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Effect of Stevia as a Natural Sweetener on the Quality of Synbiotic Pumpkin Yogurt

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Abstract: Pumpkin (*Cucurbita moschata*) is a potential base ingredient for synbiotic yogurt to its content of antioxidant compounds and Raffinose Family Oligosaccharides (RFO), which act as prebiotics to support probiotic growth. Stevia (*Stevia rebaudiana* Bertoni) offers a high-intensity, calorie-free natural sweetener, making it an alternative to conventional sugar in functional fermented dairy products. This study aimed to examine the effect of stevia concentration on the quality and antioxidant activity of synbiotic yogurt made from pumpkin juice. The novelty of this research lies in the use of stevia as a natural sweetener in pumpkin-based synbiotic yogurt, while simultaneously evaluating its chemical, microbiological, and sensory properties, as well as its antioxidant activity. This experiment employed a Completely Randomized Design (CRD) with four stevia composition treatments and three replicates: P0 (0%), P1 (0.1%), P2 (0.2%), and P3 (0.3%). Data were analyzed using One-Way Analysis of Variance (ANOVA), followed by the Least Significant Difference (LSD) test at a 5% significance level. Increasing stevia concentration significantly affected pH, TTA, Lactic Acid Bacteria (LAB) count, and antioxidant activity; it lowered pH while increasing TTA and LAB counts, all within the limits of the SNI 2981:2009 standard. Stevia concentration did not significantly affect color, aroma, or texture, but it did significantly influence taste, with treatment P2 (0.2%) achieving the highest level of sensory acceptance. The highest antioxidant activity was observed in P3 (0.3%) with an IC_{50} value of 77,388 ppm, categorizing its antioxidant activity as strong. Overall, P3 (0.3%) was determined to be the optimal formulation based on chemical, microbiological, and antioxidant properties, as well as sensory acceptance. These findings demonstrate the practical potential of stevia as a natural sweetener for the development of low-calorie functional dairy products with synbiotic and antioxidant properties.

Keywords: Antioxidant Activity; Product Quality; Pumpkin; Stevia.

Introduction

The rising prevalence of metabolic diseases such as diabetes and obesity due to diets high in sugar and fat has prompted people to switch to healthier functional foods [1]. One of the most widely developed functional foods is yogurt, a dairy product fermented by lactic acid bacteria (LAB) that contains conjugated fatty acids with anti-obesity, anticarcinogenic, and anti-diabetic properties, as well as the ability to balance the immune system [2]. Yogurt contains various types of LAB, with *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei* acting as probiotics, while *Lactobacillus bulgaricus* and *Streptococcus thermophilus* serve as starter cultures for yogurt fermentation [3]. Recent innovations have led to synbiotic yogurt, which synergistically combines probiotics and prebiotics to more effectively support the balance of gut microbiota and maintain digestive health compared to conventional yogurt [4]. The quality of the resulting synbiotic yogurt must comply with SNI 2981:2009, which requires a pH of 3.80–4.50; lactic acid content of 0.5–2.0%; and a minimum starter culture count of 10^7 colonies/g.

The development of synbiotic yogurt can be enhanced with local food ingredients that have high functional value, one of which is pumpkin. Pumpkin grows well in warm climates like Indonesia's and has potential as a food crop because it is rich in nutrients [5]. Pumpkin is rich in carotenoids, vitamins (A, B6, C, K), dietary fiber, essential minerals (K, P, Mg, Fe, Se), and phenolic compounds that may provide health benefits such as antioxidant, antidiabetic, antibacterial, and anticancer activities [6]. The flesh of pumpkin has a reddish-orange color derived from the pigment β-carotene, a carotenoid compound that acts as a natural antioxidan [7]. In addition to β-carotene, pumpkin also contains two major flavonoid compounds, namely catechin and kaempferol, where kaempferol in the form of a glycoside can be hydrolyzed by the β-glucosidase enzyme from LAB during fermentation into a bioactive aglycone form that is more easily absorbed by the body [8]. The antioxidant activity of pumpkin extract is classified as very strong, with an IC_{50} value of 12.13 ppm [9].

