Original Research

THE EFFECT OF TOTAL SOLUBLE SOLIDS AND pH ON SOME QUALITY PARAMETERS OF FERMENTED BEVERAGE FROM BLUEBERRY (*Vaccinium corymbosum* L.)

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ABSTRACT

Aims: To investigate the effects of total soluble solids and pH on the physical and chemical composition, as well as the sensory quality of fermented blueberry beverages (*Vaccinium corymbosum* L.).

Methods: The study investigated the effects of pH levels (3.8, 4.0, and 4.2) and total soluble solids (18, 20, and 22°Brix) on the quality attributes of the beverage.

Results: The optimal fermentated conditions were found to be 20°Brix and pH 4.0. Under these conditions, the beverage showed high alcohol content (4%Vol.), low residual sugar (7.43%), total acidity (0.3%), and anthocyanin content (58.36mg%) after 4 days of fermentation. The 20°Brix - pH 4 treatment received high sensory scores across all attributes: appearance (4.54), color (4.46), aroma (4.43), and taste (4.66). This resulted in a product characterized by a balanced sweet-and-sour flavor, a vibrant red color, and a moderate ethanol content. Unlike wine, the ethanol level was not overly high, aligning well with current preferences for fermented beverages.

Conclusion: Both pH and total soluble solids significantly influenced key quality indices. The fermentation process of blueberry beverages was identified with the optimal total soluble solids and pH values.

Keywords: *°Brix, pH, blueberry, Vaccinium corymbosum L., fermentation*

I. INTRODUCTION

Blueberry is a perennial flowering plant with the scientific name *Vaccinium corymbosum L.*, belongs to the genus *Vaccinium*, family *Ericaceae*, and is native to North America and Europe [1]. The plant grew upright or low to the ground, had few branches, elliptical leaves with short petioles, and produced flowers and fruits from June to September [2]. When ripe, the berries had a sweet, mild taste and grew in clusters. They appeared blue-black with a purple sheen and a round shape [2]. Blueberries were rich in anthocyanins, which benefited human health by protecting retinal cells and preventing cardiovascular diseases [3]. Bioactive compounds such as phenolics, organic acids, anthocyanins, proanthocyanidins, flavonol glycosides, vitamin C, and carbohydrates were found in blueberries, compounds have been proven to inhibit cancer growth and cardiovascular diseases, show antibacterial activity and prevent urinary tract infections, dental and gum diseases, stomach ulcers, obesity, diabetes, aging, and reduce cholesterol and biofilm formation [4, 5].

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Doi: 10.56283/1859-0381/841

Received: 15 October, 2024
Revised: 26 October, 2024
Accepted: 25 November, 2024
Online first: 5 December, 2024

Blueberries were also used in traditional medicine and exhibited important biological functions such as antioxidant, anti-inflammatory, and anti-tumor effects [6].

Fermented beverages resulted from an incomplete alcoholic fermentated process using fruit ingredients. These beverages were considered natural drinks with low alcohol content (4-6%), non-distilled, and contained many nutrients beneficial to health. They included various compounds produced during the alcoholic fermentation process, such as organic acids and fragrant esters, as well as distinctive fruit components used for fermentation. In fermented beverages, biochemical processes continued even when stored at low temperatures. Therefore, after fermentation, yeast cells were removed and pasteurized to increase shelf life [7–9]. Cider, an alcoholic beverage made from apples, became very popular in countries like France, Spain, Ireland, Switzerland, and South Africa. The cider industry saw significant development in the U.S. and Canada, with rising production levels. Although generally referred to as cider, differences in production conditions and methods led to varied tastes between countries. The taste of cider could be sweet, sour, or bitter, depending on the ingredients used for fermentation. In Vietnam, the concepts of cider and fruit wine were not distinguished, leading to some debate. In recent years, demand for fruit-based alcoholic beverages has grown in Vietnam [10].

According to Luong Duc Pham, the fermentated process involves converting carbohydrates and other organic compounds into new substances by microorganisms under the influence of enzymes [11]. Microorganisms or their enzymes were the primary agents of fermentation, and the fermentation rate and yeast growth depended heavily on the total soluble solids and the pH of the fermentation medium. These factors influenced the alcohol content, total acids, total sugars, anthocyanin levels, and the sensory quality of the product during fermentation. Selecting the appropriate initial total soluble solids concentration and pH value was critical for producing a fermented beverage with low alcohol content suitable for all ages, especially women and children. This aided digestion, enhanced meal enjoyment, and prevented intoxication, unlike other alcoholic products. Therefore, this study aimed to investigate the effects of total soluble solids and pH on the physical and chemical composition, as well as the sensory quality of fermented blueberry beverages, to identify the optimal total soluble solids and pH values for the fermentation process.

