Original Research

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

Nguyen Ngoc Trang[1], Nguyen Ngoc Lan Anh[1], Vi Nha Tran[1,✉]

Cantho University of Technology, Vietnam

ABSTRACT

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

Methods: The study investigated the impact of pH levels (3.8, 4.0, and 4.2) and total soluble solids (18, 20, and 22°Brix) on beverage quality. Sensory quality was evaluated on appearance, color, aroma, and taste of obtained products.

Results: The optimal fermentation occurred at 20°Brix and pH 4.0, yielding a beverage with high alcohol content (4%Vol.), low residual sugar (7.43%), total acidity (0.3%), and anthocyanin content (58.36mg%) after 4 days. Sensory evaluation of this treatment revealed high scores for 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 affected beverage quality, identifying identifying 20°Brix and pH 4.0 as optimal fermentation conditions for blueberry beverages.

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

I. INTRODUCTION

Blueberry (*Vaccinium corymbosum L.*,), a perennial flowering plant native to North America and Europe [1], grows as upright or low-lying shrub with elliptical leaves. Its blue-black berries, ripening from June to September, are round, have a purple sheen, and a sweet, mild taste [2]. Rich in anthocyanins, blueberries protect retinal cells and prevent cardiovascular diseases [3]. They contain bioactive compounds like phenolics, organic acids, anthocyanins, proanthocyanidins, flavonol glycosides, and vitamin C, which exhibit antibacterial and anticancer properties, and may prevent urinary tract infections, dental issues, stomach ulcers, obesity, diabetes, aging, high cholesterol, and biofilm formation [4, 5]. Blueberries, used traditionally in medicine, possess significant biological functions, including antioxidant, anti-inflammatory, and anti-tumor effects [6].

Fermented beverages are natural, low-alcohol (4-6%) drinks produced through incomplete alcoholic fermentation of fruit. These non-distilled beverages retain beneficial nutrients, organic acids,

✉ Corresponding author: Vi Nha Tran
Email: vntran@ctuet.edu.vn
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fragrant esters, and distinctive fruit components developed during fermentation. Due to ongoing biochemical processes even at low temperatures, yeast is typically removed and the beverage pasteurized after fermentation to extend shelf life [7–9].

Cider, an alcoholic apple beverage, is popular in France, Spain, Ireland, Switzerland, and South Africa. The U.S. and Canada have also seen significant growth in cider production. While generally called "cider," diverse production methods result in varied flavors, ranging from sweet to sour or bitter, depending on fermentation ingredients. In Vietnam, cider and fruit wine are often conflated, sparking debate, although demand for fruit-based alcoholic beverages is increasing [10].

Fermentation, defined by Luong Duc Pham [11] as the enzymatic conversion of carbohydrates and other organic compounds by microorganisms, is significantly influenced by total soluble solids and pH levels. These factors, acting through microorganisms or their enzymes, directly impact fermentation rate, yeast growth, and ultimately, the alcohol content, total acids, total sugars, anthocyanin levels, and sensory characteristics of the final product. Therefore, optimizing initial total soluble solids and pH is crucial for creating low-alcohol fermented beverages suitable for all ages, particularly women and children, offering digestive benefits and enhanced meal enjoyment without intoxication. This study aims to determine the ideal total soluble solids and pH values for fermenting blueberry beverages by investigating their effects on the beverage's physicochemical composition and sensory quality.

II. METHODS

2.1. Materials

Blueberries imported 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 of blueberries 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 blueberries were washed, drained, and frozen at (-20 ± 2°C), then thawed at room temperature before use. Other materials included *Saccharomyces cerevisiae* RV100 yeast (ICFOOD Company, Vietnam), sucrose (Vietnam), citric acid (China), sodium carbonate (China), and sodium hydrogen sulfite (China).

2.2. Processing procedure

As shown in Figure 1, 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. 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 saturated sodium carbonate. The juice was then pasteurized with 120 mg/L sodium bicarbonate for 2 hours to eliminate contaminating microorganisms.