The benefits of pumpkin become even more pronounced when it is fermented using BAL. Mixed fermentation with Lactobacillus helveticus and Lactobacillus bulgaricus in pumpkin juice has been shown to significantly increase the levels of vitamin C, total phenolics, flavonoids, and antioxidant capacity compared to non-fermented products [10]. This is due to LAB's ability to hydrolyze complex phenolic compounds into simpler, bioavailable units through the biotransformation process, thereby enhancing free radical scavenging activity [11]. On the other hand, pumpkin contains oligosaccharides, pectin, and soluble fiber that act as natural prebiotics because they are resistant to stomach acid and digestive enzymes, allowing them to be selectively fermented by gut microbiota to support the growth of probiotics [12]. Pumpkin also contains Raffinose Family Oligosaccharides (RFO) specifically raffinose, stachyose, and verbascose which can

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be broken down by the α-galactosidase enzyme in LAB into galactose and glucose, which then undergo glycolysis, thereby reinforcing the role of pumpkin as a prebiotic source in synbiotic yogurt [13].

The characteristic sour taste of yogurt is typically adjusted by adding sweeteners such as sucrose. However, excessive sugar consumption has been directly linked to an increased risk of metabolic diseases. According to the 11th edition of the International Diabetes Federation (IDF) data, the number of people with diabetes in Indonesia in 2024 reached 20.4 million, placing Indonesia fifth in the world with a national prevalence of 11.7%, and is projected to rise to 28.6 million by 2025. This situation underscores the need for healthier sweetener alternatives in yogurt products. Stevia (*Stevia rebaudiana Bertoni*) is a natural, non-caloric sweetener containing steviol glycosides with a sweetness level 70–400 times higher than that of cane sugar [14]. In addition to being calorie-free, steviol glycosides possess diverse biological activities, including antidiabetic, antihypertensive, anti-inflammatory, antioxidant, and anticancer properties, and have been deemed safe by the FDA and EFSA as a sugar substitute in functional foods [15].

The use of stevia in fermented dairy products has shown promising results. A study [16] reported that adding stevia extract to fermented milk can accelerate BAL growth during the fermentation process and increase antioxidant activity compared to products without stevia. A study [17] reported that adding instant stevia powder to yogurt significantly increased phenolic content and antioxidant activity during 14 days of storage at 4°C. Research on pumpkin-based yogurt has also been conducted. A study [18] reported that adding pumpkin filtrate to yogurt can produce a product with characteristics that meet quality standards and achieve good consumer acceptance. Meanwhile, [19] reported that pumpkin juice yogurt with added sucrose produced a pH value consistent with yogurt characteristics. Previous studies indicate that pumpkin has potential for use in yogurt development; however, the sweetener used was sucrose, which does not yet address the need for yogurt development using non-caloric stevia as a sweetener. In contrast, studies by [17] and [16] have demonstrated the potential of stevia in yogurt, but did not use pumpkin as a source of bioactive compounds and prebiotic properties.

Although numerous studies on synbiotic yogurt, pumpkin, and stevia have been conducted, research specifically combining stevia as a natural sweetener in pumpkin-based synbiotic yogurt remains very limited, particularly studies that comprehensively evaluate chemical, microbiological, organoleptic, and antioxidant activity. Therefore, this study was conducted to determine the effect of adding liquid stevia at different concentrations (0%; 0.1%; 0.2%; and 0.3%) on the chemical quality (pH and total titratable acidity), microbiological quality (total viable count), organoleptic quality (color, aroma, taste, and texture), and antioxidant activity of synbiotic yogurt made from yellow pumpkin juice. This study is expected to result in a low-calorie, antioxidant-rich functional food product that meets the SNI 2981:2009 standard and continues to enjoy high public acceptance.

Research Methods

Tools and Materials

Some of the materials used in this study included pumpkin, distilled water, skim milk, Beeru brand stevia extract, a commercial mixed starter culture, oxalic acid, 1% PP indicator, NaOH (Urlab), MRS agar (Merck), $CaCO_3$, cotton, aluminum foil, 0.9% NaCl (Merck), DPPH (TCI), p.a. methanol (Smart Lab), and 70% alcohol.

