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Physicochemical and colorimetric characterization of cryoconcentrated grape juices and their impact on *Saccharomyces cerevisiae* fermentation

Caracterização físico-química e colorimétrica de sucos de uva crioconcentrados e seu impacto seu impacto na fermentação de *Saccharomyces cerevisiae*

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Highlights

Cryoconcentration increased total soluble solids content by 24.14 °Brix.
High sugar and alcohol contents impaired yeast growth and fermentation performance.
Cryoconcentrated fermented beverages exhibited higher total organic acid contents.
Cryoconcentrated fermented beverages exhibited higher total phenolic compound levels.

Abstract

Cryoconcentration increases sugar concentration in grape juice, influencing alcoholic fermentation. Thus, physicochemical and colorimetric parameters of cryoconcentrated grape juices and their impact on alcoholic fermentation were evaluated. Five treatments, each with a volume of 1.5 L, were prepared. Treatments received 0.05 g L^{-1} potassium metabisulfite and 0.60 g L^{-1} *Saccharomyces cerevisiae.*

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Subsequently, treatments were incubated at 25.0 ± 2.0 °C and remained under these conditions until alcoholic fermentation was completed after 10 days (treatment 1), 18 days (treatments 2, 3, and 5), and 26 days (treatment 4). Alcoholic degree and residual reducing sugar levels were 10.56 ± 0.01% and 4.22 ± 0.42 g L^{-1} (treatment 1), 14.58 ± 0.03% and 34.53 ± 0.20 g L^{-1} (treatment 2), 14.91 ± 0.03% and 35.88 ± 0.56 g L^{-1} (treatment 3), 10.37 ± 0.03% and 76.03 ± 1.55 g L^{-1} (treatment 4), and 5.26 ± 0.02% and 208.50 ± 5.57 g L^{-1} (treatment 5), respectively. Total polyphenol content and total titratable acidity increased with higher volumes of cryoconcentrate in treatments. However, pH, volatile acidity, and phenolic profile did not show the same behavior. Cryoconcentration did not affect blue color or hue, but increased color intensity while reducing luminosity and red coloration. High concentrations of total soluble solids reduced the fermentative capacity of *Saccharomyces cerevisiae* in treatments 2, 3, 4, and 5, delaying fermentation and resulting in high residual sugar levels.
Key words: Grape. Yeast. Cryoconcentration. Alcoholic fermentation. Freeze concentration.

Resumo

A crioconcentração aumenta a concentração de açúcares no suco de uva, influenciando a fermentação alcoólica. Assim, foram avaliados os parâmetros físico-químicos e colorimétricos dos sucos de uva crioconcentrados e o seu impacto na fermentação alcóolica. Em seguida, foram preparados cinco tratamentos, com volume de 1,5 L cada. Os tratamentos receberam 0,05 g L^{-1} de metabissulfito de potássio e 0,60 g L^{-1} de *Saccharomyces cerevisiae.* Posteriormente, os tratamentos foram incubados a 25,0 ± 2,0 ºC em câmara até o término da fermentação alcoólica, por 10 (tratamento 1), 18 (tratamentos 2, 3 e 5) e 26 dias (tratamento 4). O grau alcoólico e o teor de açúcares redutores residuais foram, respectivamente, de 10,56 ± 0,01% e 4,22 ± 0,42 g L^{-1} (tratamento 1), 14,58 ± 0,03% e 34,53 ± 0,20 g L^{-1} (tratamento 2), 14,91 ± 0,03% e 35,88 ± 0,56 g L^{-1} (tratamento 3), 10,37 ± 0,03% e 76,03 ± 1,55 g L^{-1} (tratamento 4) e 5,26 ± 0,02% e 208,50 ± 5,57 g L^{-1} (tratamento 5). O teor de polifenóis totais e os níveis de acidez titulável aumentaram com a presença do maior volume de crioconcentrado nos tratamentos. O pH, a acidez volátil e o perfil fenólico não apresentaram o mesmo comportamento. A crioconcentração não alterou a cor azul e a tonalidade, mas aumentou a intensidade da cor, com redução da luminosidade e da cor vermelha. As altas concentrações de sólidos solúveis totais reduziram a capacidade fermentativa da *Saccharomyces cerevisiae* nos tratamentos 2, 3, 4 e 5, reduzindo o tempo de fermentação e deixando teores residuais de açúcares elevados.
Palavras-chave: Uva. Levedura. Crioconcentração. Fermentação alcóolica. Concentração por congelamento.

Introduction

Concentration stands as a traditional method of food preservation, reducing storage and logistics expenses while extending product shelf life. Cryoconcentration safeguards volatile and thermolabile compounds, preserves nutrients and organoleptic properties, and mitigates microbial and enzymatic degradation (Belén et al., 2012; Ribeiro et al., 2017; Miyawaki & Inakuma, 2021a). Although the degree of concentration achieved by this process is lower than that obtained by evaporation, it is higher than that achieved using membrane-based methods (Moreno et al., 2014a; Amran et al., 2016; Petzold et al., 2016).

Block cryoconcentration occurs in three stages: liquid freezing, simple gravitational thawing, and separation of cryoconcentrated and cryodiluted fractions (Aider & Halleux, 2009; Casas-Forero et al., 2021; Haas et al., 2022). Water immobilization in grape juice during ice nucleation segregates solutes, which accumulate at the solid-liquid interface, generating a system of capillaries between ice crystals occupied by a more concentrated liquid (Petzold et al., 2013; Vuist et al., 2021). Safiei et al. (2017) reported that retention of solids in the ice crystal lattice is maximized by greater proximity to the solid-liquid interface. According to Petzold et al. (2013), increased solute mass transfer combined with a low ice growth rate ensures greater efficiency in cryoconcentrate elution.

