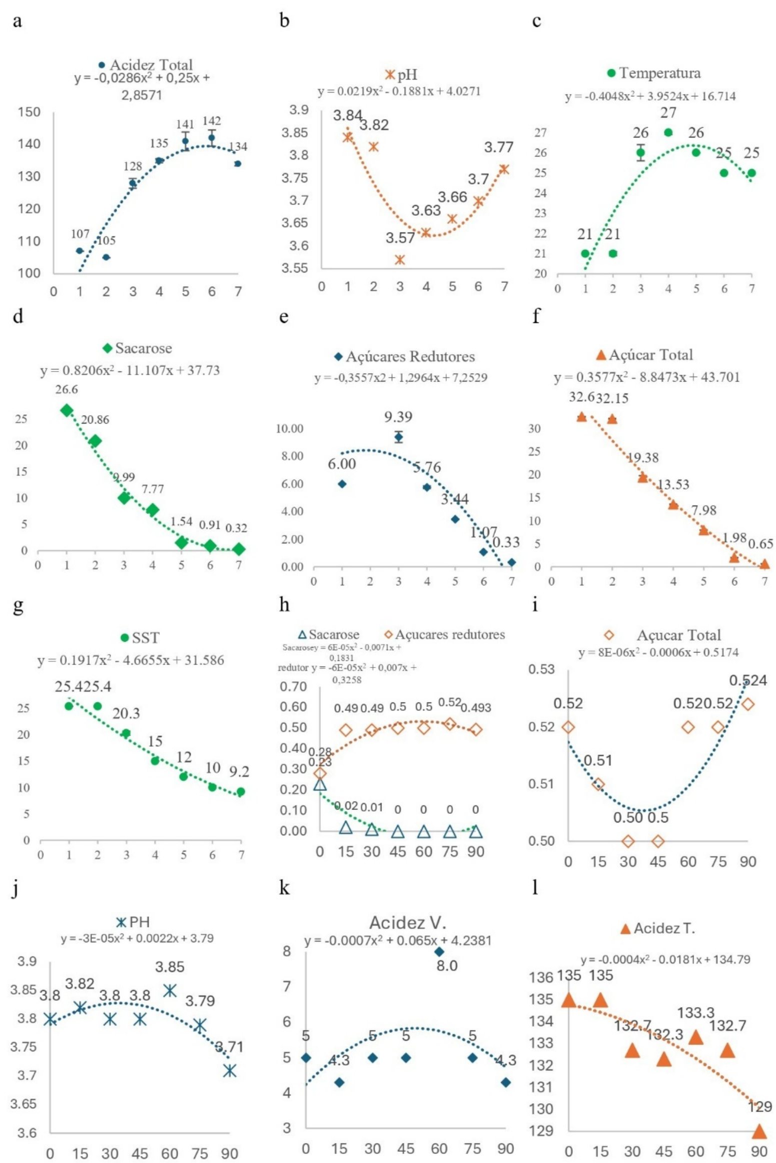

Figure 3. Physico-chemical and sugar parameters during fermentation of mandarin must: a–l: Parameters observed at different stages of fermentation, covering both vigorous and slow fermentations. Polynomial regression of fermentation parameters: **(a)** total acidity; **(b)** pH; **(c)** temperature; **(d)** sucrose content; **(e)** sugar content; **(f)** total sugar content; **(g)** total soluble solids (TSS); slow fermentation; **(h)** reducing sugar content and sucrose content; **(i)** total sugar content; **(j)** pH; **(k)** volatile acidity, and **(l)** total acidity.

Table 1. Chemical characterization of Murcott mandarin juice with standard deviation.