The equipment used in this study included a knife, a basin, a pot, a stove, a blender, a strainer, a sterile yogurt bottle, a spoon, a micropipette (DragonLab), an incubator, an analytical balance (Adventurer Ohaus), pH meter, beakers, Erlenmeyer flasks, dropper pipettes, burettes, stands, clamps, volumetric flasks, electric hot plate (D Lab MS7-H550-Pro), magnetic stirrer, autoclave (Hiramaya HI CLAVE HVE-50), Petri dishes, laminar air flow cabinet (Thermo Scientific 1300 Series A2), test tubes, test tube racks, plastic beakers, measuring beakers, centrifuge tubes, vortex mixer (Labnet), rotary evaporator (Buchi), UV-Vis spectrophotometer (Shimadzu 1800), and other glassware.

Research Procedure

Experimental Design

This study used a Completely Randomized Design (CRD) with four treatments of liquid stevia concentration, namely P0 (0%), P1 (0.1%), P2 (0.2%), and P3 (0.3%). Each treatment was replicated three times, resulting in 12 experimental units. Each experimental unit was prepared independently, starting from the extraction of pumpkin juice through the fermentation process. Samples for chemical, microbiological, organoleptic, and antioxidant activity analyses were taken from each experimental unit. Measurements taken in triplicate served as analytical replicates and were not counted as biological replicates.

Pumpkin Juice Making

Ripe pumpkins are peeled, cut, and have their seeds and fibers removed; they are then washed thoroughly and steamed for 30 minutes. Once cooled, the pumpkins are blended with water in a 1:2 ratio until a thin puree is obtained, then strained to produce pumpkin juice ready for use [20].

Preparation of Pumpkin Synbiotic Yoghurt

The pumpkin juice and 12% skim milk are placed in a sterile bottle with a total volume of 100 mL, then pasteurized at 70°C for 15 minutes and cooled to a temperature of 40–45°C [20]. Stevia was added at concentrations of 0%, 0.1%, 0.2%, and 0.3%, and the mixture was inoculated with a 0.5% lactic acid bacteria starter consisting of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei* [21]. The mixture was then incubated at 37°C for 6 hours until synbiotic yogurt formed [22].

pH Test

pH measurements were performed using a pH meter calibrated with standard buffer solutions at pH 4.01, 6.86, and 9.18 prior to measurement. A 25 mL sample was placed in a 100 mL beaker. The pH meter electrode was immersed in the sample until a stable reading was obtained. The electrode was rinsed with distilled water and dried before being used for the next sample measurement. Each sample was analyzed three times, and the results were reported as the average value [20].

Total Titratable Acidity (TTA)

The Total Titratable Acidity (TTA) test was performed to determine the lactic acid content by titration. A 10 mL sample was diluted with distilled water to a volume of 100 mL and homogenized; then, 10 mL of the diluted sample was transferred to an Erlenmeyer flask. Two to three drops of a 1% phenolphthalein indicator were added to the solution, and it was titrated with 0.1 N NaOH solution until a stable pink color developed. The test was performed in triplicate, and the total acid calculation was based on SNI [23]. The lactic acid content (%) was determined using the following equation:

$$\text{TTA}(\%) = \frac{\text{V x N x fp x BM}}{\text{W x 1000}} \text{ x } 100\%$$

Description:
V : volume of NaOH solution for titration (mL)
N : normality of NaOH
fp : dilution factor
BM : equivalent weight of lactic acid (90.08)
W : sample weight

Total Lactic Acid Bacteria (LAB)

Total lactic acid bacteria (LAB) were determined using the Total Plate Count (TPC) method on MRS Agar medium. The medium was prepared by dissolving 6.82 g of MRSA powder in distilled water to a volume of 100 mL, then sterilized in an autoclave at 121°C for 15 minutes, with the addition of 1% $CaCO_3$. A 1 mL sample was serially diluted in 0.9% physiological NaCl solution to a dilution of 10^{-7} or 10^{-8}. A 1 mL aliquot of the selected dilution was inoculated into a sterile Petri dish, which was then poured with MRS Agar medium, homogenized, and allowed to solidify. The dishes were incubated at 37°C for 48 hours in an inverted position; the number of colonies was then counted and expressed as CFU/mL [23].

