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ORIGINAL ARTICLE

Influence of must composition and clarification method on the properties of a wine made from orange juice

Influencia de la composición del mosto y método de clarificación en las propiedades de un vino elaborado a partir de jugo de naranja

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Abstract The study developed wine from orange juice, analyzing the physicochemical parameters of fresh juice and wines produced with different concentrations and must treatments. The initial juice exhibited an acidity of 0.27 g/L, a pH of 3.12, and soluble solids (5.9 °Brix), values linked to a lower sugar content likely due to insufficient fruit ripening and during fermentation, acidity increased, and soluble solids decreased due to sugar consumption, resulting in wines with high alcohol content (>12%). The pH ranged from 3.54 to 4.02, meeting established standards, although no apparent relationship with treatments was observed. Microbiological analyses confirmed product quality, showing no presence of contaminant microorganisms. Sensorially, wines with 25% juice clarified with bentonite were better accepted, while those with 100% juice received low scores due to unfavorable organoleptic characteristics. The results highlight how the must composition and treatments influenced the final properties of the wine.

Keywords orange wine, alcoholic fermentation, physicochemical parameters, clarification, sensory acceptance.

Resumen El estudio desarrolló vino a partir de jugo de naranja, analizando parámetros físico-químicos del jugo fresco y los vinos elaborados con diferentes concentraciones y tratamientos del mosto. El jugo inicial presentó una acidez de 0,27 g/L, pH de 3,12 y sólidos solubles (5,9 °Brix), valores relacionados con menor contenido de azúcares debido a una posible maduración insuficiente de la fruta. Durante la fermentación, la acidez aumentó y los sólidos solubles disminuyeron por el consumo de azúcares, resultando en vinos con alto contenido alcohólico (>12%). El pH osciló entre 3,54 y 4,02, cumpliendo normas establecidas, aunque sin relación clara con los tratamientos. Los análisis microbiológicos confirmaron la calidad del producto, mostrando ausencia de microorganismos contaminantes. Sensorialmente, los vinos con 25 % de jugo clarificados con bentonita fueron mejor aceptados, mientras que los de 100 % de jugo recibieron bajas calificaciones por características organolépticas desfavorables. Los resultados resaltan cómo la composición del mosto y los tratamientos influyen en las propiedades finales del vino.

Palabras clave vino de naranja, fermentación alcohólica, parámetros físico-químicos, clarificación, aceptación sensorial.

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PUBLIS EDITORIAL

Introduction

The European Economic Community (EEC) defines fruit wine as an alcoholic beverage obtained through the partial or complete fermentation of fresh fruit juices, concentrated or reconstituted juice, or macerated pulp with the addition of water, sugar, or honey. After fermentation, fresh, concentrated, or reconstituted juice can be added, resulting in an alcohol content between 8 and 14% (w/v). These wines can be still or carbonated, either by CO_2 injection or secondary fermentation (González, 2012). The production of these wines is highly popular in many northern European countries and regions where climatic conditions hinder the development of traditional viticulture (Valle, 2016).

The consumption of such products is high in countries like Spain, England, Switzerland, and France, where farmers historically cultivated fruits such as apples or pears primarily for wine production rather than direct consumption (Fuentes-García et al., 2024). In Latin America, fruit wine production is also evident; for example, in Ecuador, multiple studies have been conducted on blackberry and jícama wines (Petric et al., 2024).

Orange juice contains sugars that can undergo fermentation to produce alcoholic beverages such as orange wine, which is the focus of this study. However, it has been the subject of several investigations due to challenges in fermentation caused by its citric acid content (Patelski et al., 2024).

One of the main issues in wine production lies in achieving a final product with a clear and bright appearance. The end product often exhibits turbidity, color instability, or abnormal flavors and aromas, highlighting the need for clarification to achieve a translucent and polished appearance (Ailer et al., 2022). This study aimed to develop orange wine with suitable physical, chemical, and sensory characteristics.