II. METHODS

2.1. Materials

Blueberries: Imported blueberries from Peru were purchased from the Dalatshop fruit store in Ward 7, Tan Binh District, Ho Chi Minh City, and transported to the laboratory in Can Tho. The blueberries were packed in cartons of 12 boxes, each containing 125g. Selection was based on the criteria of fresh, round berries with thin skins, dark purple or blue-purple color, a stem at one end a calyx at the other, and a white bloom on the outer skin. Damaged or slimy berries were excluded.

The berries were washed, drained, and frozen at (-20 ± 2°C). Thawing occurred at room temperature before use.

Other materials: *Saccharomyces cerevisiae* RV100 yeast (ICFOOD Company, Vietnam), sucrose (Vietnam), citric acid (China), sodium carbonate (China), sodium hydrogen sulfite (China).

2.2. Processing procedure

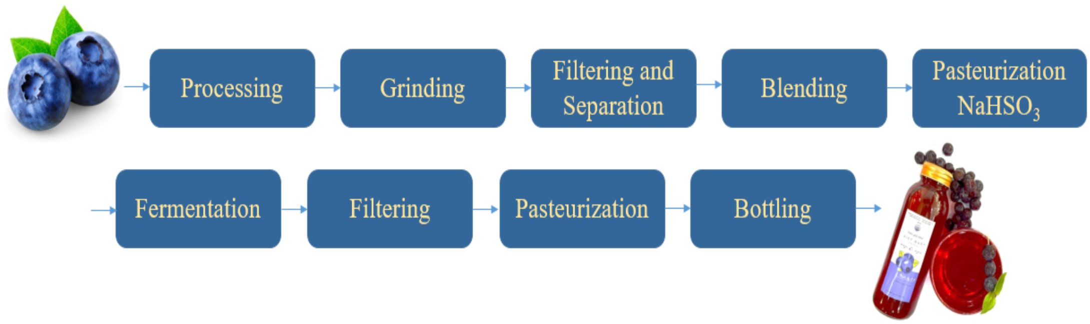

Figure 1. *Diagram of the processing procedure for fermented blueberry beverage*

Explain for procedure

The blueberries were thawed and blended with water in a ratio of 1:3.5 (w/v), then filtered and supplemented with 0.1% citric acid. The total soluble solids (18, 20, and 22°Brix) was adjusted by adding sucrose, and the pH (3.8, 4, and 4.2) was adjusted using citric acid and the saturated sodium carbonate solution. The juice was then pasteurized with the sodium bicarbonate solution at 120 mg/L for 2 hours to eliminate contaminating microorganisms. *Saccharomyces cerevisiae* yeast was added in the ratio of 0.15% w/w. The fermentation process was carried out at room temperature (28-30ºC) and monitored for the following parameters over 96 hours without shaking: alcohol content (%Vol.), total acid content (%), total sugar content (%), anthocyanin content (mg%), and total soluble solids (°Brix). After fermentation, the mixture was filtered to remove yeast residue and remaining impurities, clarifying the product. A volume of 220 ml of juice was transferred into a 300 ml glass bottle and pasteurized at 65°C in 15 minutes to inhibit and eliminate any remaining microorganisms in the fermented juice and outside the bottle. The experiment was repeated three times.

Monitoring parameters included alcohol content (%Vol.), anthocyanin content (mg%), total sugar content (%), total acid content (%), sensory evaluation.

2.3. Analytical methods

- *pH determination*: measured using a pH meter (Basic pH Meter, USA).

- *Moisture content (%)*: determined by oven-drying to a constant weight at 100-130°C [12].

- *Determination of total soluble solids (°Brix)*: measured using a refractometer (Atago Master-T 0-32% Atc Brix Refractometer, USA).

- *Anthocyanin content (mg%)*: determined by differential pH method [13].

- *Total sugar content (%)*: measured using the colorimetric method with 3,5-dinitrosalicylic acid (DNS) reagent [14].