Organoleptic Test

An organoleptic test was conducted using the hedonic method to determine the panelists' preference levels for the color, aroma, taste, and texture of synbiotic yogurt made from pumpkin juice with 0%, 0.1%, 0.2%, and 0.3% stevia added. The test involved 20 untrained UNESA chemistry students as panelists. Panelists were given plain water to neutralize their taste buds between samples. Preference levels were rated using a four-point hedonic scale: 4 = like very much, 3 = like, 2 = dislike, and 1 = dislike very much. This study did not require ethics committee approval because it involved only organoleptic testing with volunteer panelists and posed no health risks; all panelists provided their consent after receiving an explanation of the study.

Antioxidant Activity Test

Antioxidant activity testing was performed using the DPPH method with UV-Vis spectrophotometry. The samples were first concentrated using a rotary evaporator by evaporation over a water bath at 50°C until a thick extract was obtained. A 40 ppm DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of p.a. methanol. A blank was prepared using 2 mL of the 40 ppm DPPH solution and 2 mL of p.a. methanol; it was then incubated for 30 minutes in the dark, after which the wavelength at which the maximum absorbance was observed was determined over the 400–700 nm range until 516 nm was reached. A 1000 ppm stock solution was prepared from the concentrated extract of the synbiotic yogurt with pumpkin juice, which was then diluted to concentrations of 50, 100, 150, 200, and 250 ppm. A 2 mL sample was reacted with 2 mL of 40 ppm DPPH, incubated for 30 minutes in the dark, and then its absorbance was measured in triplicate [23]. The percentage of inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{Ac-A}{Ac} \text{ x } 100\%$$

Description:
Ac = Blank absorbance value
A = Sample absorbance value
The IC_{50} value is determined using a linear regression curve based on the percentage of inhibition obtained, which is then used to classify antioxidant activity

Statistical Analysis

The test results were analyzed using parametric methods; data normality was tested using the Shapiro–Wilk test, and homogeneity of variances was tested using Levene's test. Data that met the assumptions of normality and homogeneity were analyzed using one-way ANOVA at a 5% significance level. If significant differences were found, the analysis continued with a post hoc Least Significant Difference (LSD) test at a 5% significance level to determine differences between treatments. Meanwhile, the organoleptic data were analyzed using the nonparametric Kruskal–Wallis test, followed by the Mann–Whitney test if significant differences were found. All statistical analyses were performed using IBM SPSS Statistics software, version 27. Differences were considered significant if $p < 0.05$.

Results and Discussion

Determination of pH, TTA, and Total LAB

The results of the study on synbiotic pumpkin yogurt with added stevia sweetener indicate a correlation between a decrease in pH, an increase in TTA, and LAB growth during the fermentation process. As the concentration of stevia sweetener increases, pH decreases and TTA and total LAB increase. The following shows the correlations among pH, TTA, and total LAB for each stevia concentration variant (Figure 1).

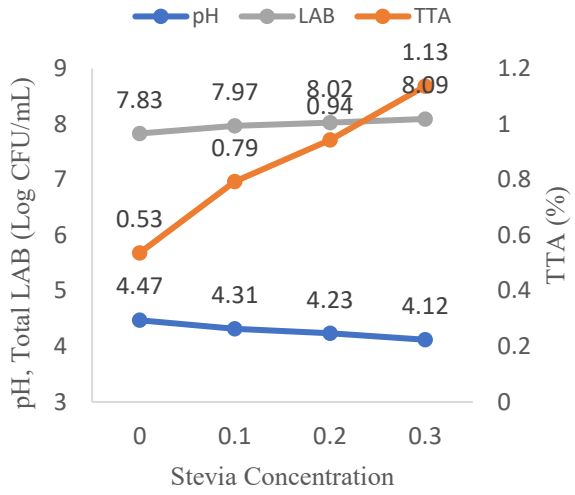


Figure 1. Correlation Graph of Decrease and Increase in pH

Data from the analysis of pH, TTA, and total LAB in synbiotic pumpkin yogurt sweetened with stevia are presented in Table 1. The results of a one-way ANOVA showed $p < 0.05$, indicating that the stevia concentration variants had a significant effect on pH, TTA, and total LAB. The analysis was then continued using the LSD post hoc test to determine significant differences between treatments.