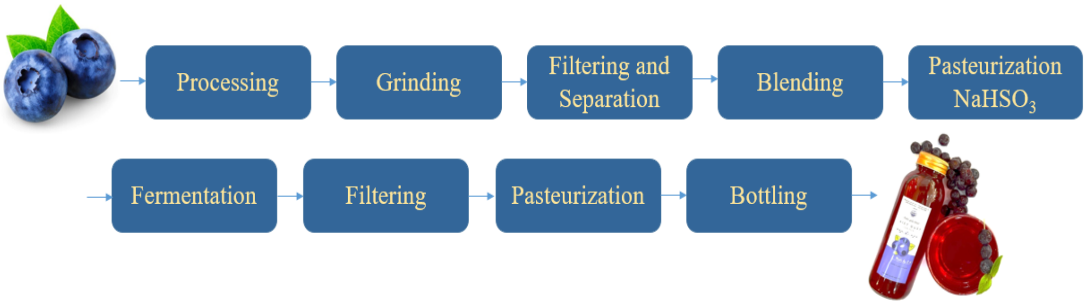

Figure 1. *Diagram of the processing procedure for fermented blueberry beverage*

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* was measured using a pH meter (Basic pH Meter, USA).

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

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

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

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

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

- *Total acid content (%)* was 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 thawed blueberries used in the experiment had the following composition: moisture (89.30 ± 1.42%), total sugar (4.22 ± 0.21%), reducing sugar (2.32±0.12%), total soluble solids (7°Brix), total acid (0.60±0.02%), anthocyanin (152.20±2.24 mg%), vitamin C (18.3±1.27 mg%), and pH (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 variations likely stem from differences in 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].

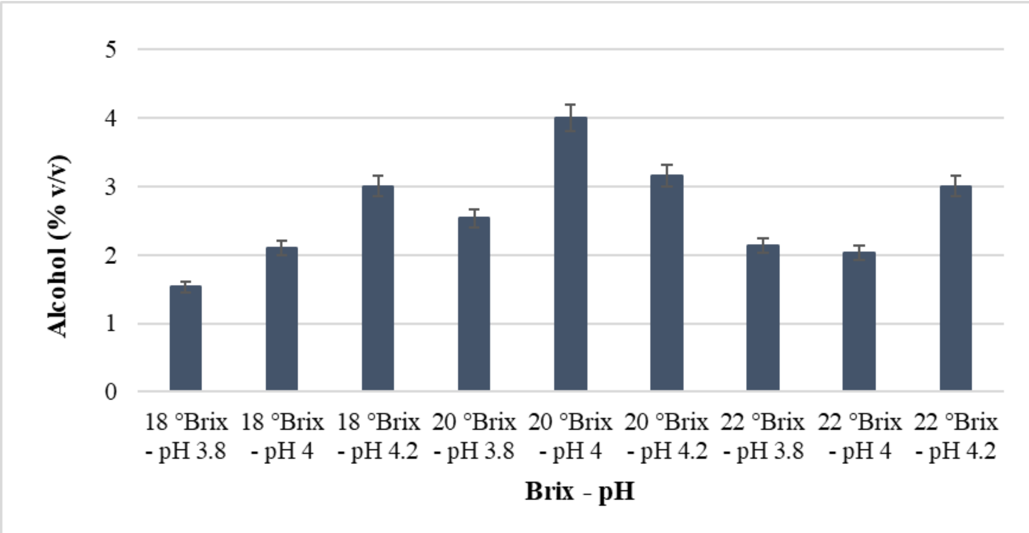

Figure 2. *Alcohol content of the product influenced by the total soluble solids and pH value in the initial fruit solution*

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 4%Vol. ethanol, which was not significantly higher than the fermentation of dragon fruit juice using the *Saccharomyces cerevisiae* RV100 strain (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, fermented blueberry juice (18°Brix, pH 3.8) produced the lowest ethanol content at 1.53%Vol. Increasing total soluble solids to 22°Brix at pH 4 significantly decreased ethanol content (2.03%Vol.) compared to 20°Brix at pH 4 (4%Vol.), while ethanol yield remained unchanged 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 cerevisiae* 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 4.0, 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, due to differences in total soluble solids and the chemical composition between 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° compared to before fermentation under normal alcoholic fermentation conditions [15].