Concentrated products of superior quality, with darker color and higher contents of total soluble solids, total phenolic compounds, and vitamin C, were obtained through vacuum-assisted block cryoconcentration (Orellana-Palma et al., 2017, 2018). Safiei et al. (2017) reported that progressive freeze concentration represents a promising alternative for producing grape juice with higher levels of total phenolic compounds and other bioactive compounds. Adorno et al. (2017) reported higher levels of antioxidant activity, phenolic compounds, and total anthocyanins in cryoconcentrated strawberry products.

Brazil was estimated to produce and consume 3.6 and 4.0 million hectoliters of wine, respectively, in 2023 (International Organisation of Vine and Wine [OIV], 2024). Studies involving novel wine processing techniques that maintain quality, increase bioactive compounds, and enhance commercial value are of interest to the wine industry. Therefore, this study aimed to evaluate physicochemical and colorimetric characteristics of cryoconcentrated grape juices and their effects on alcoholic fermentation.

Materials and Methods

Chemicals

Sodium potassium tartrate tetrahydrate, copper sulfate pentahydrate, sodium thiosulfate, and sulfuric acid were purchased from Merck (Darmstadt, Germany). Potassium iodide, sodium hydroxide, gallic acid, sodium carbonate, and Folin–Ciocalteu phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Soluble starch was purchased from Contemporary Chemical Dynamics LTDA (Indaiatuba, SP, Brazil). Purified water was obtained using a Milli-Q® system (Millipore, Bedford, MA, USA).

Application of cryoconcentration and development of alternative and innovative beverages

For this study, fourteen liters of experimental grape juice (produced using a blend of *Vitis labrusca* varieties) from the 2021 harvest were used. The experiment was conducted in triplicate with volumes of 4.67 ± 0.06 L. Juice samples were subjected to block cryoconcentration. In the first step, after freezing (255 K/48 h) juice samples in a horizontal freezer (model CHB53, Consul,

Brazil) and thawing (279 K/12.2 h) in a refrigerator (model DC41, Electrolux, Brazil), 2.33 ± 0.03 L of each cryoconcentrated sample (C1) and the same volume of cryodiluted samples (D1) were obtained. In the second step, after freezing (255 K/72 h) C1 samples in a horizontal freezer (model CHB53, Consul, Brazil) and thawing (279 K/5.3 h) in a refrigerator (model DC41, Electrolux, Brazil), 1.12 ± 0.01 L of each cryoconcentrated sample (C2) and the same volume of cryodiluted samples (D2) were obtained. Subsequently, five treatments were formulated, each with a volume of 1.5 L.

Treatment 1 consisted of 100.00% grape juice, treatment 2 of 36.15% grape juice and 63.85% cryoconcentrate 1, treatment 3 of 64.42% grape juice and 35.58% cryoconcentrate 2, treatment 4 of 100.00% cryoconcentrate 1, and treatment 5 of 100.00% cryoconcentrate 2 (Figure 1).

Treatments 2 and 3 were prepared using the equation below to achieve approximately 27.0 °Brix.

$$TSS_f \; x \; V_f = (TSS_{gj} \; x \; V_{gj}) + (TSS_c \; x \; V_c) \quad \text{(Equation 1)}$$

where: TSS_f = total soluble solids of the formulation (°Brix); V_f = formulation volume (L); TSS_{gj} = total soluble solids of grape juice (ºBrix); V_{gj} = grape juice volume (L); TSS_c = total soluble solids of the cryoconcentrate (°Brix); V_c = cryoconcentrate volume (L).

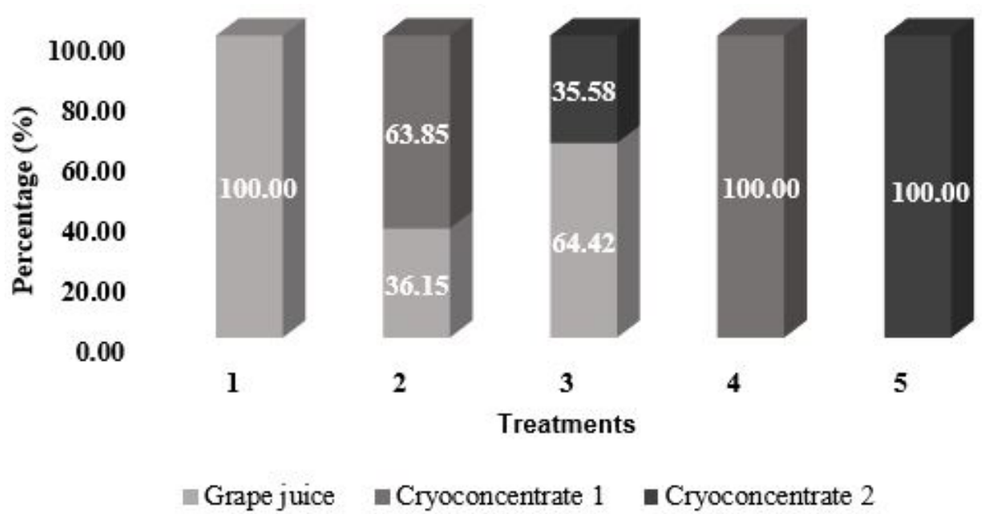


Figure 1. Treatments formulated with different proportions of initial juice, cryoconcentrate 1, and cryoconcentrate 2.

