Beetroot Stalk Extract as a Functional Colorant for Stirred Yogurt Beverages: Effect on Nutritional Value and Stability during Storage

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Abstract: Betalains are natural red colorants characterized by their stability to anthocyanins, particularly in acidic foods. Beetroot stalks are a good source of betalains, with higher bioactive components than the whole root. Hence, the current study aims to investigate the potential use of beetroot stalk water extract (BSE) as a functional colorant for raspberry-flavored stirred yogurt. For this purpose, the betalains of BSE and their stability at pH 4 and 5 were investigated in addition to the phenolic and flavonoid content. Furthermore, the antioxidant and antimicrobial activities of BSE were characterized. Subsequently, BSE was added to raspberry-flavored stirred yogurt at concentrations of 1 (T1), 2 (T2), and 5% (T3) to study the stability of betalains, the physicochemical properties, the nutritional value, and the viability of lactic acid bacteria during storage (14 days/4 °C). BSE showed a considerable amount of betalains (456.82 mg/L) and phenolics (139.87 mg/g), with a high content of chlorogenic and ferulic acids. The betalains showed greater stability at pH 4 than pH 5 after 14 days of cold storage (275.05 and 247.00 mg/L, respectively). Applying BSE resulted in a functional beverage with high phenolic content (116.55 ± 1.23 mg/g) and flavonoids (71.77 ± 0.57 mg/g) in T3 (5%) compared to the control (95.11 ± 1.12 and 64.72 ± 0.29 mg/g, respectively). The beverages shared high DPPH scavenging activity (IC50 = 71.68 ± 1.30–69.18 ± 0.48) compared with the control (78.47 ± 3.27 µL/mL). BSE significantly increased the betalain level in yogurt from 44.19 ± 0.05 mg/L to 67.86 ± 0.54 mg/L, resulting in pale red beverages with a redness value of 6.38–9.68 on day 1. By day 14, the redness of the treatments decreased by 6–18% compared with the first day, reaching 5.25 ± 0.03 (T1), 7.87 ± 0.03 (T2), and 8.43 ± 0.05 (T3) due to the degradation of betalains. Generally, BSE is a promising natural colorant when added to stirred yogurt, and it has preferable physical and sensory properties, as it improves the stability of the red color throughout cold storage and increases the nutritional quality. The use of beet stalks as a natural and functional colorant is presented for the first time in the current investigation.

Keywords: betalains; phenolic compounds; flavonoids; antioxidant potential; physical properties; beverages

1. Introduction

Yogurt is a functional, probiotic, and palatable beverage consumed worldwide [1]. It is a lactic-acid-fermented milk product [2] with favorable health benefits. Yogurt is a successful vehicle for producing functional and nutraceutical products. In addition to
its ability to balance the intestinal microflora [3], the fortification of yogurt with various plant-based additives could prevent and treat chronic diseases, such as diabetes [4,5] and renal diseases [6]. On the other hand, additives affect the sensory characteristics of yogurt; therefore, it is a challenge to develop acceptable and palatable functional products with stable sensory characteristics. Color is one of the sensory attribute pillars that influences consumer opinion by 62 to 90%. As consumers are aware of the negative impacts of synthetic colorants, food manufacturers are striving to use natural colorants to improve the color and quality of foods [7].

Anthocyanins and betalains are the most common natural, safe red colorants; however, their low stability limits their use [7]. pH is one of the most contributing factors in pigment degradation [8]. Anthocyanin is unstable at an acidic pH (4–5), as it converts to a colorless carbinol pseudobase, resulting in a faded red color [9]. Betalains, on the other hand, are more stable than anthocyanins over a wide pH range (3–7), with higher stability at pH 4–6 [8], so betalains can be used in various foods. Yet, betalains from beetroot have an unpleasant, earthy taste [10]; red pitahaya [11] and Alternanthera brasiliana [12] could be suitable alternatives to betalains, but they are expensive. Therefore, it is important to find other feasible betalain sources.

Beetroot (Beta vulgaris L.) is one of the edible roots, and it yielded nearly 42 million tons worldwide in 2021 [13]. Beetroot stalks account for 35% of the total beetroot weight, as shown by preliminary experiments in a previous study [14]. Accordingly, about 14 million tons of beetroot stalks were produced, which is a massive agricultural waste. Various studies have demonstrated the high nutritional value of beetroot stalks. The stalks are rich in phenolics, including rutin, kaempferol, quercetin, catechin, vanillic acid, vanillin, syringic acid, and ellagic acid [15–17], and contain a considerable amount of betalains (up to 6.59 mg/g) [18–20]. Thus, beetroot stalks are a huge underutilized agricultural waste that could be a cheap, sustainable food additive, providing products with a natural red color and a high content of bioactive components. In addition, beetroot stalks could overcome the flavor limitations of using beetroots in the food industry. Beetroot stalk juice was acceptable at up to 20% in orange juice [14], while beetroot syrup at 20% in yogurt [10] and beetroot peel extract at 5% in a strawberry–whey beverage [21] resulted in unacceptable sensory attributes. However, studies on the utilization of beetroot stalks in food and dairy products have not yet been conducted.

Raspberry is considered one of the preferred yogurt flavors [12], and its anthocyanin is mainly composed of unstable aglycone cyanidin, whose color fades at a yogurt pH (4–5) [9]. Therefore, betalains from beetroot stalks could be a sustainable, inexpensive, nutritious, and novel functional colorant to improve the color stability of raspberry-flavored yogurt and reduce the environmental problems associated with waste disposal. In this context, the present study aimed to investigate the effect of adding beetroot stalk extract on the color stability, sensory attributes, nutritional value, and physicochemical properties of raspberry-flavored yogurt during 14 days of storage at 4 °C. For this purpose, the water extract from beetroot stalks and its biological activity were characterized, and the stability of betalains was studied. Moreover, the effect of adding the extract (1, 2, and 5%) was studied in developing a functional stirred yogurt with the assessment of physicochemical properties, sensory characteristics, bioactive components, and betalain content during storage.