Table 1. Research Results for Synbiotic Pumpkin Yogurt

Conc. (%)	Total LAB (CFU/mL)	TTA (%)	pH
0	$6.79 \times 10^7 \pm 0.01^a$	0.53 ± 0.04^a	4.47 ± 0.01^a
0.1	$9.37 \times 10^7 \pm 0.03^b$	0.79 ± 0.06^b	4.31 ± 0.02^b
0.2	$1.07 \times 10^8 \pm 0.05^c$	0.94 ± 0.04^c	4.23 ± 0.01^c
0.3	$1.24 \times 10^8 \pm 0.02^d$	1.13 ± 0.06^d	4.12 ± 0.02^d

Note: Different letters in the table indicate significant differences at the 5% significance level.

The pH, TTA, and total LAB data obtained above are normally distributed and homogeneous; therefore, they were tested for using one-way ANOVA, followed by LSD post hoc analysis. The results indicate significant differences among the stevia concentration treatments in total LAB, TTA, and pH.

The total BAL results above indicate that the bacterial starter culture comprising *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus plantarum,* and *Lactobacillus casei* utilizes nutrients from the pumpkin extract and the sugar in steviol glycosides for its growth. This increase in total BAL is related to the presence of steviol glycosides in stevia as a nutrient source for lactic acid bacteria, particularly *Lactobacillus plantarum*, which is capable of cleaving a single glucose residue bound to the steviol glycoside backbone and converting it into rubusoside as the main product without damaging the structure of the steviol aglycone, through the activity of the enzyme β-glucosidase [24]. The increase in the number of LAB within the stevia concentration range of 0%–0.3%, from 6.79×10^7 CFU/mL to 1.24×10^8 CFU/mL is consistent with previous studies [25], which showed that an increase in stevia concentration correlates with increased total BAL growth.

This study is consistent with recent research by [22], which specifically evaluated the interaction between stevia and inulin on the fermentation profile using *Lactobacillus acidophilus* in a milk system and in vitro, demonstrating that stevia enhances the survival of *Lactobacillus acidophilus* in both in vitro systems, and can be classified as a potential prebiotic source and sugar substitute for low-sugar dairy products. According to the results of this study, an increase in total BAL was directly observed as stevia concentration increased; however, based on a literature review, the mechanism of glucose residue hydrolysis in steviol glycosides by the β-glucosidase enzyme produced by *Lactobacillus plantarum* is thought to contribute to the breakdown of the steviol glycoside structure [24]. This mechanism provides the basis for explaining the potential role of BAL enzymatic activity in this study, even though β-glucosidase activity was not measured directly. These findings also indicate that the increase in total BAL in this study was most likely not solely due to the direct utilization of steviol glycosides as the primary carbon substrate, but was also influenced by the availability of simple sugars and Raffinose Family Oligosaccharides (RFO) components from pumpkin juice as the primary energy source, with stevia acting as a secondary supporting factor that requires further confirmation through direct measurement of β-glucosidase activity in future studies.

In addition to stevia, pumpkin also contains carbohydrates known as Raffinose Family Oligosaccharides (RFO), namely raffinose, stachyose, and verbascose, which can be utilized as prebiotics with the help of the α-galactosidase enzyme from lactic acid bacteria to break down into galactose and glucose, which are then subjected to glycolysis [20]. Lactic acid bacteria such as *Lactobacillus plantarum* and *Lactobacillus acidophilus* possess α-galactosidase enzyme activity capable of hydrolyzing the bonds in these RFO carbohydrates [26]. This aligns with the theory that pumpkin contains oligosaccharides, pectin, and soluble fiber that have prebiotic potential because they support the growth of beneficial bacteria in the gut. These components consist primarily of galactose, glucose, and fructose, which are highly soluble, resistant to stomach acid and digestive enzymes, and easily fermented by gut microbiota, allowing them to reach the large intestine and support the growth of probiotic bacteria [12].