Materials and methods

Orange juice, the main raw material for wine produc-tion, was obtained from sweet oranges (*Citrus sinensis*) of national production with appropriate maturity, harvested in January 2016. The fruits were randomly selected, but they did not present any mechanical damage or bruises caused by post-harvest processing and showed no signs of insect dam-age or excessive sun exposure.

Before preparing the juice, the oranges were washed, brushed, then cut, and extracted using a Philips Cucina electric juicer. The juice was free of seeds and peel particles. The quality of the orange juice was evaluated as the primary raw material used, as a good product starts with quality raw material. The orange juice was tested for titratable acidity (NC-ISO 750, 2001), pH (NC-ISO 1842, 2001), and soluble solids (NC ISO 2173, 2001).

Three musts, each 5 L in volume, were made with different percentages of orange juice (25, 50, and 100%). The soluble solids were then adjusted to 23 ºBrix by adding sucrose (refined sugar), and the pH was adjusted to 4.00.

The fermentation process was carried out under static conditions at 25 ºC for 12 days, using a *Saccharomyces cerevisiae* strain under anaerobic conditions. After the stated period, 0.1 g/L of $NaHSO_3$ was added to stop the fermentation, and the wine was left to rest for 2 days.

Each fermented must was clarified using three different methods. The first was clarification with bentonite: 0.4 g/L of bentonite were added, dissolved, and left to rest for 14 days. Finally, racking was performed to remove the sediments. The second method was filtration through plates, which involved vacuum filtration through nitrocellulose packs. The third method was racking, where the product naturally sedimented particles, which were then separated by a racking process. In each group, one must be maintained as a control sample. The musts were standardized to 23 ºBrix and pH 4.00.

The nine orange wine samples were left to rest and mature for 60 days at 25 ºC, protected from light. The orange wines were characterized by determining acidity (NC 291, 2009), alcohol content (NC 290, 2010), soluble solids content (NC 707, 2013), and pH (NC 83-34, 1983).

The microbiological quality of the fermented beverage was determined by counting aerobic mesophiles (NC ISO 4833-1, 2014), molds and yeasts (NC-ISO 7954, 2002), total coliforms (NC-4832, 2010), and fecal coliforms (NC 38-02, 2014).

An affective sensory test of the level of preference was used to evaluate the general acceptance of the formulations and compare each one among them. Forty untrained judges (potential consumers) participated, and a tasting was conducted using a 7-point verbal hedonic scale, ranging from “I like it a lot” (maximum value, 7) to “I dislike it a lot” (minimum value, 1). Using the criteria provided by the consumers, the arithmetic mode of the general acceptance of each formulation was calculated and processed mathematically according to the design used.

The results were processed using descriptive statistics to determine the arithmetic mean, standard deviation, maximum and minimum values, and the mode. ANOVA and Duncan’s multiple ranges were also used to detect significant differences between the evaluated samples. The statistical software SPSS version 22 was employed.

Results and discussion

Table 1 shows the results of the physicochemical parameters of fresh orange juice. The orange juice had an acidity value of 0.27 g/L, similar to the minimum values reported by Ferreyra (2006) but lower than those reported by Hoyos et al. (2010), who characterized different varieties of Valencia oranges in Colombia.

Table 1. Physicochemical parameters of orange juice

Indicator	Mean (Standard deviation)
Titratable acidity (g/L)	0.27 (0.01)
pH	3.12 (0.01)
Soluble solids (°Bx)	5.9 (0.06)

*Expressed as citric acid.

The pH of the juice was 3.12, a value similar to that reported by Ferreyra (2006), who characterized oranges from four varieties in the Concordia area of Argentina intended for winemaking based on this parameter. Alvarenga (2004) obtained pH values of 3.92 in orange juice intended for winemaking, which is higher than the value determined in this study. This value is favorable for winemaking, as it inhibits the development of most bacteria that could cause contamination and also promotes yeast flocculation in a moderately acidic medium (Vion et al., 2024).

The low soluble solids levels may be related to an inadequate maturity state of the fruit used, as soluble solids levels in oranges increase as maturity progresses (Tiencheu et al., 2021).