2. Materials and Methods

2.1. Materials

Fresh whole milk (protein 2.6%, fat 3.1%, and carbohydrates 4.6%), raspberries (protein 15.2%, fat 0.31%, and carbohydrates 8.3%), and beetroot stalks (protein 2.5%, fat 0.4%, and carbohydrates 2.8%) were obtained from a local market in Alexandria, Egypt (March 2022). The raw materials were kept at 4 °C until their preparation (within 4 days). Freeze-dried lactic culture YC-X11 (Lactobacillus delbrueckii spp. bulgaricus and Streptococcus thermophilus) was procured from Christian Hansen, Denmark, to produce the stirred yogurt. To determine the antimicrobial activity of beetroot stalk extract, pathogens (Staphylococcus aureus NCTC...
10788, *Escherichia coli* BA 12296, and *Salmonella senftenberg* ATCC 8400) were provided by Dr. Amira Darwish, City of Scientific Research and Technological Applications (SRTA-City), New Borg El Arab, Alexandria, Egypt. The starter culture and pathogens were stored at −18 °C at the Faculty of Agriculture (Saba Basha), Alexandria University, Egypt, until use.

Fine chemicals: Folin–Ciocalteu reagent, DPPH• (2,2-diphenyl-1-picrylhydrazyl), ABTS• (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and phenolic standards (gallic acid, chlorogenic acid, catechin, methyl gallate, caffeic acid, syringic acid, pyrochatechol, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, daidzein, quercetin, cinnamic acid, apigenin, kaempferol, and hesperetin) were purchased from Merck, Darmstadt, Germany. The solvents ethanol and methanol, as well as sodium carbonate, sodium nitrate, sodium hydroxide, aluminum chloride, and trichloroacetic acid (TCA), were purchased from Aljomhoria Company, Alexandria, Egypt.

2.2. Preparation of Beetroot Stalk Water Extract (BSE)

Fresh, high-quality, red beetroot stalks were collected. The green leaves were removed, and then the stalks were washed thoroughly under running water to remove any adhering dust. Afterward, the stalks were dried with paper towels, cut into small pieces (~3 mm), and subjected to water extraction. The stalk pieces were vortexed with water (20 ± 2 °C) at a ratio of 1:10 (w/v) for 1 min. Subsequently, the mixture was centrifuged at 7000 rpm at 20 °C/10 min. The pellets were re-extracted twice under the same conditions [22]. The collected supernatants were combined, filtered, sterilized at 60 °C/3 min using a water bath [10], and stored at −18 ± 2 °C for further analysis and application.

2.3. Biological Characterization of BSE

2.3.1. Determination of Total Phenolics

Briefly, 200 µL of the extract was thoroughly mixed with 1 mL of Folin–Ciocalteu reagent (0.2 N), and then, a sodium carbonate solution (800 µL; 7.5%) was added. The mixture was then incubated in the dark at a room temperature of 20 ± 2 °C/2 h before the absorbance of the mixture was measured at 760 nm using a spectrophotometer (Jenway 6405UV/VIS, Stone, Staffordshire, UK) [23]. The total phenolic content was expressed as mg gallic acid/g stalks.

2.3.2. Determination of Total Flavonoids

In a tube, 1 mL of the extract, 4 mL of d.H2O, and 300 µL of sodium nitrate (5%) were mixed and incubated at 20 ± 2 °C/5 min. Afterwards, 300 µL of aluminum chloride (10%) was added, and the mixture was allowed to stand at 20 ± 2 °C/6 min. Later, 2 mL of NaOH was added, and the volume of the mixture was adjusted to 10 mL with dd H2O. The absorbance of the samples was measured at 510 nm; the total flavonoids were expressed as mg catechin/g stalks [24].

2.3.3. HPLC Profile

HPLC (Agilent, Agilent 1260 series, Stevens Creek BLVD, San Jose, CA, USA) equipped with an Eclipse C18 column (4.6 × 250 mm i.d., 5 µm) was used to identify the phenolics of BSE, as reported by Mansour et al. [25]. In brief, 5 µL of BSE was injected into the HPLC; the separation was performed at 40 °C using water and 0.05% trifluoroacetic acid in acetonitrile as solvents A and B, respectively. The mobile phase was successively programmed at a flow rate of 0.9 mL/min in a linear gradient, as follows: at 0 min (82% A), 0–5 min (80% A), 5–8 min (60% A), 8–12 min (60% A), 12–15 min (82% A), 15–16 min (82% A), and 16–20 (82%A). The multi-wavelength detector was monitored at 280 nm.
2.3.4. Betalain Content

The absorbance of the extract was measured at 353 nm and 438 nm to calculate the betacyanins and betaxanthins, respectively, as mg/L (Equation (1)). The betalains were calculated as mg/L, as shown in Equation (2) [21].

\[
\text{Betacyanins/ Betaxanthins (mg/L)} = \frac{A \times DF \times MW \times V}{\varepsilon L} \quad (1)
\]

where (A) is the sample’s absorbance, (DF) is the extract’s dilution factor, (MW) is the molecular weight of the betacyanins (550 g/mol) and betaxanthins (308 g/mol), (V) is the volume of the extract in mL, (\(\varepsilon\)) is the extinction coefficient of the pigments in water, which is 60,000 L/mol cm (betacyanins) and 48,000 L/mol cm (betaxanthins), and (L) is the length of the cuvette path (1 cm).