Microvinification

After grape juice enrichment, 0.05 g L^{-1} of potassium metabisulfite was added to treatments. After four hours, approximately 0.60 g L^{-1} of *Saccharomyces cerevisiae* La Claire C58 (DANSTAR FERMENT A.G., Denmark/PERDOMINI-IOC SPA, Italy [exporter]), previously activated according to the manufacturer's instructions, was added to each glass tank, which was then placed in a climate-controlled room at 25.0 ± 2.0 °C for alcoholic fermentation. After the alcoholic fermentation period (approximately 8 days), treatments were transferred to a cold chamber at 0.0 ± 2.0 °C for 30 days for tartaric stabilization. Wines did not undergo malolactic fermentation. After the stabilization period, wines were bottled in 0.75 L bottles and subjected to the appropriate laboratory analyses.

Classical enological parameters

According to methods described in the Compendium of International Methods of Wine and Must Analysis of the International Organization of Vine and Wine (OIV, 2022), relative density (OIV-MA-AS2-01) and total soluble solids content (OIV-MA-AS2-01A/2021) were determined using a concentration densimeter (Anton Paar DMA 4500M, Austria); pH (OIV-MA-AS313-15) was measured using a pH meter (HI 3221, Hanna Instruments, Romania); titratable acidity (OIV-MA-AS313-01) was determined by titration with 0.1 N NaOH; total reducing sugar content (OIV-MA-AS311-01A) was determined by hot alkaline reduction of copper sulfate; and chromatic characteristics were determined according to CIELab methodology (OIV-MA-AS2-11:R2006). Alcohol content (OIV-MA-AS312-01) and volatile acidity (OIV-MA-AS313-02) were analyzed using a digital enological distiller (SUPER DEE model, Gibertini, Italy) and determined using a densimeter (Anton Paar DMA 4500M, Austria) and titration with 0.1 N NaOH, respectively.

Concentration factor and efficiency

According to Aider and Ounis (2012), the concentration factor (CF) for each concentration step was calculated using the following equation:

$$CF = 100 \times \frac{CC_c \text{ ou } CC_d}{CC_i} \quad \text{(Equation 2)}$$

where: CC_c is the compound content of the cryoconcentrate, CC_d is the compound content of the cryodilute (ice), and CC_i is the compound content of the initial sample.

To evaluate cryoconcentration efficiency (E) at each stage, the methodology proposed by Belén et al. (2012) was used according to the following equation:

$$E\ (\%) = 100 \text{ x } \frac{(CF_c - CF_d)}{CF_c} \quad \text{(Equation 3)}$$

where: CF_c is the cryoconcentrated concentration factor; CF_d is the cryodiluted concentration factor.

Cryoconcentration validation

According to Petzold et al. (2015), mass was balanced and compared with a theoretically predicted value (Equation 4) to validate the experimental results obtained.

$$W_p = \frac{C_c - C_0}{C_c - C_i} \quad \text{(Equation 4)}$$

where: C_0 is the total soluble solids content of the initial sample; C_c is the total soluble solids content in the cryoconcentrated fractions, and C_i is the total soluble solids content in ice fractions.

According to Marafon et al. (2024), experimental W (W_e) is defined as the ratio between the initial mass of solids (m_0; in kg) and the mass of solids in ice (m_i) (kg).

$$W_e = \frac{m_0}{m_i} \quad \text{(Equation 5)}$$

Root mean square (RMS) was used to assess the goodness of fit between predicted and experimental data across N experimental repetitions for each cryoconcentration stage.

$$RMS\ (\%) = 100\ x \sqrt{\frac{\frac{(W_e - W_p)^2}{W_e}}{N}} \quad \text{(Equation 6)}$$

Estimated alcoholic potential

The alcoholic potential of fermented grape juice was estimated through calculations.

$$T_{TSS} = \frac{T_{treatments}}{100} \quad \text{(Equation 7)}$$

where: $T_{treatments}$ corresponds to the percentage value of total soluble solids content (º Brix) of treatments; T_{TSS} is the mass titer of total soluble solids content (g 100 g^{-1}).

$$M_{glucose} = 1000 \times \frac{T_{TSS}\ x\ d_{tsu}}{MM_{glucose}} \quad \text{(Equation 8)}$$

where: $M_{glucose}$ is the molarity of glucose (mol L^{-1}); T_{TSS} is the mass titer of total soluble solids content (g 100 g^{-1}); d_{tsu} is the density of grape juice treatment (g cm^{-3}); and $MM_{glucose}$ is the molar mass of glucose (g mol^{-1}).

According to Cardoso (2019), the Gay-Lussac equation determines the stoichiometric conversion of glucose into ethanol and carbon dioxide during alcoholic fermentation.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$
$$M_{ethanol} = 2 \times MM_{glucose} \quad \text{(Equation 9)}$$

where: $MM_{ethanol}$ is the molar mass of ethanol (g mol^{-1}), and $MM_{glucose}$ is the molar mass of glucose.

$$T_{ethanol} = \frac{M_{ethanol}\ x\ MM_{ethanol}}{1000 \times d_{tsuf}} \quad \text{(Equation 10)}$$

where: $T_{ethanol}$ is the volume fraction of ethanol content (mL 100mL^{-1}); $M_{ethanol}$ is the molarity of ethanol (mol L^{-1}); $MM_{ethanol}$ is the molar mass of ethanol (g mol^{-1}); and d_{tsuf} is the density of fermented grape juice treatment (g cm^{-3}).

$$PA_{ethanol} = 100 \times T_{ethanol} \quad \text{(Equation 11)}$$

where: $PA_{ethanol}$ is the alcoholic potential (%); and $T_{ethanol}$ is the volume fraction of ethanol content (mL 100mL^{-1}).