- *Ethanol content (%v/v)*: determined by distillation and measuring alcohol content using a hydrometer at 20°C [15].

- *Total acid content (%)*: measured by titration with 0.1N NaOH using phenolphthalein as an indicator [16].

- *Sensory evaluation*: preference rating from 0 to 5 points among the samples, comparing differences in appearance, color, aroma, and taste according to TCVN3215-79 [17]. The sensory evaluation was conducted by a panel of 10 university students who possessed knowledge of food and were well-versed in the sensory evaluation procedure using a scoring system. The parameters evaluated included apperance, color, aroma, and taste.

2.4. Data processing methods

Data are processed using Microsoft Excel 2013 and Statgraphics Centurion XV.1. All data are presented as means ± SD, with significant differences indicated at a 5% significance level.

III. RESULTS AND DISCUSSION

3.1. Determination of blueberrie compositions

Blueberries stored for a short period at a stable temperature retain much of their nutritional value, such as vitamin C and anthocyanins, making them suitable for research into fermented beverage production. The results of chemical composition analysis after thawing were presented in Table 1.

Table 1. *Physicochemical properties and chemical composition of blueberries after thawing*

Chemical composition	Unit	Value
Moisture content	%	89.30 ± 1.42
Total sugar content	%	4.22 ± 0.21
Reduced sugar content	%	2.32 ± 0.12
Anthocyanin content	mg%	152.20 ± 2.24
Total acid content	%	0.60 ± 0.02
Vitamin C content	mg%	18.30 ± 1.27
pH value	-	2.75 ± 0.01
Total soluble solids	°Brix	7 ± 0.00

The values in the table were the averages of three repetitions

The chemical composition of the blueberries after thawing used in the experiment included: moisture content of 89.30 ± 1.42%, total sugar content of 4.22 ± 0.21%, reduced sugar content of 2.32 ± 0.12%, total soluble solids of 7°Brix, total acid content of 0.60 ± 0.02%, anthocyanin content of 152.20 ± 2.24 mg%, vitamin C content of 18.3 ± 1.27 mg%, and pH of 2.75 ± 0.01. According to research by Michalska et al., fresh blueberries contain 84% water [18]. The average vitamin C content in blueberries was 8.6 mg/100 g [1]. The total sugar and reducing sugar contents in the thawed blueberries were relatively low (4.22% and 2.32%) while the acid content was high (0.60%), likely because the fruit had not reached physiological ripeness, contributing to the characteristic sour taste. These differences could be attributed to factors such as variety, location, and harvest time. Based on the analyzed properties and chemical composition of the blueberry raw material, alcohol content, total acid, total sugar, reducing sugar, and anthocyanin were monitored after fermentation, while varying the added sugar content and adjusting the pH of the diluted blueberry juice.

3.2. Study of Influence on alcohol content and pH of the product

The total soluble solids, primarily composed of sugars, became a necessary substrate for yeast growth and the fermentation process. Variations in the initial total soluble solids content significantly affected alcohol yield after fermentation; approximately 10% of the sugars were utilized for biomass production, while the remaining sugars were converted into ethyl alcohol and by-products such as glycerol and pyruvate [19]. According to Luong Duc Pham (2010), higher sugar concentrations led to an excess of substrate after fermentation, prolonging the fermentation time. Conversely, if the sugar concentration was too low, the conversion of sugar into alcohol might have been insufficient, resulting in yeast mortality due to nutrient competition and ultimately causing low alcohol content [11].