\[
\text{Betalains (mg/L)} = \text{Betacyanins (mg/L)} + \text{Betaxanthins (mg/L)} \quad (2)
\]

2.3.5. Antioxidant Activity

**DPPH• Scavenging Activity**

Different concentrations of the extract (0.5 mL) were mixed with 0.5 mL of a freshly prepared DPPH• in methanol (0.3 mM); the mixture was incubated at 25 °C for 20 min. Later, the absorbance of the mixture was measured at 517 nm [26]; the DPPH• inhibition% was calculated according to Equation (3) to determine the IC\(_{50}\) concentrations.

\[
\% \text{ Inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100 \quad (3)
\]

**ABTS• Scavenging Activity**

To prepare the ABTS• working reagent, a mixture of ABTS• solution (7 mmol/L) and potassium persulfate (2.4 mmol/L) (1:1 \(v/v\)) was incubated at 10 ± 2 °C/12 h in the dark. The mixture was then diluted with d.H\(_2\)O (1:60 \(v/v\)) to obtain an absorbance of 0.701 ± 0.01 at 734 nm. Subsequently, 4 mL of the working reagent was added to 10 µL of the extract, and the mixture was incubated for 6 min before the absorbance was read at 734 nm [27]. The ABTS• scavenging activity was calculated using Equation (4) to determine the IC\(_{50}\) concentrations.

\[
\% \text{ Inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100 \quad (4)
\]

2.3.6. Antimicrobial Effect of BSE

The antimicrobial activity of BSE was investigated using the agar well diffusion assay, as described by Gonelimali et al. [28]. In the present study, the antimicrobial activity of BSE was tested against three bacterial strains at the Faculty of Agriculture Saba Basha, Alexandria University, Alexandria, Egypt: one G+ strain (Staphylococcus aureus NCTC 10788) and Escherichia coli BA 12296 and Salmonella senftenberg ATCC 8400 as G− strains. After the overnight incubation of the bacterial strains in a nutrient broth medium at 37 °C, 1 mL of each freshly cultured bacterial strain (10\(^8\) CFU/mL) was placed in sterile Petri dishes. Then, a plate count agar (PCA) medium (Lab M Ltd., Lancashire, United Kingdom) was poured into the plates and mixed with the inoculum, and the plates were allowed to solidify. Subsequently, four wells were formed in each plate using a sterile 6 mm cork borer; 100 µL of the extract was pipetted into each well at different concentrations (0, 5, 10, and 15 mg/mL). The plates were kept at 4 °C/30 min to allow the extract to diffuse into the plates before incubation at 37 °C/18 h. The zone of inhibition was measured and expressed in mm.
2.4. Stability of Betalains at pH 4–5

Yogurt has an acidic pH (~4.6); therefore, we estimated the stability of betalains from beetroot stalks at pH 4–5, as described by Rocha et al. [29]. The extract was mixed with a citric acid buffer (pH 4 and 5) and stored for 14 days/4 °C in the absence of light. Betalains were determined on days 1, 7, and 14 of storage, as previously depicted in Section 2.3.4.

2.5. Preparation of Raspberry Flavor and Stirred Yogurt

To prepare the raspberry flavor, raspberries were washed, pureed with a hand blender, mixed with sugar (1:1), and concentrated to obtain a raspberry concentrate with 65 °Brix. Subsequently, the milk was flavored with the raspberry concentrate (10%), pasteurized in a water bath at 82 °C/20 min, and immediately cooled to 42 °C. The milk was then mixed with the BSE to prepare the treatments (Table 1); the treatments were simultaneously inoculated with the starter culture (2%) and incubated at 42 °C/4–5 h until coagulation was achieved (pH 4.69 ± 0.04) [1]. Later, the yogurt samples were stirred and stored in the refrigerator for 14 days at 4 °C. The analysis was performed on the 1st, 7th, and 14th day of storage.

Table 1. The ingredients of the prepared stirred yogurt samples (%).

<table>
<thead>
<tr>
<th>Whole Milk</th>
<th>Raspberry Concentrate</th>
<th>BSE *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>T1</td>
<td>89</td>
<td>10</td>
</tr>
<tr>
<td>T2</td>
<td>88</td>
<td>10</td>
</tr>
<tr>
<td>T3</td>
<td>85</td>
<td>10</td>
</tr>
</tbody>
</table>

*BSE: beetroot stalk water extract; the concentrations of the BSE were chosen based on preliminary experiments.

2.6. Sensory Evaluation

The color, flavor, odor, taste, consistency, appearance, acidity, and total acceptance of the raspberry-flavored stirred yogurt enriched with BSE were evaluated by 25 panelists (18 females and 7 males, 24–65 years old) on days 1, 7, and 14. The panelists were asked to rate each sensory attribute with nine points on the hedonic scale: 1 = extremely disliked and 9 = extremely liked. Randomly coded BSE-enriched yogurt samples were served to the panelists at 7 ± 1 °C after the control [30].

2.7. Physical Properties of Yogurt

2.7.1. pH and Acidity

The pH of the yogurt was measured at 20 °C during storage. The total titratable acidity (TTA) was determined and calculated according to Equation (5) and expressed as g lactate/100 mL yogurt [31].

\[
\% \text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 0.09 \times 100}{\text{Sample weight}}
\]  

2.7.2. Viscosity

The viscosity was determined at 20 °C ± 2 using a viscometer set to 100 rpm/20 °C and spindle number L3. After 50 s of shearing, the results were reported in mPa·s [32].