Total metabolized reducing sugar content

Metabolized reducing sugar content was obtained from the difference between reducing sugar content of treatments before and after alcoholic fermentation (Equation 9). Results were expressed as g of total reducing sugars per liter of sample (g L^{-1}).

$$TMRS = TRS_{gj} - TRS_{gjf} \quad \text{(Equation 12)}$$

where: TMRS is the total metabolized reducing sugar content (g L^{-1}); TRS_{gj} is the total reducing sugar content of grape juice (g L^{-1}); and TRS_{gjf} is the total reducing sugar content of fermented grape juice (g L^{-1}).

Total phenolic compound content

Total phenolic compound content in samples was determined at 765 nm after reaction with Folin–Ciocalteu reagent using a UV–Vis spectrophotometer (Shimadzu 1800, Kyoto, Japan), according to the colorimetric method described by Lazzarotto et al. (2021). Gallic acid was used as the standard, and total phenolic compound levels were expressed as mg gallic acid equivalents per liter of sample (mg GAE L^{-1}).

Statistical analysis

All analyses were performed in triplicate, and data were subjected to analysis of variance followed by Tukey's test at $p < 0.05$ using IBM SPSS Statistics version 20.0 (Boston, MA, USA).

Results and Discussion

Ice impurity index and concentration efficiency of grape juice

Total soluble solids and total reducing sugar contents of control, cryoconcentrated, and cryoconcentrated-enriched musts differed statistically, whereas treatments 2 and 3 were statistically similar (Figure 2).

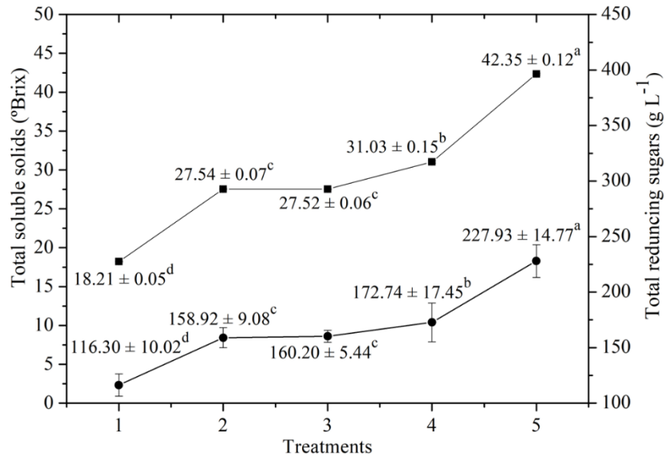


Figure 2. Effects of cryoconcentration on total soluble solid and total reducing sugar contents in grape juice treatments. Treatments identified by different letters indicate statistically significant differences (Tukey's test at 5.0%).
Note: Treatments: 1 = 100.00% grape juice; 2 = 36.15% grape juice + 63.85% cryoconcentrate 1; 3 = 64.42% grape juice + 35.58% cryoconcentrate 2; 4 = 100.00% cryoconcentrate 1; and 5 = 100.00% cryoconcentrate 2.

As shown in Figure 2, cryoconcentration resulted in increases in total soluble solids content of 12.08 °Brix in stage 1 and 11.32 °Brix in stage 2, totaling a cumulative increase of 23.40 °Brix compared with the initial juice (18.21 °Brix). The increases reported by Sun et al. (2007) (5.00 °Brix), Hernández et al. (2010) (16.40 °Brix), Petzold et al. (2016) (17.00 °Brix), Zhang et al. (2016) (9.00 °Brix), and Miyawaki et al. (2020) (8.40 °Brix) were lower than those obtained in the present study when considering the combined results of both cryoconcentration stages. However, when analyzed separately, the first and second stages yielded lower values than those reported by Hernández et al. (2010) and Petzold et al. (2016), but higher values than those reported by Zhang et al. (2016) and Miyawaki et al. (2020).

Cryoconcentration increased reducing sugar content by 56.44 g L^{-1} in the first stage and 55.19 g L^{-1} in the second stage, resulting in a total increase of 111.63 g L^{-1} (Figure 2). Higher values were reported by Wu et al. (2017), who used a scraped-surface cryoconcentrator and increased sugar content in Cabernet Sauvignon must by 234.47 g L^{-1}, approximately doubling the initial sugar content. These results support the use of cryoconcentrates as must enhancers, adding quality and value to wines produced using this approach, as reported by Sun et al. (2007), Hernández et al. (2010), Petzold et al. (2016), Zhang et al. (2016), Wu et al. (2017), and Miyawaki et al. (2020).

Similar results have been reported for other raw materials, including aqueous yerba mate extract (Boaventura et al., 2013), skimmed milk (Aider & Ounis, 2012), tofu whey (Benedetti et al., 2015), apple juice (Zielinski et al., 2019), whey (Cochachin-Carrera et al., 2023), and strawberry extract (Adorno et al., 2017). Following extraction of the frozen fraction (D1), cryoconcentrate 1 showed increased viscosity, reducing solute mobility during thawing and promoting greater retention of solutes in the frozen fraction (D2) (Dunaway et al., 2010; Nakagawa et al., 2010; Moreno et al., 2014b; Osorio et al., 2018; Meneses et al., 2021).