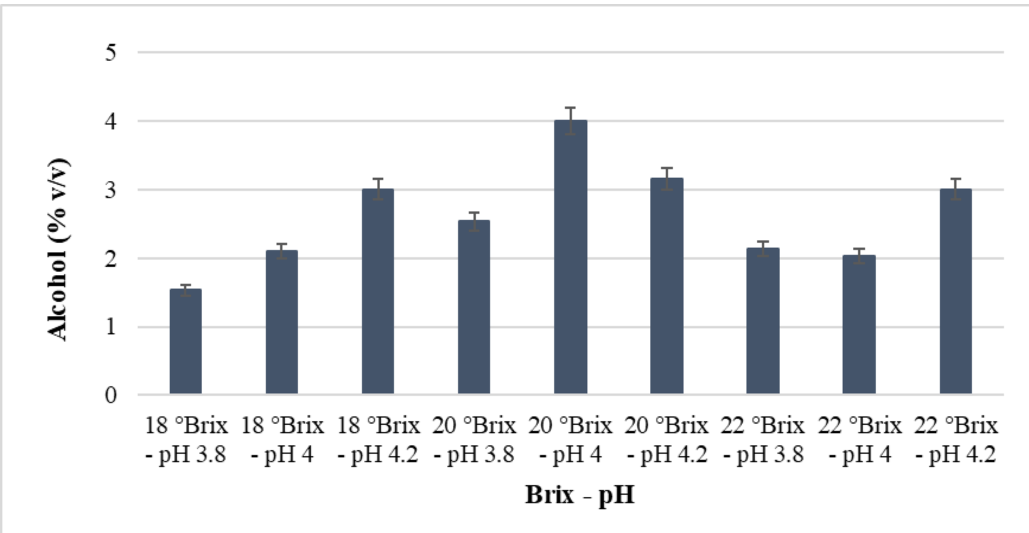

Figure 2. *Alcohol content of the product influenced by the total soluble solids and pH value in the initial fruit solution*

As shown in Figure 2, the ethanol concentration increased from 18°Brix to 20°Brix after fermentation in 4 days. However, when the concentration was raised to 22°Brix, no further increase in alcohol content was observed, and no significant difference was noted at the 5% level compared to 18°Brix. According to Vu Cong Hau (2004), the optimal total soluble solids for the fermentation process was between 20 and 22°Brix. Furthermore, the total soluble solids affected both the ethanol yield and the reduced sugar content in the final product [20]. Additionally, blueberry juice fermented with an initial total soluble solids of 20°Brix at pH 4 yielded an ethanol content of 4%Vol., which was not significantly higher than the fermentation of dragon fruit juice using the *Saccharomyces cerevisiae* RV100 strain, as reported by Huynh Thi Ngoc Mi and Doan Thi Kieu Tien (2021), which produced an alcohol content of 3.51%Vol. with a post-fermentation total soluble solids of 14°Brix, resulting in a sweet-sour flavor that was easy to drink [21]. In contrast, blueberry juice fermented with an initial total soluble solids of 18°Brix at pH 3.8 produced the lowest ethanol content at 1.53%Vol.. Increasing the total soluble solids to 22°Brix at pH 4 led to a significant decrease in ethanol content (2.03%Vol.) compared to 20°Brix at pH 4 (4%Vol.), with no difference observed in ethanol yield at 18°Brix. This suggested a potential substrate surplus or the high initial total soluble solids concentration may have inhibited yeast metabolism and growth, leading to inefficient fermentation.

Moreover, pH was a critical factor affecting the formation of primary and secondary products during fermentation and influenced the yeast's fermentation substrate utilization. The optimal pH range for *Saccharomyces cerev*isiae was 4 to 6, although it functioned well at pH 3.8 to 4 [11]. The initial pH of the fermentation medium at 3.8, 4, and 4.2 showed a slight decrease during fermentation, dropping to 3.5 to 3.8 due to the production of CO_2 and acids. This pH range was appropriate for inhibiting bacterial growth; a high initial pH promoted bacterial growth, leading to spoilage, poor color, and overall quality degradation [22]. At 20°Brix at pH 4, the highest ethanol content (4%Vol.) was observed, while at 20°Brix at pH 3.8 and 20°Brix at pH 4.2, the ethanol concentrations were only 2.53% and 3.16%Vol., respectively. In comparison, the fermented cherry juice product by Tran Thi Ngoc Mai, under similar yeast strain conditions and an initial pH of 4, yielded an average alcohol content of 1.4%Vol. [23]. The fermented blueberry juice (average 4%Vol.) was much higher than the fermented cherry juice, which was attributed to differences in total soluble solids concentration and the chemical composition of the fruits used in the fermentation process.

3.3. Study of Influence on the total acid and total sugar content of the product

As shown in Figure 3, it became evident that at the same °Brix, the total acid content generated after fermentation tended to decrease as pH increased. When the total soluble solids increased from 18°Brix to 22°Brix, the total acid also increased. The decrease in pH or the increase in acidity of the environment was attributed to the formation of CO_2 and various organic acids (acetic acid, lactic acid, citric acid, pyruvic acid, and succinic acid) during fermentation [24]. According to Nguyen Dinh Huong and Nguyen Thanh Hang (2007), the acidity only increased by about 0.2^{o} compared to before fermentation under normal alcoholic fermentation conditions [15].