2.7.3. Syneresis

The yogurt (5 g) was placed in a #45 Whatman paper in a funnel; the samples were left at 4 °C/120 min. The collected liquid was weighed; the syneresis was calculated according to Equation (6) [10].

\[
\% \text{Syneresis} = \frac{\text{weight of the collected liquid}}{\text{weight of the yogurt sample}} \times 100
\]
2.7.4. Color

The L* (brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) of yogurt were measured to calculate the chroma (C*), hue angle (h), and change in color during storage (ΔE^*ab), according to Equations (7), (8), and (9), respectively [33]. Additionally, the browning index (BI) was calculated based on Equation (10) [34].

\[
C^* = \left( a^{*2} + b^{*2} \right)^{1/2}
\]

\[
h = \left( \tan^{-1} \frac{b^*}{a^*} \times 360 \right) / \left( 2 \times \pi \right)
\]

\[
\Delta E_{ab} = \left[ (L^* I - L^* 0)^2 + (a^* I - a^* 0)^2 + (b^* I - b^* 0)^2 \right]^{1/2}
\]

where L^*0, a^*0, and b^*0 are color values on day 1, and L^*i, a^*i, and b^*i are color values during storage.

\[
BI = 100 \times \frac{x - 0.31}{0.17}
\]

where \( x = \frac{a^* + 1.75L^*}{(5.645L^* + a^* - 0.3012b^*)} \).

2.8. Bioactive Components, Betalains, and Antioxidant Activity of Fortified Yogurt

The bioactive components of the yogurt were extracted, as described by Flores-Mancha et al. [35], to determine the total phenols and flavonoids and the DPPH• scavenging activity, as previously described in Section 2.3.1, Section 2.3.2 and Section DPPH• Scavenging Activity. Additionally, the betalains were determined according to Section 2.3.4 after the supernatant was filtered through 0.45-mm porous paper, mixed with 4% TCA (1:1 v/v), homogenized for 3 min, centrifuged at 10,000 rpm at 25 °C/10 min, and filtered through a 0.45-mm porous paper.

2.9. Viability of Lactic Acid Bacteria (LAB)

The LAB viability in the yogurt was evaluated using the dilution method in 0.1% sterile peptone water (1:9 w/v). The dilutions were then cultured on the MRS agar; the plates were incubated at 37 °C/24 h [3], and the colonies were expressed as cfu/mL.

2.10. Shelf Life and Microbial Load of Yogurt

Ten-fold serial dilutions were prepared from the yogurt; the dilutions (1 mL) were transferred to Petri dishes filled with violet red bile agar (VRBA) and rose Bengal agar-based media to enumerate coliform and yeasts and molds, respectively. The colonies were expressed as Log10 cfu/mL [21].

2.11. Statistical Analysis

Statistical analysis was performed with the program IBM SPSS 25; the data obtained were expressed in means ± SDs. The data were analyzed using one-way ANOVA to determine the significance between means. The means were compared using Duncan’s test at a 95% confidence level (p < 0.05).

3. Results and Discussion

3.1. Characteristics of Beetroot Stalk Extract (BSE)

3.1.1. Phenolic and Flavonoid Content

Beetroot stalks are an abundant source of bioactive components; they contain more phenolics than the whole root [15,16]. BSE showed a high phenolic content of 139.87 ± 0.45 mg/g, with a considerable content of flavonoids (31.17 ± 2.24 mg/g), accounting for almost 23% of the detected phenols (Table 2). In the current work, the water extract from beetroot stalks contained more phenolic and flavonoid content than the 10.40–14.58 mg/g phenols [15,16].
and 4.84 mg/g flavonoids [16] obtained from an ethanol extract of dried stalks. The discrepancy between these results suggests that the drying of stalks, the extraction conditions, and the variety of the beetroots might significantly affect the content of phenolics and flavonoids in stalks. The findings of the current study emphasize that beet stalks are a good source of phenolic compounds, which might play an effective functional role when added to food products.

Table 2. Characterization of beetroot stem water extract (BSE).

<table>
<thead>
<tr>
<th>Bioactive Components</th>
<th>Value (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolics (mg/g)</td>
<td>139.87 ± 0.45</td>
</tr>
<tr>
<td>Total flavonoids (mg/g)</td>
<td>31.17 ± 2.24</td>
</tr>
<tr>
<td>Betacyanins (mg/L)</td>
<td>254.38 ± 13.61</td>
</tr>
<tr>
<td>Betaxanthins (mg/L)</td>
<td>202.45 ± 4.99</td>
</tr>
<tr>
<td>Betalains (mg/L)</td>
<td>456.82 ± 18.60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phenolics profile (µL/mL)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>36.52</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>17.57</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>8.43</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>4.46</td>
</tr>
<tr>
<td>Methyl gallate</td>
<td>0.66</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.43</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>0.12</td>
</tr>
<tr>
<td>Catechin</td>
<td>8.89</td>
</tr>
<tr>
<td>Rutin</td>
<td>8.65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antioxidant activity (µL/mL)</th>
<th>Value (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH• IC₅₀</td>
<td>207.33 ± 4.19</td>
</tr>
<tr>
<td>ABTS• IC₅₀</td>
<td>64.69 ± 0.71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antimicrobial activity (mm)</th>
<th>Value (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em> NCTC 10788</td>
<td>13.10 ± 0.14</td>
</tr>
<tr>
<td><em>Escherichia coli</em> BA 12296</td>
<td>14.70 ± 0.42</td>
</tr>
<tr>
<td><em>Salmonella senftenberg</em> ATCC 8400</td>
<td>19.80 ± 0.42</td>
</tr>
</tbody>
</table>

Data are presented as means ± SDs.