Characteristics	Average
Soluble solids content (°Brix)	10.46 ± 0.46
pH	3.8 ± 0.01
Total acidity (meq/L)	108.0 ± 1.10
Tannin (mg/100 mL)	0.22 ± 0.01
R. sugars (%)	5.99 ± 0.11
Sucrose (%)	4.97 ± 0.41
Total sugar (%)	10.96 ± 0.48
Moisture (%)	89.97 ± 0.03
Ash (%)	1.29 ± 0.06
Lipids (%)	0.16 ± 0.01
Proteins (%)	0.45 ± 0.02
Carbohydrates (%)	8.13 ± 0.02

for proteins, and 0.5–1.5% for ash. These small variations may be due to factors such as harvests or seasons, regional aspects, as well as the characteristics of each mandarin variety (Teixeira et al., 2017).

As shown in Figure 3, the soluble solids content was 10.46°Brix, which is within the range mentioned in the literature for fresh mandarin juice. This range corresponds to the data presented by Teixeira et al. (2017), which ranged from 9.11 to 14.33°Brix. Authors such as Alcântara et al. (2018) also confirm these values, suggesting that the fruit sold has a sweeter taste.

The pH value for the juice was 3.8. These were similar to those found in the literature by Santos et al. (2013), who in their study found pH values of 3.1–3.3 (Detoni et al., 2009). Other authors went further in their research and concluded that factors such as the incidence of sunlight have an impact on the pH, acidity, and total soluble solid parameters; the pH was found to be close to that of this study at 3.75 (Patrício et al., 2022).

The total acidity of the juice was 108 meq/L, slightly higher than the results obtained by Couto and Canniatti-Brazaca (2010), who in their study found values of 80 for acidity, and Detoni et al., (2009), who in turn found values of 0.57–0.78 meq/L. These values for acidity in the Murcott mandarin are attributed to its stage of ripeness. During the ripening process in climacteric fruit, the citric acid content in the fruit tends to decrease.

Sucrose is the predominant sugar in citrus fruits and is found in abundance. In citrus juices such as mandarin, sugars generally make up around 80% of the total soluble solids (Darjazi et al., 2020). The results for total sugars are in line with the values found for the soluble solids content; however, there was a higher concentration of reducing sugars present in this juice, with 5.99 and 4.97% for the sucrose values, these results coinciding with the 4.42–7.94% found by the authors Darjazi et al. (2020). In addition, other factors such as stage of ripeness, acidity, and seasons contribute to oscillations in these values.

The result found of 0.22% for the tannin content is due to the fact that tannins are phenolic compounds that are not very present in citrus fruit juices such as mandarin, which makes it difficult to quantify and compare with other studies, being a compound commonly found in grape stems, which play a protective role when in high concentrations in fruits, leaves, seeds, or stems (Monteiro et al., 2005).

Vigorous fermentation of the mandarin must peaked in the first 24 h and continued for the next 6 days. During the fermentation process, ethanol is continuously generated as sugar is consumed. All the parameters were monitored for a total of 7 days (Figure 3), ensuring a detailed understanding of the process.

It can be seen that the parameters for pH, total acidity, and temperature had no significant difference in the first 24 h, respectively. The pH after the first 24 h showed a significant difference ($p < .05$) each day, with a sharp decline from 3.84 to 3.57 with the start of fermentation and stabilizing at a pH of 3.77. Following similar routes for all the profiles evaluated, it was possible to see that the desired biochemical process occurred, i.e., alcoholic fermentation, and not another route, such as the production of acetic acid. In addition, it can be seen that the small fluctuations in pH resulted in an increase in total acidity in the drink compared to the start of fermentation.

The acidity and pH varied during vigorous fermentation, starting at 107 meq/L and 3.84, respectively, and ending tumultuous fermentation with values of 135 meq/L and 3.8. During the fermentation process, pH and total acidity show opposite behavior: while pH decreases, total acidity increases. This is due to the production of organic acids by fermentable microorganisms, which increases the concentration of hydrogen ions, reducing the pH of the medium (Barbosa, 2014).

The temperature of the must at the start of fermentation remained stable at 21 °C. From the second day onward, along with the reduction in total sugars and total soluble solids, a daily increase in temperature was observed in the bioreactor, with an increase of 5 °C on the second day with a significant difference of 5% each day; after the third day, the temperature of the must tended to decrease, varying between 21 and 27 °C. During this fermentation process, this temperature range allowed for complete fermentation, since yeasts are mesophilic and operate up to 33 °C (Aquarone et al., 2001).