The effect of pH on total acid content after fermentation showed a statistically significant difference with 95% confidence. As illustrated in Figure 3, increasing the pH from 3.8 to 4 while maintaining the same total soluble solids resulted in a gradual decrease in total acid content after fermentation. At a pH of 3.8

in the pre-fermentation solution, the total acid content after fermentation was relatively high because the alcohol produced at this pH was lower than at the other two pH values, causing a slower fermentation process and allowing bacteria to infiltrate and make a large amount of acid. The results were consistent with the findings regarding fermented dragon fruit juice, where the pH fluctuated between 3.5 and 3.6 after fermentation compared to a pre-fermentation pH of 4 [21]. It was indicated that H^+ ions affected yeast activity. However, a certain correlation existed between H^+ ions and acid content in any solution [15]. Furthermore, acidity could vary significantly while the pH value of a solution remained relatively stable, acidity depended on the degree of contamination of the raw materials and the fermentation solution. pH was less affected by contamination since it led to the production of weakly dissociated organic acids. Therefore, in the production of fermented beverages, it was essential to determine not only the pH but also to measure the total acid content.

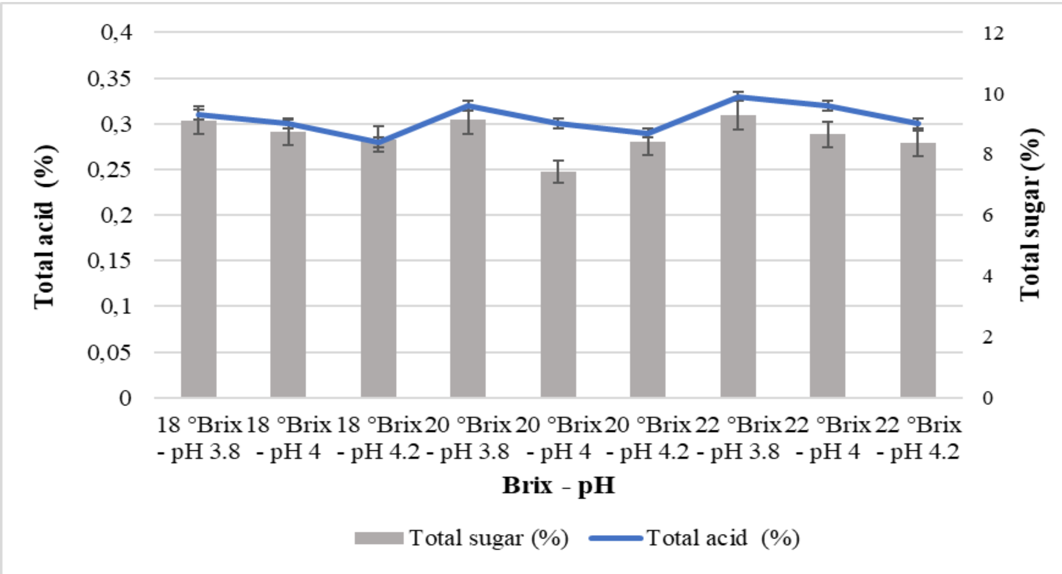

Figure 3. *The total acid content of the product varies according to total soluble solids and pH value in the initial fruit solution*

The total soluble solids and pH interacted with each other, as presented in Figure 3. At the same total soluble solids, when pH increased, the total sugar content decreased. Conversely, at the same pH, an increase in total soluble solids led to an increase in total sugar content. The residual sugar content at the end of the fermentation process ranged from 7.43% to 9.28%, depending on the different °Brix and pH levels, representing a significant reduction of about 70% compared to the sugar content before fermentation, which was 27.6%, 28.8%, and 30% for total soluble solids of 18, 20, and 22°Brix, respectively. During fermentation, sugars and nutrients were absorbed into the yeast cells, where enzymes acted through multiple intermediates, ultimately producing the main products of alcohol and CO_2 [15]. The greater the difference in sugar content after fermentation, the higher the amounts of alcohol and CO_2 produced, indicating stronger yeast activity. Sugar plays a crucial role in providing nutrients for yeast growth and metabolism. The total soluble solids after fermentation was lower than that at the start of the fermentation process, indicating that the substrate was actively utilized [24].