3.1.2. Phenolics Profile of BSE

Among the detected phenolic compounds, chlorogenic acid dominated, accounting for about 40% of the phenols (Table 2, Figure S1), which is consistent with the previous literature [15,17]. Similarly, high concentrations of ferulic acid (20%), followed by rutin (10%), catechin (10%), gallic acid (9%), and syringic acid (5%), were detected in BSE. However, previously, Abdo et al. [16] reported that rutin and catechin were dominant in the ethanol extract of dried stalks, while chlorogenic and gallic acids were absent. Moreover, coumaric acid, methyl gallate, and caffeic acid were detected in BSE, but at very low concentrations. The high content of chlorogenic, ferulic, rutin, and other compounds make the beet stalk extract a good source of antioxidants and antimicrobial agents, which may play a crucial role in extending the shelf lives of food products.

3.1.3. Betalain Content

Betalains are a water-soluble, nitrogenous pigment with potent free radical scavenging activity, consisting of red–violet betacyanins and yellow–orange betaxanthins [36]. BSE contains a considerable amount of betalains (456.82 ± 18.60 mg/L) (Table 2), which was within the range of 0.21 mg/g to 6.59 mg/g reported in the literature [16,19,20].

The red–purple betacyanins accounted for the majority of betalains (56%) compared to the yellow–orange betaxanthins (44%), reflecting the red color of BSE. The obtained result is consistent with the ratio of betacyanins to betaxanthins determined in the ethanol extract of the stalks [16]. However, the results are in contradiction with the high content of
betaxanthins in beet stalks (5.31 mg/g) reported by Maran and Priya [20], which accounts for about 80% of betalains; this might be due to the variance in beetroot cultivars and extraction conditions. Generally, due to the high antioxidant power of betalains with their extensive red color, they can exhibit a dual function in food products as a colorant and antioxidant agent at the same time.

3.1.4. Antioxidant Activity

In addition to betalains, beetroot stalks contain a significant concentration of phenols and flavonoids, which boost the antioxidant activity of BSE. BSE had considerable radical scavenging activity; 207.33 and 64.69 µL/mL of the extract scavenged 50% of the DPPH• and ABTS• radicals, respectively (Table 2). Our results agree with those of Ben Haj Koubaier et al. [15] and Lasta et al. [17], who reported that 200 µg/mL and 364 µg/mL of beetroot stalk extract scavenged 85% and 50% of DPPH• radicals, respectively. The scavenging activity of BSE might be related to the high content of chlorogenic acid (Table 2), which has strong antioxidant activity due to its high polyphenolic structure that directly eliminates hydroxyl radicals and superoxide anions [37,38]. In addition, BSE contains large amounts of ferulic acid, which has great potential to scavenge hydroxyl groups [39], as well as fair amounts of the potent antioxidants catechin and rutin [40] and betalains, which exhibit strong scavenging activity for superoxide anions. Betalains scavenged three times more DPPH than vitamin C, reflecting their potent antioxidant activity [40,41].

3.1.5. Antimicrobial Activity

Food spoilage due to microorganisms is one of the biggest problems for food producers. Apart from economic losses, foodborne contamination is also a food safety concern that should be avoided. The addition of natural antimicrobials to extend the shelf lives of products is a new trend due to growing health awareness. The potent antioxidant activity of beetroot stalks could imply antimicrobial activity against various microbial species.

Generally, extracts reveal intermediate and efficient antimicrobial activity when they inhibit bacterial growth by 11–15 mm and ≥16 mm, respectively [42]. In this regard, BSE efficiently inhibited the growth of G− Salmonella senftenberg, while it showed intermediate antimicrobial activity against G+ Escherichia coli and G+ Staphylococcus aureus, respectively (Table 2). The current results reveal the promising antimicrobial activity of BSE: the water extract of stalks showed higher antimicrobial activity than the water extract from beetroot peel, which showed no antimicrobial activity against Staphylococcus aureus and achieved a lower inhibition of Salmonella senftenberg and Escherichia coli [21]. Additionally, methanol extracts from beetroot stalks showed a lower inhibitory effect against Staphylococcus aureus (IZ = 10) [43]. The antimicrobial effect of the BSE extract might be attributed to the ability of phenolic compounds to damage the bacterial membrane, inhibit virulence factors such as enzymes and toxins, and suppress bacterial biofilm formation.