During the tumultuous fermentation, the values of reducing sugar and sucrose varied throughout the process. At the start of fermentation, sucrose levels were higher than reducing sugar levels, at 26.6% sucrose and 6.01% reducing sugar.

The total soluble solids content varied from 25.4 °Brix at the start to 9.2 °Brix at the end of fermentation. During the first 24 h there was no variation in the values, but during the same period, there was a decrease in the sucrose content, which was hydrolyzed into reducing sugars. After the adaptation phase (lag), the soluble solids values began to decrease on a daily basis, indicative of tumultuous fermentation.

In the first 24 h of fermentation, there was a reduction in sucrose levels, while total sugars remained unchanged. This is due to the hydrolysis of sucrose into reducing sugars during the fermentation process. On the second day, there was a marked reduction in sucrose in relation to reducing sugars, and on the fifth day of fermentation, sucrose and reducing sugar values were below 1%, signaling the end of alcoholic fermentation.

From the second day onward, there was a marked reduction in sucrose compared to reducing sugars. On the fifth day of fermentation, both sucrose and reducing sugar levels were below 1%, indicating the end of alcoholic fermentation.

During the 90 days of fermentation, it was possible to observe that all the parameters underwent little variation, including pH values, which remained between 3.80 and 3.71. According to Balmaseda et al. (2024), malolactic fermentation is a desirable transformation to take place in fermented beverages with high acidity, consisting of the process in which malolactic bacteria catalyze the decarboxylation of malic acid (dicarboxylic) into lactic acid (monocarboxylic), releasing CO_2 through the action of these bacteria, which contribute to reducing acidity and increasing pH (Zhang et al., 2024).

The pH and temperature parameters for the fermented products are in line with the guidelines of Betteridge et al. (2015), who suggest a pH between 4.8 and 5.5 and a temperature close to 25 °C. However, the alcohol content of 13 °GL exceeded the 5% limit recommended by the same authors for optimal development of the desired cultures.

According to Asquieri et al. (2004), pH is a crucial factor in the quality of fermented beverages, directly impacting taste, stability, and resistance to unwanted microorganisms. In this study, the mandarin juice had an initial pH of 3.8, while the fermented product reached a pH of 3.54.

Although legislation does not stipulate an exact pH for fermented fruit beverage products, several authors, such as Asquieri et al. (2004) and Aquarone et al. (2001), have reported that a pH of 3.3–4.0 allows for a greater sensation of freshness and good storage stability. In addition, a more acidic pH helps preserve the product for longer periods, maintaining its sensory characteristics over time.

4 CONCLUSIONS

This study achieved its objectives by demonstrating the technological viability of the Murcott mandarin as a raw material for the production of a fermented alcoholic beverage, characterizing it using physicochemical parameters. The research confirms the little-exploited potential of this fruit, offering an important alternative for the development of new products with added value.

Fermentation with *Saccharomyces cerevisiae* proved efficient, culminating in a final alcohol content of 13 °GL (v/v). The pH of the product was stabilized at 3.54, which is within the ideal range to guarantee both the stability and freshness of the drink.

Sucrose hydrolysis and sugar conversion were effective, with reducing sugars reduced to less than 1% on the fifth day of fermentation, signaling complete alcohol production. Total acidity reached 129 meq/L at the end of fermentation, which contributes to the sensory quality and stability of the product.

The rigorous conduct of the process, including decanting and vacuum filtration steps, ensured a clear, stable product of good quality in appearance, taste, and aroma. Furthermore, the use of the Murcott mandarin not only adds value to the production chain, but also represents an effective strategy for reducing waste in agricultural production.

This study opens up new perspectives for the use of tropical fruits in biotechnological processes, stimulating innovation and diversification in the fermented alcoholic beverage market. The results obtained not only fill gaps in knowledge about the fermentative potential of Murcott mandarin, but also present possibilities for application in new products and investments in the area.

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