From Figure 3, it became evident that as the concentration of total soluble solids increased, the residual sugar content after fermentation also increased. The residual sugar content at 20°Brix was lower than at 18°Brix and 22°Brix, as it was just enough for the yeast to proliferate and ferment to produce the highest alcohol content (4% v/v). The residual sugar content at 22°Brix was relatively high, indicating a surplus of the substrate, or the initial concentration of total soluble solids may have been too high, inhibiting the growth and metabolism of the yeast, resulting in lower alcohol production. Therefore, a higher concentration of total soluble solids was not necessarily an ideal condition for yeast development; rather, a high concentration could inhibit yeast and exceed the tolerance threshold, prolonging the fermentation time and leading to incomplete fermentation. The residual sugar content after fermentation at 20°Brix - pH 4.0 was observed to be the most suitable for yeast growth and metabolism, with the residual sugar content (7.43%) being lower than at pH 3.8 and 4.

3.4. Study of Influence on anthocyanin content of the product

According to Hyo-Nam et al. (2018), the higher the sugar concentration, the lower the color intensity, indicating a reduction in anthocyanin content [25]. Different types of sugars and sugar concentrations affected anthocyanin levels; sucrose, maltose, and fructose significantly influenced color intensity, while glucose and galactose had minimal effects. The studied results indicated in Figure 4, the total soluble solids increased, the anthocyanin content decreased, with a significant difference at the 5% level. According to Maarit's study, sugar reduces the stability of anthocyanins. Anthocyanins reacted with sucrose to form polymers that produced brown pigments, and as the added sugar content increased, anthocyanins were increasingly degraded, resulting in a gradual decrease in color intensity [26].

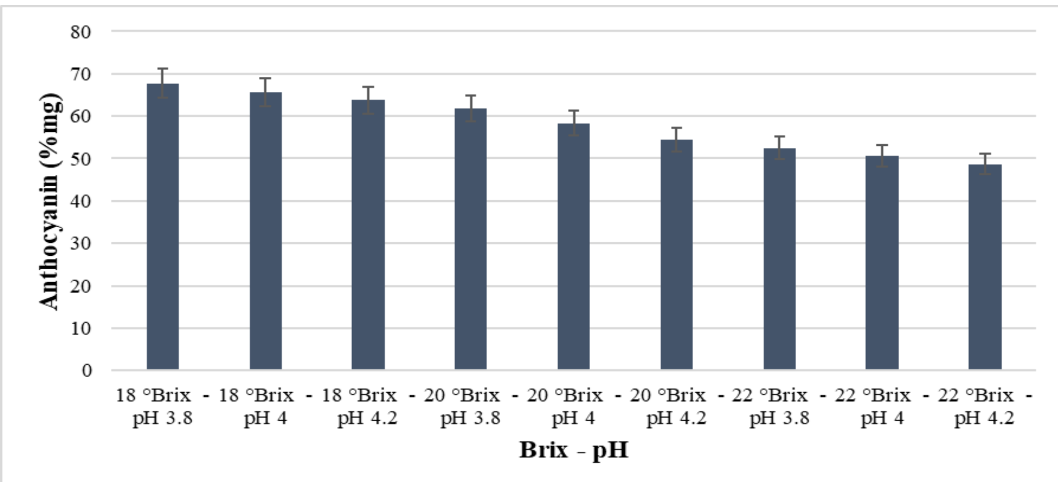

Figure 4. *Anthocyanin content of the product changes according to the total soluble solids and pH value in the initial fruit solution*

At different pH values, anthocyanins changed color, leading to different hues at various pH levels. The anthocyanin content during the production of refreshing drinks was influenced by many factors and needed consideration to optimize the production process, as the color of the extract changed across different pH ranges, gradually transitioning from dark red to light red between pH values of 1 to 4 [27]. At the same °Brix, the pH values ranging from 3.8 to 4.2 showed a decreasing trend in color intensity due to the increasing pH of the fermentation solution, with a significant difference at the 5% level. It was observed that the color stability of anthocyanins decreased as both the total soluble solids and pH increased.