The soluble solids content did not meet the specifications defined in NC 903 (2012), and the value was lower than those reported in the literature. Hoyos et al. (2010) conducted a study of oranges' maturation process, obtaining a value of 11.0 °Brix at full maturity, close to the value determined by Alvarenga (2004), who reported 10.0 °Brix.

Table 2 shows the results of the physical-chemical analyses of the final product for each formulation. The determined parameters showed increasing or decreasing trends in relation to the composition of the must, which was the variable with the greatest influence on the characteristics of the wines.

Table 2. Physical-chemical parameters of orange wine

Formulation	Acidity (g/L)	Soluble solids (°Brix)	Alcohol content (°GL)	pH
25% OJ control	0.70 e	7.03 b	13.5	3.54 d
25% OJ bentonite	0.69 e	7.10 a	12.9	3.54 d
25% OJ filtered	0.77 d	7.13 a	13.1	3.55 d
50% OJ control	1.02 c	4.43 d	15.0	3.89 b
50% OJ bentonite	0.99 c	4.53 c	15.0	3.85 c
50% OJ filtered	0.98 c	4.53 c	14.2	3.84 c
100% OJ control	1.64 b	3.06 f	15.9	3.96 ab
100% OJ bentonite	1.93 a	3.16 e	15.6	4.02 a
100% OJ filtered	2.00 a	3.06 f	15.4	3.92 b

The acidity of all nine wine formulations increased, relative to the acidity of the orange juice from which they originated, due to the formation of acid by yeasts during fermentation (Vion et al., 2024). The increase in acidity could be due to the action of the yeasts during fermentation, as there are Saccharomyces species, including *S. bayanus*, that are capable of producing citrates (acidity regulators) through their metabolism during alcoholic fermentation (Maicas, 2020). The production of malic acid by Saccharomyces yeasts raises the acidity levels in wine, a hypothesis widely accepted under the concept of oxaloacetate reduction to malic acid due to CO_2 fixation on pyruvate (Vion et al., 2024).

On the other hand, the increase in acidity could have been facilitated by the action of lactic acid bacteria from the fruit, mainly from the genera Lactobacillus, Leuconostoc, and Pediococcus. These bacteria are capable of metabolizing part of the sugars in the must, producing lactic acid and acetic acid (Wang et al., 2021).

An increase in acidity was observed as the concentrations of orange juice in the must be increased. A direct relationship existed between the percentage of orange juice used in the must and the total acidity of the wine (Ferreyra, 2006). This trend may have been related to the contribution of micronutrients such as water-soluble vitamins from the juice (Mitchell et al., 2020). The presence of these micronutrients could promote the development of bacteria and yeasts different from the ones used for fermentation, which produce high levels of organic acids such as acetic and succinic acids (Atasoy et al., 2024), the latter having a negative impact on wine quality at high levels (Torres-Guardado et al., 2024).

A significant difference was observed in the acidity of the wine with 25% orange juice with bentonite compared to other treatments. In wines with 50% orange juice, no significant differences were found between the clarification treatments. The control wines with 100% orange juice had a significantly lower ($p \leq 0.05$) acidity compared to the other two variants. Due to the variability in the results, no relationship between the clarification method and the total acidity of the wine could be established; the behavior of this parameter was different in each treatment.

As expected, residual soluble solids (RSS) decreased throughout the fermentation process due to the yeast strains consuming sugar. The musts had an initial value of 23 °Brix, and the results obtained for each formulation are shown in Table 2.

Wines made from 25% orange juice must showed residual soluble solids (RSS) values slightly above 7 (Table 2). In this case, the control treatment showed a significantly different ($p \leq 0.05$) value compared to the others. These results coincided with those reported by Sepúlveda (2009) for Pinot Noir grape wines, where musts with approximately 23 °Brix resulted in wines with around 7 °Brix. For treatments with 50% orange juice in the must, the RSS content was lower ($p \leq 0.05$) than the 25% wines. Additionally, the control treatment showed lower RSS values, possibly due to the presence of dormant yeasts, which consumed residual sugars after the primary fermentation (Ferreyra, 2006).