3.2. Stability of Betalains at pH 4–5

Generally, the content of betalains was not affected by the change in pH on days 1 and 14 (p > 0.05) (Table 3). However, on day 7, the betalains were higher at pH 5 than at pH 4 (p < 0.05), as at a low pH, betacyanins undergo dehydrogenation and decarboxylation, which change their structure, shift their maximum absorption wavelength, and decrease their content [12]. On the other hand, the behavior of betalains during storage is affected by pH. At pH 4, the content of betalains decreased by almost 45% to 250.75 ± 6.68 mg/L on day 7, but on day 14, the betalains regenerated by roughly 25% and reached a value of 275.05 ± 12.12 mg/L compared to day 7. However, at pH 5, the betalains decreased by 30% and 40% during storage to 285.22 ± 2.01 (on day 7) and 247 ± 0.97 mg/L (on day 14), respectively. The obtained results reflect the high stability of betalains in the pH of the prepared yogurts and during storage. Similarly, Rocha et al. [29] reported that increasing the pH from 3 to 5 increased the stability of betalains during storage.
### Table 3. Betalains’ stability at pH 4 and 5 during storage.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH 4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betacyanins (mg/L)</td>
<td>247.5 ± 12.96</td>
<td>147.13 ± 7.13</td>
<td>152.17 ± 11.67</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>Betaxanthins (mg/L)</td>
<td>199.88 ± 8.62</td>
<td>103.63 ± 0.45</td>
<td>122.88 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>c,*</td>
<td>b</td>
</tr>
<tr>
<td>Betalains (mg/L)</td>
<td>447.38 ± 21.58</td>
<td>250.75 ± 6.68</td>
<td>275.05 ± 12.12</td>
</tr>
<tr>
<td><strong>pH 5</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betacyanins (mg/L)</td>
<td>214.5 ± 1.3</td>
<td>157.21 ± 0.65</td>
<td>138.88 ± 5.83</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td>Betaxanthins (mg/L)</td>
<td>191.54 ± 2.27</td>
<td>128.01 ± 1.36</td>
<td>108.12 ± 6.81</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b,*</td>
<td>c</td>
</tr>
<tr>
<td>Betalains (mg/L)</td>
<td>406.04 ± 0.97</td>
<td>285.22 ± 2.01</td>
<td>247.00 ± 0.97</td>
</tr>
</tbody>
</table>

Data are presented as means ± SDs. Means with different superscripts (a–c) are statistically different at \( p < 0.05 \); * means of the same parameter at a different pH are statistically different at \( p < 0.05 \).

### 3.3. Sensory Evaluation

Sensory evaluation showed that BSE enhanced the acceptability of the treatments more than the control. On day 1, BSE insignificantly improved the odor, taste, flavor, consistency, appearance, and acidity of the yogurt (Figure 1A). However, increasing BSE significantly improved the color of the treatments compared to the control, which was correlated with the improvement in the overall acceptance of the treatments \( r = 0.95 \). The increase in the red color’s intensity could influence mouthfeel and taste due to color–taste synesthetes [44]. Additionally, the stalks contain a low but considerable amount of sugar [16], which could partially affect the taste.

![Figure 1](image_url)

**Figure 1.** Sensory evaluation of raspberry-flavored stirred yogurt enriched with BSE at 0% (control), 1% (T1), 2% (T2), and 5% (T3) on day 1 (A), day 7 (B), and day 14 (C) of storage. Sensory attributes that share different lowercase letters (a–c) are statistically different \( p < 0.05 \); attributes without letters are not statistically different \( p > 0.05 \).
After 7 days, the color sharpness decreased (Figure 1B) with no statistically significant difference between the control (6.8) and the treatments (T1 (6.6), T2 (7.4), and T3 (7.4)). On the other hand, the acceptability of the other attributes was statistically different between the samples. The appearance of T2 and T3 was very acceptable (score = 9) compared to T1 (8) and the control (7.4). The acidity also changed during storage: the control recorded the lowest acceptability (6.6) compared to T3 (8.2), reflecting the low taste acceptability of the control compared to T3. Thus, T3 and T2 recorded the highest acceptability scores (8.4 and 8.2, respectively) compared to T1 (7.4) and the control (7.4). The acidity scores revealed a strong negative correlation with TTA on day 7 ($r = -0.84$). After 14 days, the attributes revealed a significant decrease in their appeal. However, T3 and T2 remained the most preferred beverages (Figure 1C). The odor, taste, and appearance were better for the treatments than for the control ($p < 0.05$); the acidity was also rated better for the treatments, which correlated negatively with the TTA values ($r = -0.92$). The color of T3 was the best (8.2) compared to the control (6), T1 (6.2), and T2 (7.8). Therefore, the acceptability of T3 was higher than that of T2, T1, and the control.

Generally, the overall beverage acceptability was influenced by storage; acceptability correlated more strongly with color and appearance ($r = 0.97$ and 0.98, respectively). Our result suggests that BSE can be added at higher concentrations (>5%) without affecting flavor and taste.

3.4. Physicochemical Properties

3.4.1. pH and Acidity

BSE caused a slight increase in pH to 4.70 ± 0.00 (T1), 4.71 ± 0.01 (T2), and 4.75 ± 0.03 (T3) compared with the control (4.63 ± 0.06) (Figure 2A), which was due to the neutral pH of BSE (pH = 6.55) and the low LAB growth rate in the treatments because of the antimicrobial effect of the extract. However, during storage, the pH decreased in all samples ($p < 0.05$). By day 7, the pH of the treatments and the control ranged from 4.45 to 4.47, with no statistically significant difference among the samples. On day 14, the pH decreased further by 8–11% compared to day 1, with pH values of 4.07 (the control), 4.20 (T1), 4.32 (T2), and 4.23 (T3).

The acidity of the control yogurt (0.71%) was higher than that of T1 (0.68%), T2 (0.67%), and T3 (0.65%) (Figure 2B). As noted, BSE significantly decreased the acidity of the beverages due to inhibiting the growth of LAB on day 1. On day 7, the acidity of all samples was significantly increased, reaching 0.78, 0.72, 0.71, and 0.68% in the control, T1, T2, and T3, respectively. At the end of storage, the acidity reached its highest values in all samples; the acidity increased by 14, 13, 12, and 11% in the control, T1, T2, and T3, respectively, compared to day 1.

The decline in pH and increase in acidity during storage are due to the post-acidification of LAB, as *Lactobacillus bulgaricus* are acid-tolerant bacteria that constantly produce lactic acid during storage [45].