The increase in total BAL was consistent with the increase in total titratable acidity (TAT) and the decrease in pH in the synbiotic pumpkin yogurt product, as a result of BAL metabolic activity during fermentation, which produced lactic acid from the available sugar substrate. Increased lactic acid production leads to a rise in TAT because this parameter indicates the total amount of acid formed during fermentation, while increased BAL growth produces more lactic acid, leading to the accumulation of H^+ ions and a decrease in pH [27].

Organoleptic Test

Organoleptic acceptance testing was conducted using a hedonic test questionnaire to assess panelists' preferences regarding aspects such as color, aroma, taste, and texture of the pumpkin synbiotic yogurt product. This test was conducted with 20 untrained panelists consisting of UNESA chemistry students. Sensory evaluation was conducted using

a rating scale of 1–4, where 1 indicates “strongly dislike,” 2 indicates “dislike,” 3 indicates “like,” and 4 indicates “strongly like.” The following table shows the effect of adding stevia as a natural sweetener on the organoleptic characteristics of the synbiotic yogurt made from pumpkin juice, as presented in Figure 2.

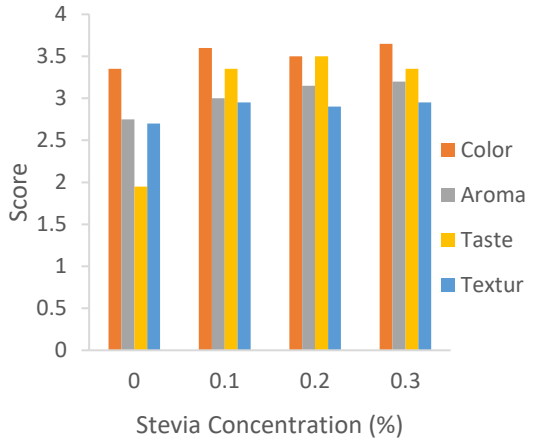


Figure 2. Results of the Organoleptic Evaluation of Color, Aroma, Taste, and Texture of Synbiotic Pumpkin Yogurt

Color

The hedonic test results indicate that the panelists’ level of preference for the color of the synbiotic pumpkin juice yogurt tended to increase with higher stevia concentrations. The highest color score was obtained in the 0.3% stevia treatment, at 3.65, while the lowest score was recorded in the 0% stevia treatment, at 3.35. The color of the resulting product did not change significantly upon the addition of the natural sweetener stevia; this is because the stevia used also has a yellowish hue. Consequently, the product retained its natural yellow color derived from the carotenoid pigments in pumpkin [7]. The bright, uniform yellow colour gave the product a fresher, more appealing appearance to the panellists. Furthermore, the fermentation process during yogurt production does not cause noticeable colour changes, so the pumpkin's visual characteristics remain dominant and enhance the product’s appeal.

Aroma

Hedonic test results showed an improvement with increasing stevia concentration, with the highest score of 3.20 in the 0.3% stevia treatment and the lowest of 2.75 in the 0% stevia treatment. The aroma of yogurt generally originates from volatile compounds produced during fermentation, such as acetaldehyde, diacetyl, lactic acid, and a number of other organic acids produced by lactic acid bacteria. The addition of stevia does not impart a dominant aroma but can help improve panelists acceptance of the product’s aroma. The combination of the characteristic aroma of fermentation and pumpkin juice produced a more preferred aroma profile in treatments with higher stevia concentrations.