3.5. Influence on the sensory values of the product

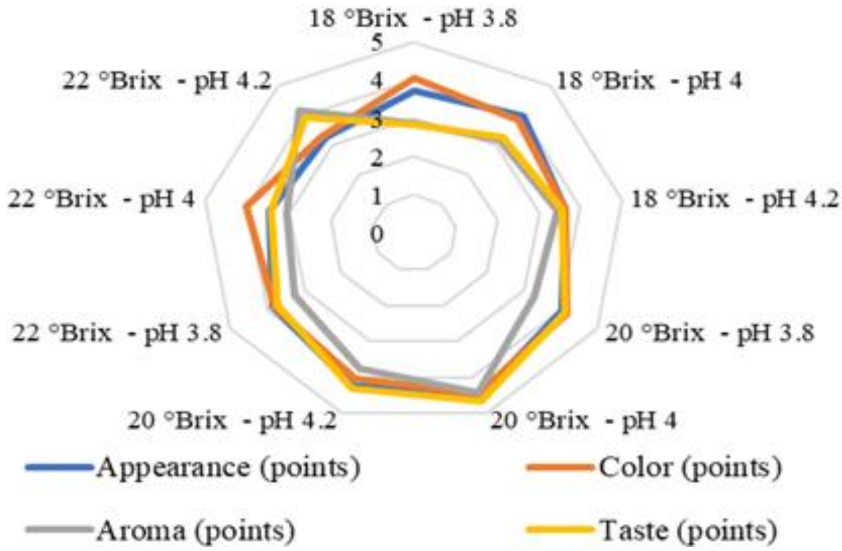

Figure 5. *Sensory scores of the product at different levels of total soluble solids and initial pH values*

In addition, sensory factors are also crucial in determining the quality of the product. The results of the sensory evaluation were affected by the concentration of total soluble solids and the pH of the initial solution (Figure 5).

For the appearance criterion, the treatment of 20°Brix - pH 4 received the highest sensory score for appearance (4.56). The product exhibited a liquid, homogeneous state with no sediment, distinctly different from the other treatments. The varying conditions of °Brix and pH resulted in incomplete fermentation, leaving residual yeast, which caused the product to have sediment and led to a less favorable uneven appearance.

From the results shown in Figure 5, higher concentrations of total soluble solids and pH resulted in a gradual decrease in color intensity. The treatment at 22°Brix - pH 4.2 had the lowest sensory score for color (3.33). However, the treatment at 18°Brix - pH 3.8 presented a color that was not harmonious, appearing slightly too dark and thus received less preference. The treatment at 20°Brix - pH 4 received the highest score (4.46), featuring a moderately bright red color that was visually appealing.

Regarding the aroma criterion in Figure 5, the treatment at 18°Brix - pH 3.8 had the lowest ethanol content (1.53%Vol.), resulting from insufficient sugar for yeast biomass increase, which may have caused yeast death due to nutrient competition. This led to a low aroma score (2.86). The treatment at 20°Brix - pH 4 achieved the highest score (4.43) and the highest alcohol content (4%Vol.), providing a pleasant blend of alcoholic and natural blueberry aromas that many found appealing.

Statistical results for the taste criterion indicated significant differences across treatments. The 18°Brix - pH 3.8 treatment had the lowest alcohol content, leaving a substantial amount of residual sugar, resulting in an overly sweet flavor and receiving the lowest score (2.83). Additionally, the other two treatments at 18°Brix were also less preferred due to a slightly sour taste and low sweetness. The three treatments at 22°Brix had relatively low scores of 3.73, 3.43, and 3.96, primarily because the high total soluble solids led to excessive residual sugar, creating a somewhat sweet and unbalanced flavor that was less popular. In contrast, the three treatments at 20°Brix received relatively high scores of 4.13, 4.66, and 4.3 for pH values of 3.8, 4, and 4.2, respectively. The reason for these higher scores was the moderate soluble solid content that produced a balanced sweetness, making it easy to drink. However, the 20°Brix - pH 4 treatment was rated the highest due to its harmonious sweet-sour flavor profile, pleasant aftertaste, and alignment with contemporary trends in fermented beverages.

V. CONCLUSION

The fermented beverage product from blueberries with suitable technical conditions as an initial total soluble solids of 20°Brix and pH 4 resulted in a product with an alcohol content of 4%Vol., total acid content of 0.3%, total sugar content of 7.43%, and anthocyanin content of 58.36 mg%. The sensory scores for the 20°Brix - pH 4 treatment were all high: appearance (4.54), color (4.46), aroma (4.43), and taste (4.66), resulting in a product with a harmonious sweet-sour flavor, red color, and appropriate ethanol content, which was not excessively high like wine, making it suitable for current trends in fermented beverages.

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