3.4.2. Viscosity

Viscosity reflects the consistency and firmness of yogurt [1]. Generally, the viscosity of the treatments was lower than that of the control during the storage days ($p < 0.05$) (Figure 2C). On day 1, the viscosity of T1, T2, and T3 was 16, 22, and 28% lower than the control (62.33 ± 3.21 mPa.s) ($p < 0.05$). BSE probably affected the microstructure of the yogurt’s gel, resulting in network breakup and low viscosity [46]. The viscosity of the final product was affected by the properties of the additives: adding phycocyanin extract [46] and beetroot juice [35] decreased the viscosity of the yogurt compared to the control, while adding flaxseed [1] and passion fruit [47] increased the viscosity of the yogurt.

On day 7, the viscosity of the samples increased slightly compared to day 1 ($p > 0.05$). After 14 days, the viscosity increased significantly compared to day 1, reaching 72.33 ± 2.52 (the control), 61.33 ± 1.15 (T1), 64.33 ± 4.04 (T2), and 60.00 ± 5.57 (T3). The increase in the viscosity of yogurt during storage could be attributed to the constant LAB metabolic activity [46]; the rise in acidity increases the firmness of the protein and, thus, the quality.
of the yogurt [1]. Besides, the gel structure of yogurt may rearrange during storage, which increases the viscosity [48]. The obtained result is consistent with the previous literature [46–48].

Figure 2. Physical characteristics of raspberry-flavored stirred yogurt enriched with BSE at 0% (control), 1% (T1), 2% (T2), and 5% (T3) on days 1, 7, and 14 of storage. (A) pH value, (B) total titratable acidity (TTA) expressed as g lactic acid/100 mL, (C) viscosity in mPa.s, and (D) syneresis %. Different lowercase letters (a–c) indicate statistical differences between the different beverages on the same storage day ($p < 0.05$); different uppercase letters (A–C) indicate the statistical differences between each beverage during the storage days ($p < 0.05$).

3.4.3. Syneresis

Syneresis is a crucial quality indicator for yogurt [11]. Syneresis increased in the yogurt samples with the increase in BSE concentration ($p < 0.05$). The syneresis of the treatments was higher than that of the control ($19.50 \pm 0.71\%$), ranging from $20 \pm 0.00\%$ to $25 \pm 0.00\%$ (Figure 2D), which could be due to the low viscosity of the treatments compared to the control [48]. The state of the additive affects syneresis: adding powders decreases syneresis, while adding liquids increases syneresis. Gengatharan et al. [11] reported that betacyanins powder from pitahaya stabilized milk proteins and decreased the syneresis of yogurt, while the addition of beetroot juice increased syneresis [35].

During storage, syneresis increased significantly in all yogurt samples due to post-acidification [11], as the change in pH leads to a breakup of the casein network and increases syneresis [46]. T1 was similar to the control (syneresis $= 22.50 \pm 0.00\%$ and $22.50 \pm 0.65\%$ on day 7 and $25.50 \pm 0.70\%$ and $24.50 \pm 0.71\%$ on day 14). However, T2 and T3 had the highest syneresis values during storage ($25.50 \pm 0.00\%$ and $26.50 \pm 0.70\%$ on day 7 and $26.50 \pm 0.71\%$ and $29.50 \pm 0.71\%$ on day 14, respectively).
3.4.4. Color

On day 1, the color of the control was pale yellow; it had the most lightness and yellowness ($L^* = 81.56 \pm 0.08; b^* = 6.38 \pm 0.11$) and low redness ($a^* = 1.85 \pm 0.03$) (Figure S2). BSE reduced the lightness and yellowness of the treatments compared with the control ($p < 0.05$), reaching 77.65–73.67 and 5.32–1.9, respectively. On the other hand, the redness of the treatments increased significantly, reaching 6.38 ± 0.04 (T1), 8.41 ± 0.06 (T2), and 9.68 ± 0.03 (T3) (Figure S1B), which was strongly correlated with the noticeable color change of the treatments compared with the control ($p < 0.05$) ($r = 0.99; \Delta E^{*ab} = 6.08 \pm 0.05$ (T1), 10.52 ± 0.02 (T2), and 10.78 ± 0.03 (T3)) (Figure S3). Similarly, beetroot syrup [29] and beetroot extract [49] led to similar changes in the color parameters of yogurt samples, as betacyanins darkened their color [33]. In this regard, chroma ($C^*$) significantly increased from 8.31 ± 0.01 to 9.86 ± 0.03 in the treatments compared to the control (6.64 ± 0.11) (Figure 3A). Similarly, BI was increased by adding BSE of 6.51–9.29 to the treatments compared to 2.40 ± 0.04 in the control (Figure 3C). The hue angle ($h$) declined and reached 11.10 ± 0.05 in T3 compared to 73.83 ± 0.04 in the control (Figure 3B), reflecting the change of color toward red, which is strongly correlated with betalains ($r = 1.00$).

By day 7, redness ($a^*$) was slightly reduced in the treatments (5.54 ± 0.01–8.93 ± 0.04; $p < 0.05$) but higher than in the control (1.81 ± 0.01) (Figure S2B). Similarly, yellowness decreased in the control and all treatments except T3, in which it was significantly higher than on day 1 (2.38 ± 0.03) (Figure S2C). The change in yellowness and redness resulted in a decrease in chroma and an increase in hue angle ($p < 0.05$). $C^*$ increased significantly with an increasing BSE concentration in the treatments and ranged from 7.45 ± 0.00 to 9.24 ± 0.03.
compared to the control (5.53 ± 0.05) (Figure 3A). On the other hand, color sharpness decreased, with hue angles of 70.91 ± 0.33 (the control), 41.95 ± 0.15 (T1), 25.19 ± 0.23 (T2), and 14.92 ± 0.24 (T3) (Figure 3B). The BI values of the samples declined during storage \((p < 0.05)\); however, the BI of the treatments was 59–73% higher than that of the control (2.31 ± 0.01) (Figure 3C). The change in color during storage was more pronounced in the control after 7 days \((\Delta E^{*ab} = 3.33 ± 0.15)\) than in the treatments \((\Delta E^{*ab} = 1.38 ± 0.06 \text{ (T1)}, 0.77 ± 0.14 \text{ (T2)}, 1.15 ± 0.10 \text{ (T3)})\) (Figure 3D).