Migration and retention of total reducing sugars in cryoconcentrates occurred with greater efficiency during the first cryoconcentration stage, exceeding the concentration efficiency observed for total soluble solids at the same stage. (Figure 3).

In contrast, during the second cryoconcentration stage, concentration of total soluble solids was more efficient than concentration of total reducing sugars. However, the first stage showed greater concentration efficiency than the second stage for both total soluble solids and total reducing sugars. Bredun et al. (2023) reported similar results when studying three-stage block cryoconcentration of grape pomace extract and observed that subsequent stages exhibited reduced efficiency. Bredun et al. (2023) observed an increase in total soluble solids content of 17.80 °Brix, with an average efficiency of 83.20%. Similarly, Miyawaki et al. (2020) subjected must to progressive cryoconcentration using a tubular ice system with a circulation pump and reported a total soluble solids concentration efficiency of 60.20%.

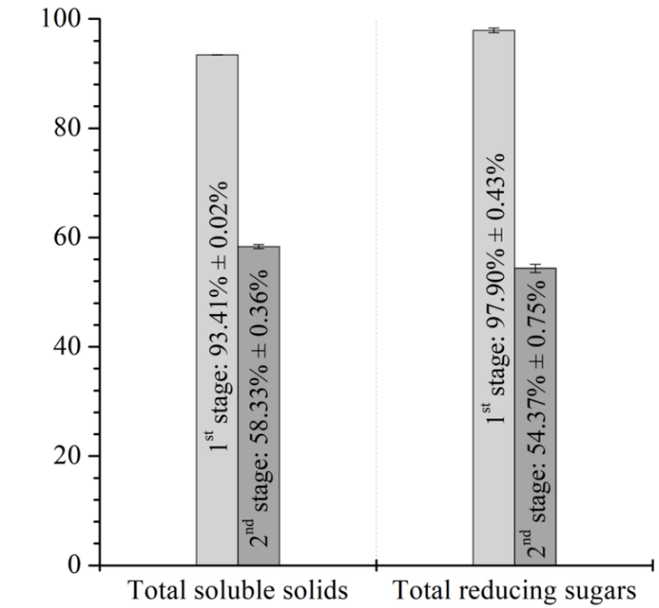


Figure 3. Concentration efficiency of total soluble solids and total reducing sugars during the first and second stages of the cryoconcentration process.

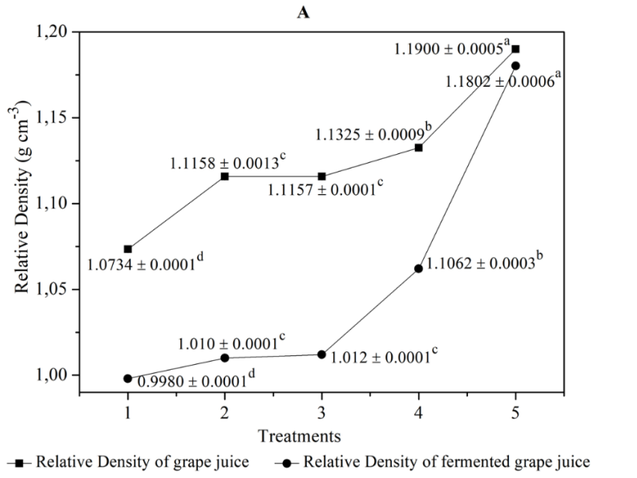
Root mean square (RMS) values for cryoconcentration were 17.4% in the first stage and 8.5% in the second stage. Petzold et al. (2015) studied cryoconcentration of blueberry and pineapple juices and found RMS values of 4.4% and 6.5%, respectively. Gil et al. (2023) studied cryoconcentration of three whey types (fresh cheese, mató cheese, and blue cheese) and reported an RMS value of 6.50% for all three. In a study of acerola pulp cryoconcentration, Marafon et al. (2024) recorded RMS values of 4.71% in the first stage, 9.11% in the second stage, and 12.35% in the third stage. All of these studies demonstrated good adjustment of the cryoconcentration process, with RMS values below 25.0% (Sánchez et al., 2009).

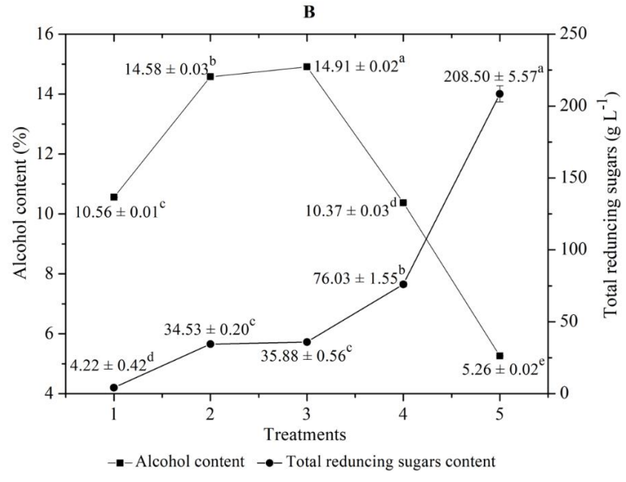
Characterization of fermented grape juices obtained through cryoconcentration

Alcoholic fermentation of different grape juices showed varying durations. Treatment 1 completed alcoholic fermentation after ten days, whereas treatments 2, 3, and 5 required eighteen days, and treatment 4 required twenty-six days, reaching final relative density values as shown in Figure 2A.