Taste

The results of the hedonic test for the taste aspect showed an increase in panelists’ liking scores with the addition of stevia. The highest score was obtained at a stevia concentration of 0.2% (3.50), while the lowest was at 0% (1.95). These results indicate that the addition of stevia enhances panelists acceptance of the taste of the synbiotic pumpkin juice yogurt by creating a balance between sweet and sour flavors. The sour taste in this product is produced by lactic acid formed during fermentation by lactic acid bacteria. In the treatment without stevia, the sour taste tended to be more dominant, making it less preferred by the panelists. The addition of 0.2% stevia provided a sufficient level of sweetness to balance the sourness without masking the yogurt’s distinctive characteristics. In contrast, the addition of 0.3% stevia resulted in a decrease in the score to 3.35, presumably because the sweetness was too strong, thereby disrupting the product’s flavor balance. These results indicate that panelists acceptance of the flavor profile does not necessarily increase with higher stevia concentrations but is instead influenced by the appropriate balance between the sweetness and sourness produced.

Texture

The results of the hedonic test for texture in the synbiotic pumpkin juice yogurt product showed that the highest score, 2.95, was obtained for the 0.1% and 0.2% stevia treatments. These results indicate that the addition of stevia does not significantly affect the product’s texture. The texture of the yogurt is more influenced by the fermentation process and the formation of milk protein gels than by the concentration of stevia used. Furthermore, the use of pumpkin juice in a relatively higher amount compared to skim milk resulted in a more liquid yogurt consistency, making the textural differences between treatments less pronounced. Nevertheless, panelists tended to prefer the texture of the treatments containing stevia because they offered a balance between thickness and ease of consumption.

Antioxidant Activity

The results of the study on synbiotic pumpkin yogurt with added stevia as a sweetener, as measured by antioxidant activity parameters, showed a decrease in the IC_{50} value. Statistical analysis of these results using one-way ANOVA revealed a p-value < 0,05, indicating that variations in stevia concentration had a significant effect on antioxidant activity. The analysis was then continued using the LSD post hoc test to determine significant differences between treatments. As shown in the following table, which illustrates the effect of adding stevia as a natural sweetener on the antioxidant activity characteristics of synbiotic yogurt made from pumpkin juice (Table 2).

The research results show that adding stevia sweetener to synbiotic yogurt made from pumpkin juice can increase antioxidant activity, as indicated by a decrease in the IC_{50} value from 102,393 ppm in the control treatment (0%) to 77,388 ppm at the highest stevia concentration (0.3%), which shifts the antioxidant strength category from moderate to strong based on the classification [28]. Pumpkin is known to contain bioactive compounds with natural antioxidant potential, such as β-carotene, tocopherol, catechin, and kaempferol, which work to stabilize free

radicals through a hydrogen atom donor mechanism [29]; [30]; [8].

Table 2. Antioxidant Activity Test Data for the Pumpkin Synbiotic Yogurt Product

Stevia Concentration (%)	Nilai IC_{50} (ppm)
0	102.393[a]
0.1	90.597[b]
0.2	85.051[c]
0.3	77.388[d]

Note: Different letters in the table indicate significant differences at the 5% significance level.

During the fermentation process, BAL can produce various enzymes that actively hydrolyze complex phenolic compounds into simpler phenolic units that are more easily absorbed by the body. This mechanism is consistent with the findings [11], which revealed that BAL's ability to hydrolyze complex phenolic compounds into simpler units directly enhances its free-radical scavenging capacity. The health benefits of pumpkin are further enhanced after fermentation by BAL, as reported by [10], who found that fermenting pumpkin juice using *Lactobacillus helveticus* and *Lactobacillus bulgaricus* significantly increased vitamin C content, total phenolic content, and antioxidant capacity compared to unfermented pumpkin juice. An approach combining functional ingredients to enhance the antioxidant activity of synbiotic yogurt was also demonstrated [31], who developed synbiotic yogurt with Lacticaseibacillus rhamnosus and aloe vera gel; the addition of 5% aloe vera gel resulted in a functional food with a 68% acceptability rating that retained its functional properties, including enhanced antioxidant and antipathogenic activity, for at least 14 days of refrigerated storage. This comparison demonstrates that the strategy of enhancing antioxidant activity by combining natural functional ingredients in a fermented matrix is not a new phenomenon in the pumpkin-stevia system but rather a consistent pattern observed across various plant-based synbiotic systems, thereby strengthening the external validity of this study's findings.