In musts with 100% orange juice, the RSS was lower than in formulations with lower juice proportions. The wine clarified with bentonite showed significantly higher ($p \leq 0.05$) values than the other treatments. This behavior could be attributed to the depletion of sugars in the control, as this formulation had the lowest added sugar content.

The clarification and plate filtration treatments did not significantly affect the RSS, except for the 100% orange juice wine with bentonite, where it increased. As the proportion of orange juice decreased, the RSS was higher since the sugar added to the must be increased with the percentage of juice. Wines made from 50 and 100% juice musts were classified as "dry," while the wines with 25% were considered "semidry."

The alcohol content was high in all treatments, exceeding 12%. The higher the orange juice content, the higher the alcohol content, showing a direct relationship. Since the orange juice was not clarified, it favored yeast development during fermentation, possibly due to a component that protected the cells, allowing more sugar depletion and generating elevated ethanol levels (Patelski et al., 2024).

The 25% wine met the parameters established by the European Economic Community's Cider and Fruit Wine Produc-ers Association (8-14% ethanol), while the 50 and 100% wines exceeded these values. Plate filtration reduced the alcohol content compared to the control while adding ben-tonite showed variable behavior depending on the juice pro-portion. The pH values ranged from 3.54 to 4.02, meeting the Colombian standard for fruit wines (NTC 708, 2000), which establishes a range of 2.8 to 4.0. The pH increased with the proportion of orange juice in the must.

The clarification and plate filtration operations did not significantly affect the pH in wines with 25% orange juice in the must. The control treatment of the 50% orange juice treatments had a higher pH ($p \leq 0.05$) than the others.

In wines made from must composed entirely of orange juice, it was observed that the pH of the wine clarified with bentonite was significantly higher ($p \leq 0.05$) compared to the wine filtered with plates. None of the treatments showed significant differences ($p \leq 0.05$) compared to the control. This study established no consistent relationship between the wine pH and the clarification treatment due to the variations observed in each case.

The pH has a marked effect on the microorganisms, color, and flavor of wines (Tofalo et al., 2021) and has been related to the turbidity present in wines and resistance to oxidation (Gutiérrez-Escobar et al., 2021).

Table 3 shows the results of microbiological tests (microorganisms at 30 °C, fungi and yeasts, total and fecal coliforms) for the orange wines. The values of the microbiological determinations indicated proper hygiene throughout the process and the quality of the remaining raw materials used, such as sugar and water.

In wine production, it is recommended that a clarification and filtration system be implemented. Ideally, clarification should be performed first, followed by filtration, to ensure maximum cleanliness and stability of the product (Mierczynska-Vasilev & Smith, 2015). In this study, the clarification treatments were applied separately; however, none showed microbiological deficiencies, particularly concerning fungi and yeasts. This demonstrates that the clarification methods effectively removed the yeast residues left in the wine after fermentation. These residues could cause problems during the wine's shelf life, such as re-fermentation and turbidity (Espejo, 2021).

Table 3. Microbiological indicators of orange wine

Treatment	Microorganisms at 30 °C (CFU/ml)	Fungi and yeasts (CFU/ml)	Total coliforms (CFU/ml)	Fecal coliforms (CFU/ml)
25% OJ control	< 10	< 10	< 10	< 10
25% OJ bentonite	< 10	< 10	< 10	< 10
25% OJ filtered	< 10	< 10	< 10	< 10
50% OJ control	< 10	< 10	< 10	< 10
50% OJ bentonite	< 10	< 10	< 10	< 10
50% OJ filtered	< 10	< 10	< 10	< 10
100% OJ control	< 10	< 10	< 10	< 10
100% OJ bentonite	< 10	< 10	< 10	< 10
100% OJ filtered	< 10	< 10	< 10	< 10

The control sample did not undergo a filtration process but was clarified using traditional decanting, so the yeast involved in the fermentation process of the product remained dormant. The microbiological indicators (Table 3) were negative for fungi and yeasts, influenced by various factors, such as the antimicrobial action of sodium bisulfite (Maj et al., 2024) and the elevated ethanol levels typically found in such products. The determination of total and fecal coliforms was negative, indicating the microbiological safety and quality of the wines (NC 585, 2013).