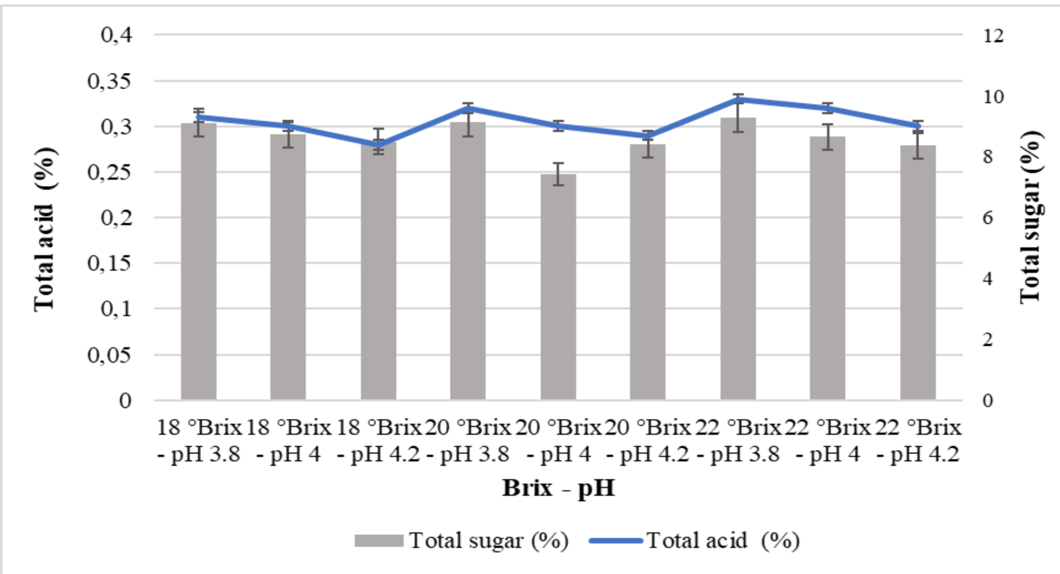

Figure 3. *The total acid content of the product varies according to total soluble solids and pH value in the initial fruit solution*

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 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 mirrored those of fermented dragon fruit juice, showing a post-fermentation pH of 3.5-3.6 compared to a pre-fermentation pH of 4 [21]. It indicated that H^+ ions affected yeast activity. However, a certain correlation existed between H^+ ions and acid content in any solution [15]. While pH might remain stable, acidity in fermented beverages can vary significantly depending on the contamination levels of raw materials and the fermentation solution. Contamination primarily affects acidity due to the production of weakly dissociated organic acids, while pH is less impacted. Consequently, both pH and total acid content should be determined during the production of fermented beverages.As shown in Figure 3, total soluble solids and pH had an interactive effect on total sugar content. At a constant total soluble solids level, increasing pH decreased total sugar content; conversely, at a constant pH, increasing total soluble solids increased total sugar content. Residual sugar content after fermentation ranged from 7.43% to 9.28%, depending on °Brix and pH, representing approximately a 70% reduction from initial levels of 27.6%, 28.8%, and 30% for 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]. Greater sugar content difference after fermentation yields more alcohol and CO_2, reflecting stronger yeast activity. This highlights sugar's role in yeast nutrition and metabolism. Lower total soluble solids post-fermentation confirm active substrate utilization [24].

Figure 3 shows that residual sugar increased with total soluble solids concentration. The residual sugar content was lowest at 20°Brix, which facilitated optimal yeast proliferation and fermentation, resulting in 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. Fermentation at 20°Brix and pH 4.0 yielded the lowest residual sugar (7.43%), indicating optimal conditions for yeast growth and metabolism compared to pH 3.8 and 4.2.

3.4. Study of influence on anthocyanin content of the product

Hyo-Nam et al. (2018) [25] observed that higher sugar concentrations correlated with lower color intensity, suggesting reduced anthocyanin content. While sucrose, maltose, and fructose significantly decreased color intensity, glucose and galactose had minimal impact. As Figure 4 illustrates, total soluble solids increased while anthocyanin content significantly decreased ($p < 0.05$). Consistent with Maarit's findings [26], sugar can destabilize anthocyanins. The reaction between anthocyanins and sucrose forms brown pigments, and increasing sugar content leads to greater anthocyanin degradation and a subsequent decline in color intensity.