The increase in antioxidant activity is related to stevia's role in supporting BAL growth during fermentation. Research data show that an increase in stevia levels was consistently accompanied by an increase in total BAL from 7,83 log CFU/mL (0%) to 8,09 log CFU/mL (0.3%). During fermentation, BAL actively breaks down nutrient substrates into lactic acid, acetic acid, and other organic acids, making the product environment increasingly acidic, as indicated by a decrease in pH and an increase in TAT. These acidic conditions contribute to increased antioxidant activity through a proton transfer mechanism, in which organic acids produced by BAL metabolism act as sources of hydrogen ions (H^+) that react with DPPH free radicals, converting them from an unstable radical form into stable, non-reactive DPPH-H [23].

Research Limitations and Implications for Product Development

This study has several limitations that must be explicitly acknowledged. The study was conducted on a laboratory scale with limited production volume; therefore, it cannot yet reflect the consistency of results at an industrial production scale, which involves more complex process variability. Furthermore, this study did not evaluate storage stability, as the parameters of BAL viability, pH, TAT, and antioxidant activity are likely to change significantly during the product's shelf life. A study by [32] showed that goat milk yogurt fermented with *Lactobacillus acidophilus, Bifidobacterium animalis, Lactobacillus casei*, and *Lactobacillus plantarum* underwent significant changes in textural, organoleptic, and biological parameters during 4 weeks of storage at refrigeration temperature, indicating that the positive results obtained in this study (day 0) may not persist consistently throughout the product's shelf life without further evaluation.

From an application perspective, the synbiotic yogurt formulation made from pumpkin juice and stevia has the potential to be developed as a low-calorie functional food because it integrates plant-based ingredients, BAL cultures, and a high-intensity sweetener into a single product. The research results show that a 0.3% stevia concentration yields the best antioxidant activity and chemical and microbiological characteristics, while the organoleptic characteristics remain acceptable. However, commercialization potential cannot be sufficiently determined based on laboratory results alone. Development toward an industrial scale requires formulation optimization, standardization of raw materials and starter cultures, control of the fermentation process, shelf-life evaluation, microbiological safety testing, physicochemical characterization, production cost analysis, and consumer acceptance testing on a larger scale.

Overall, this study contributes by demonstrating that the use of pumpkin juice as a base for functional foods, the synbiotic fermentation process using BAL, and stevia as a low-calorie sweetener can produce yogurt with favorable chemical and microbiological properties, as well as increased antioxidant activity as stevia concentration rises. These findings serve as a foundation for the development of synbiotic yogurt based on local ingredients as an alternative to low-calorie fermented dairy products, while also highlighting the need for further research on the mechanisms of compound changes during fermentation, stability during storage, physical and chemical characteristics, and testing on a larger production scale.

Conclusion

Based on the research results, increasing the stevia concentration to 0.3% had a significant effect on the chemical and microbiological quality and antioxidant activity of synbiotic yogurt made from pumpkin juice, without reducing its overall organoleptic acceptability. A stevia concentration of 0.3% is the optimal formulation because it yields the most favorable chemical and microbiological quality characteristics as well as antioxidant activity, with a BAL count that meets the requirements of SNI 2981:2009 and antioxidant activity classified as strong. These findings indicate that the role of pumpkin extract as a source of functional components, synbiotic fermentation by BAL, and stevia as a low-calorie sweetener has the potential to be developed into a functional fermented dairy product with good quality characteristics and consumer acceptance. Practically, the results of this study can serve as a foundation for the development of low-calorie synbiotic yogurt based on

local ingredients for application in the functional food industry. However, commercial-scale application still requires further research on stability and shelf life, quality changes during storage, consumer acceptance with a larger panel of participants, characterization of the phytochemical profile after fermentation, and process validation at the pilot scale. Further research is also needed to confirm the product's health benefits through biological or clinical testing before health claims can be made.

Author's Contribution

Azizah Sava Aliyah Imawan: responsible for conducting research, collecting data, analysing results, and drafting manuscripts. Rudiana Agustini: acted as corresponding author, reviewing and revising article drafts.

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