This density behavior (Figure 4A) mainly resulted from alcohol content and residual sugars remaining after fermentation, as shown in Figure 4B, along with other wine characteristics. Geraldine et al. (2012) demonstrated that high sugar concentrations

exerted greater osmotic pressure on *Saccharomyces cerevisiae* MTCC 2918, destabilizing cytoplasmic balance and compromising cellular homeostasis. A similar effect may have occurred with *Saccharomyces cerevisiae* La Claire C58 in treatments 2, 3, 4, and 5, consequently prolonging alcoholic fermentation and preventing complete fermentation. Addition of a yeast activator, as well as greater oxygenation of fermentation media, could potentially improve alcoholic fermentation performance. However, Wu et al. (2017) observed higher levels of glycerol and cellular osmoprotectors in wines enriched with cryoconcentrates, allowing yeast to perform satisfactorily (1.45 ± 0.07 g L^{-1} residual sugars), even under hyperosmotic conditions.





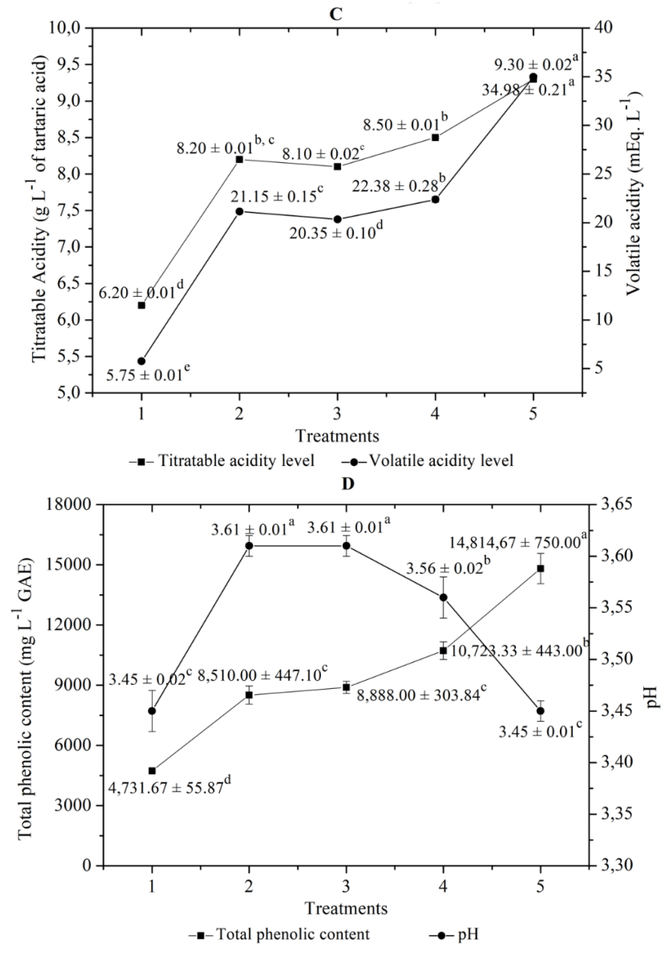


Figure 4. Effects of alcoholic fermentation on relative density, alcohol content, total reducing sugar content, titratable acidity, volatile acidity, pH, and total phenolic content of fermented grape juice. Treatments identified by different letters indicate statistically significant differences (Tukey's test at 5.0%).
Note: Treatments: 1 = 100.00% grape juice; 2 = 36.15% grape juice + 63.85% cryoconcentrate 1; 3 = 64.42% grape juice + 35.58% cryoconcentrate 2; 4 = 100.00% cryoconcentrate 1; and 5 = 100.00% cryoconcentrate 2.

Alcohol content and residual sugar levels in fermented beverages ranged from 5.26 ± 0.02% to 14.91 ± 0.02% among the five treatments (Figure 4B). Similarly, cryoconcentrated wines produced by Sun et al. (2007), Zhang et al. (2016), Wu et al. (2017), and Miyawaki et al. (2021b) showed alcohol contents within this same range, varying from 9.20% to 14.50%. In contrast, cryoconcentrated wines produced by Petzold et al. (2016) and Miyawaki et al. (2020) exceeded these values, reaching 17.67 ± 0.58% and 17.10%, respectively.

Residual sugar content in fermented beverages ranged from 4.22 ± 0.42 g L^{-1} to 208.50 ± 5.57 g L^{-1} among treatments (Figure 4B). We observed lower residual sugar levels than those reported by Wu et al. (2017), whose Cabernet Sauvignon wines contained 1.45 ± 0.07 g L^{-1} residual sugars. Within a range comparable to the present study, Sun et al. (2007) produced cryoconcentrated wines containing 9.14 g L^{-1} residual sugars. Similarly, Hwang et al. (2011) reported residual sugar contents ranging from 9.07 g L^{-1} to 14.0 g L^{-1}.

The control treatment (Figure 4C) remained within the acceptable volatile acidity limit established by current Brazilian legislation (< 20.0 meq L^{-1}). However, all treatments containing cryoconcentrated must exceeded this limit (Figure 4C). The absence of malolactic fermentation in fermented grape juices may have favored proliferation of acetic bacteria and metabolism of citric and malic acids (Vavřiník et al., 2022). Nevertheless, cryoconcentrated wines of Vitis vinifera Cabernet Sauvignon evaluated by Wu et al. (2017) exhibited volatile acidity of 8.59 ± 0.03 meq L^{-1}, which was lower than values observed in all treatments in the present study (Figure 4C).