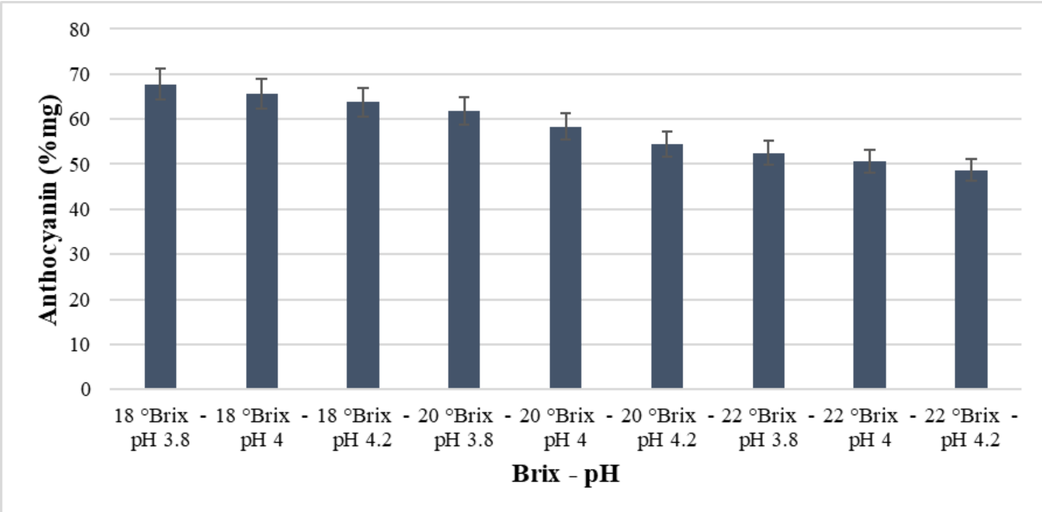

Figure 4. *Anthocyanin content of the product changes according to the total soluble solids and pH value in the initial fruit solution*

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 ($p < 0.05$). 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

Sensory factors are crucial in determining the quality of the product. The sensory scores 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.

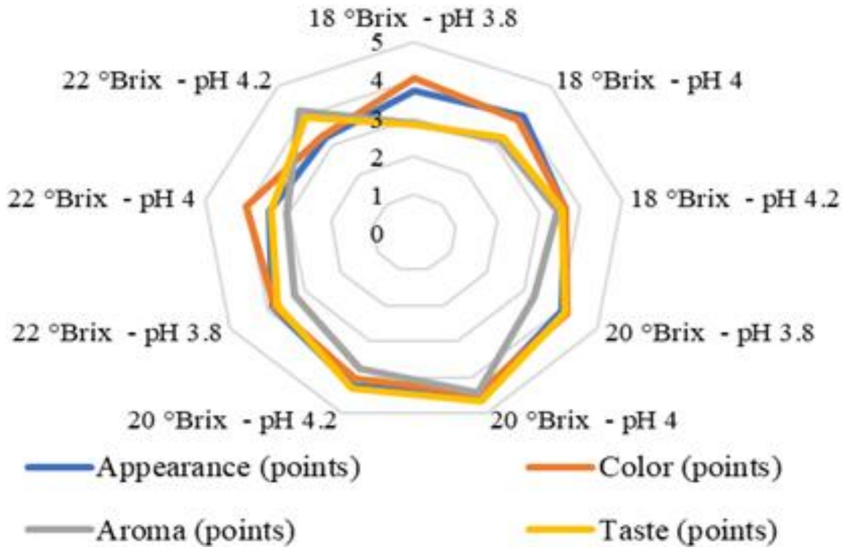

Figure 5. *Sensory scores of the product at different levels of total soluble solids and initial pH values*

Higher concentrations of total soluble solids and pH resulted in a gradual decrease in color intensity (Figure 5). 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 18°Brix - pH 3.8 treatment had the lowest ethanol content (1.53% Vol.) due to limited sugar hindering yeast growth, potentially causing cell death from nutrient competition and resulting in a low aroma score (2.86). Conversely, the 20°Brix - pH 4 treatment achieved the highest score (4.43) with the highest ethanol content (4% Vol.), delivering a desirable balance of alcoholic and natural blueberry aromas.

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

Blueberry fermentation at 20°Brix and pH 4 yielded a beverage with 4% alcohol, 0.3% total acidity, 7.43% total sugar, and 58.36 mg% anthocyanins. Sensory evaluation of this treatment showed high scores for appearance (4.54), color (4.46), aroma (4.43), and taste (4.66), resulting in a balanced sweet-sour flavor, red color, and moderate ethanol content, aligning with current trends in fermented beverages.

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