The results of this study were aligned with those reported by Bonilla (2009) for honey sweet wine. The main factor in these results is the inhibitory effect of ethanol on microorganisms, preventing cell multiplication (Bonilla, 2009). This phenomenon was favored in the wines developed in this study due to their high alcohol content, which exceeded the established value for such products (Table 2).

Affective sensory testing of the level of liking was conducted to assess the overall acceptance of the formulations using a verbal hedonic scale. Figure 1 shows the scores for the different treatments. The most accepted wine, with the highest rating of “I like it a lot,” was the formulation with 25% orange juice clarified with bentonite. Following this, the control and filtered wines with 25% orange juice and wines with 50% orange juice treated with bentonite and filtered received “I like it.” The control beverage with 50% orange juice was rated “I neither like nor dislike it,” while the control and filtered wines with 100% orange juice received ratings of “I slightly dislike it” and “I really dislike it,” respectively.

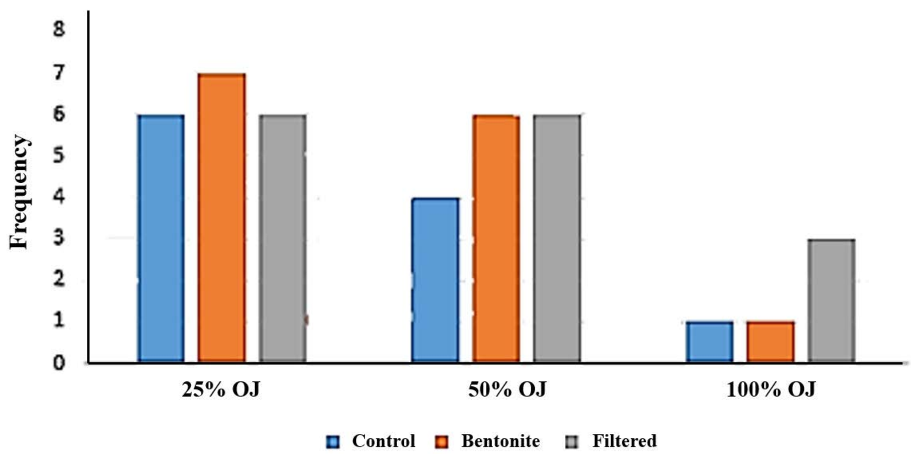

Figure 1. Sensory acceptance of orange juice wines.

The sensory evaluation showed a direct relationship between the percentages of orange juice used and the organoleptic characteristics of the final product, indicating that as the JN content increased in the must, preference decreased. However, no clear relationship could be established with the clarification methods used, as their behavior was variable in each case.

The control samples, in all three cases, received the lowest scores, possibly related to the lack of clarification, an expected factor since color and brightness are key organoleptic characteristics of wines (Gutiérrez-Escobar et al., 2021); these results suggest that filtration improves the appearance of the wine. Among the most accepted samples (25% JN) was the one treated with bentonite, which stood out for presenting a better balance between sweetness and acidity. The 100% filtered formulations were rated as "Slightly disliked" due to their higher acidity and lower residual soluble solids (SSR) content. The control samples and those treated with benton-ite had higher alcohol content and an increased sensation of sharpness (Ubeda et al., 2021).

Conclusions

The proportions of orange juice used in the preparation must increase the acidity, residual soluble solids, and alcohol content of the orange wines. The plate clarification treatment reduces the alcohol content of the wines compared to the control sample. The physicochemical characteristics of the wines remained within the established parameters, except for the alcohol content of the wines with 50 and 100% orange juice, which exceeded the recommended values for this type of product. The wines showed negative results in all the microbiological indicators analyzed. The wine with 25% orange juice and clarification treatment with bentonite received the highest rating of "I like it a lot." A direct relationship was observed between the increase in juice concentrations in the preparation of the must and the decrease in the acceptability of the wines.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statement on the use of AI

The authors acknowledge the use of generative AI and AI-assisted technologies to improve the readability and clarity of the article.

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