Titratable acidity of all treatments fell within limits established by Brazilian legislation (3.00–9.75 g L^{-1} tartaric acid) (Figure 4C). Similarly, Wu et al. (2017) and Petzold et al. (2016) reported Cabernet Sauvignon wines with titratable acidity levels of 7.79 ± 0.03 g L^{-1} and 8.95 ± 0.04 g L^{-1} tartaric acid, respectively. Petzold et al. (2016) reported values higher than those observed in treatments 1–4 but lower than treatment 5. In contrast, values reported by Wu et al. (2017) were lower than those observed in treatments 2–5 and exceeded only treatment 1. However, Sun et al. (2007) reported values of 11.40 g L^{-1} tartaric acid in most Rose Honey cryoconcentrated wines, exceeding limits established by Brazilian legislation.

Treatments showed pH values ranging from 3.45 to 3.61 (Figure 4D), whereas Wu et al. (2017) reported higher pH values in cryoconcentrated wines (4.07 ± 0.18 after malolactic fermentation and 4.14 ± 0.16 after one year of aging). In contrast, Sun et al. (2007) obtained cryoconcentrated wines with lower pH values (2.81).

Total phenolic content increased with higher concentrations of cryoconcentrates, as shown in Figure 4D. Treatments 2 and 3, composed of initial juice combined with cryoconcentrates 1 and 2, were statistically similar. Treatments 4 and 5 showed the highest total phenolic contents. Similarly, Petzold et al. (2016) and Zhang et al. (2016) observed that increasing cryoconcentrate levels in must produced wines with higher phenolic contents than controls. Phenolic compound levels in white and red wines

produced from cryoconcentrated musts also exceeded those observed in wines produced from chaptalized musts reported by Zhang et al. (2016). Petzold et al. (2016) found that total polyphenol content in cryoconcentrated wine (6.59 ± 0.03 mg L^{-1} GAE) exceeded that of control wine (3.70 mg L^{-1} GAE). Park et al. (2020, 2019) reported total polyphenol contents ranging from 61.39 to 64.62 mg L^{-1} GAE and from 117.06 to 118.40 mg L^{-1} GAE in cryoconcentrated Cheong-soo and Doonuri wines, respectively.

Alcoholic potential values of fermented grape juice were 10.07 ± 0.03% (treatment 1), 15.62 ± 0.27% (treatment 2), 15.58 ± 0.02% (treatment 3), 16.30 ± 0.20% (treatment 4), and 21.89 ± 0.09% (treatment 5). Estimated alcoholic potential values of control, cryoconcentrated, and cryoconcentrated-enriched musts differed statistically, whereas treatments 2 and 3 were similar. Nevertheless, experimentally produced ethanol values differed, as shown in Figure 4B. Figure 5 a positive correlation (R = 0.9502) between metabolized reducing sugars and ethanol production among the five treatments.

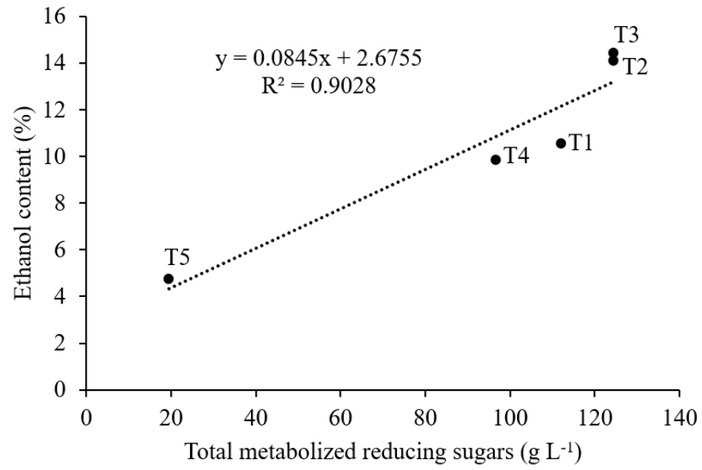


Figure 5. Scatterplot and Pearson correlation coefficient between metabolized reducing sugars and ethanol content.
Note: Treatments: 1 = 100% grape juice; 2 = 36.15% grape juice + 63.85% cryoconcentrate 1; 3 = 64.42% grape juice + 35.58% cryoconcentrate 2; 4 = 100% cryoconcentrate 1; and 5 = 100% cryoconcentrate 2.

Increasing solute concentration, together with water removal through cryodilutes, reduced free water levels and water activity in cryoconcentrates (Aider et al., 2009b). As cryoconcentration stages progressed, total soluble solids and total reducing sugar concentrations increased, and a greater number of water molecules

became associated with these compounds. These factors compromised metabolism of *Saccharomyces cerevisiae* La Claire C58. Estimated alcoholic potential was not reached in treatments containing cryoconcentrates (Figure 4B). This effect was most evident in treatment 5, which had an estimated ethanol potential of 21.89 ± 0.09%, whereas fermentation yielded only 5.26 ± 0.02% ethanol. Similar behavior was observed by Sun et al. (2007), who reported higher total reducing sugar levels in cryoconcentrated ferments (9.14 g L^{-1}) than controls (5.09 g L^{-1}).

Colorimetry of fermented grape juice produced from cryoconcentrates

All treatments showed statistically significant differences in colorimetric parameters, with treatment 1 showing greater luminosity and clarity (Table 1). These values were lower than those reported by Hwang and Park (2009), who observed *L values of 39.70 (OC2), 40.42 (Fermivin), and 41.70 (W3). However, Hwang et al. (2011) reported lower luminosity and clarity values than those observed in the present study, with *L values of 6.89 (D8), 6.97 (Fermivin), and 6.99 (S13).

Table 1
Colorimetric parameters of fermented treatments

Parameter	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
L* (au)	24.91 ± 0.01 [a]	24.89 ± 0.60 [b]	24.82 ± 0.01 [c]	24.65 ± 0.01 [d]	24.40 ± 0.01 [e]
a* (au)	0.15 ± 0.01 [b]	0.25 ± 0.01 [a]	0.18 ± 0.01 [b]	0.11 ± 0.02 [c]	0.08 ± 0.02 [c]
b* (au)	-0.72 ± 0.01 [a]	-0.75 ± 0.01 [a,b]	-0.72 ± 0.02 [a]	-0.73 ± 0.01 [a,b]	-0.73 ± 0.01 [a,b]
C* (au)	0.74 ± 0.01 [b]	0.80 ± 0.01 [a]	0.74 ± 0.01 [b]	0.74 ± 0,01 [b]	0.74 ± 0.02 [b]
h° (au)	281.96 ± 0.36 [c]	288.61 ± 0.62 [a]	284.25 ± 0.22 [b]	278.36 ± 1.20 [d]	276.01 ± 1.02 [e]

L*: luminosity; a* and b*: CIELAB color coordinates; C*: chroma (color saturation); h*: hue. Different letters within a row indicate statistically significant differences according to Tukey's test ($p < 0.05$).
Note: Treatments: 1 = 100.00% grape juice; 2 = 36.15% grape juice + 63.85% cryoconcentrate 1; 3 = 64.42% grape juice + 35.58% cryoconcentrate 2; 4 = 100.00% cryoconcentrate 1; and 5 = 100.00% cryoconcentrate 2.

Treatment 2 stood out among the other treatments (Table 1), exhibiting a higher concentration of red-colored compounds, indicating a greater presence of anthocyanins. Similarly, red wines produced by Hwang and Park (2009) and Hwang et al. (2011) showed *a values of 44.74 (OC2), 43.20 (Fermivin), and 40.98 (W3), and 18.46 (D8), 18.89 (Fermivin), and 18.90 (S13), respectively.

According to coordinate b, treatments did not differ significantly and indicated predominance of blue coloration. Nevertheless, wine samples analyzed by Hwang and Park (2009) and Hwang et al. (2011) showed predominance of yellow coloration, with *b values of 22.30 (OC2), 22.25 (Fermivin), and 23.01 (W3), and 4.37 (D8), 4.54 (Fermivin), and 4.53 (S13), respectively.

Treatment 2 showed higher hue and chroma values than treatments 1, 3, 4, and 5, which exhibited similar characteristics. Comparable results were reported by Hwang and Park (2009) and Hwang et al. (2011), who obtained hue values of 0.91 (OC2), 0.97 (Fermivin), and 0.95 (W3), and 1.03 (D8), 1.03 (Fermivin), and 1.02 (S13), respectively. In contrast, Wu et al. (2017) observed greater color saturation in cryoconcentrated wines after malolactic fermentation. However, after one year of aging, chroma values of control and cryoconcentrated wines became statistically similar.

All treatments showed significant differences, although they exhibited the same color pattern (violet). Hwang and Park (2009) and Hwang et al. (2011) reported predominance of red coloration in their samples, with values of 2.91 (OC2), 2.83 (Fermivin), and 2.75 (W3), and 3.50 (D8), 3.52 (Fermivin), and 3.52 (S13), respectively. Petzold et al. (2016) observed that control red wine exhibited greater brightness and an orange hue, whereas wines enriched with cryoconcentrates showed a red hue. Similarly, after malolactic fermentation and one year of aging, cryoconcentrate-enriched wines studied by Wu et al. (2017) demonstrated a more pronounced red hue than control wines.

Conclusions

Alcoholic fermentation of beverages with high total soluble solids concentrations presents new business opportunities. High levels of natural sugars in grape juice modify the fermentation medium. Results obtained for the control (T1) and intermediate concentrations (T2 and T3) showed behavior similar to that observed in wine fermentations using *Saccharomyces cerevisiae* La Claire C58.

However, notable differences were observed in more concentrated solutions exceeding 31 °Brix (T4 and T5), in which yeast performance was impaired. Under these conditions, ethanol production by yeast was compromised because of the low availability of free water.

Cryoconcentrated grape juice with 42.45 °Brix (T5) showed an ethanol concentration of only 5.26 ± 0.02%, with 208.00 g L^{-1} of total reducing sugars remaining unconverted. Future studies are needed to improve grape juice fermentation and prevent high volatile acidity levels that may limit commercial application.

Strategies such as supplementation with nitrogen-rich activators to support yeast growth and incorporation of lactic acid bacteria to promote malolactic fermentation may contribute to production of higher-quality fermented cryoconcentrated grape juices. Another alternative would be the use of selected yeast strains with greater tolerance to elevated sugar and alcohol concentrations. This approach could reduce residual sugar levels and limit the activity of potential acetic bacteria in fermented grape juices.

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Credit authorship contribution statement

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. All authors read and approved the final manuscript.

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