At the end of storage, the redness of the treatments decreased significantly to 5.25–8.43 compared with day 1 (Figure S2B), which correlated with the change in betalains \((r = 0.91)\). On the other hand, yellowness increased in all treatments, especially T3, in which it increased by almost 40% to 3.06 ± 0.06 compared with day 1 (Figure S2C), which was due to the degradation of betacyanins to yellow components \([2]\). The change in yellowness and redness increased the hue angles of the treatments to 45.91 ± 0.00 (T1), 24.70 ± 0.09 (T2), and 19.95 ± 0.40 (T3) (Figure 3B), and the chroma decreased to a range of 7.53–8.97 (Figure 3A). The color change was more pronounced in the control than in the treatments, \(\Delta E^{*ab} = 3.78 ± 0.10 \text{ (the control)}, 1.97 ± 0.02 \text{ (T1)}, 0.69 ± 0.07 \text{ (T2)}, \text{ and } 1.88 ± 0.07 \text{ (T3)}\) (Figure 3D). Our results are consistent with those of Flores-Mancha et al. \([2]\) and Ghasempour et al. \([49]\), who reported that adding beetroot to yogurt resulted in differences in chroma, hue angle, and color during storage. Similarly, Schneider-Teixeira et al. \([12]\) found that the color change of yogurt with beetroot was negligible during storage, indicating the high stability of betalains due to the regenerative ability of the pigment. The BI was lower on day 14 in the control and the treatments than on day 1, reaching 2.38 ± 0.02 (the control), 5.38 ± 0.03 (T1), 8.03 ± 0.03 (T2), and 8.18 ± 0.01 (T3) (Figure 3C).

3.5. Bioactive Components of Yogurt and Their Stability during Storage

3.5.1. Total Phenolic and Flavonoid Content

BSE in T1 and T2 had no effect on phenolic content compared to the control \((p > 0.05)\); however, BSE significantly increased the phenolic content of T3 compared to the other samples (Figure 4A). The control, T1, T2, and T3 had 95.11 ± 1.12, 99.78 ± 3.24, 101.20 ± 6.60, and 116.55 ± 1.23 mg/g phenolics, respectively, on day 1. The flavonoids of T1 (67.94 ± 1.43 mg/g), T2 (69.35 ± 0.57 mg/g), and T3 (71.77 ± 0.57 mg/g) were higher than those of the control (64.72 ± 0.29 mg/g) on day 1 \((p < 0.05)\) (Figure 4B). Our results are consistent with the findings of Schneider-Teixeira et al. \([12]\), Asiimwe et al. \([47]\), and Kulaitiėnė et al. \([50]\), who reported that enriching yogurt with \textit{Alternanthera brasiliana}, passion fruit, and beetroot powder increased the phenolic content.

Interestingly, the phenolic content of T1 and T3 increased significantly on day 7 compared with day 1, reaching 104.18 ± 2.09 and 125.45 ± 2.46, respectively (Figure 4A). However, the increase in the phenolic content of the control (98.83 ± 2.57 mg/g) and T2 (103.97 ± 4.25 mg/g) was insignificant compared with day 1. Likewise, the flavonoid content of T2 and T3 increased significantly on day 7, reaching 79.64 ± 3.14 and 79.84 ± 0.59 mg/g, respectively. Meanwhile, the flavonoids slightly increased in T1 and slightly decreased in the control compared with day 1 \((p > 0.05)\) (Figure 4B). By day 14, the phenolics decreased by 21, 5, 4, and 10%, while the flavonoids declined by 17, 26, 25, and 8% in the control, T1, T2, and T3, respectively, compared with day 1 \((p < 0.05)\).

Similarly, the phenols increased during storage in yogurt containing turmeric, blue pea \([45]\), and beetroot \([2]\), as the high metabolic activity of LAB detected on day 7 resulted in new phenolic acids and increased the total phenolic content \([51]\). Additionally, hydrolysis of the polyphenol conjugate of the additive could contribute to the recovery of phenols during storage \([2]\).
Figure 4. Bioactive components of raspberry-flavored stirred yogurt enriched with BSE at 0% (control), 1% (T1), 2% (T2), and 5% (T3) on days 1, 7, and 14 of storage. (A) total phenolics (mg/mL), (B) flavonoid content (mg/mL), (C) betacyanins (mg/L), (D) betaxanthins (mg/L), (E) betalains (mg/L), and (F) IC50 of DPPH• (µL/mL). Different lowercase letters (a–d) indicate statistical differences between the different beverages on the same storage day (p < 0.05); different uppercase letters (A–C) indicate the statistical differences between each beverage during the storage days (p < 0.05).

3.5.2. Betalain Content

BSE enriched T1, T2, and T3 with a concentration-dependent amount of betalains (p < 0.05); 44.19 ± 0.05, 62.32 ± 2.30, and 67.86 ± 0.54 mg/L, respectively (Figure 4E). The betacyanins (Figure 4C) and betaxanthins (Figure 4D) also increased among the treatments via increasing BSE. On day 1, the betacyanins were 29.56 ± 0.32 (T1), 47.21 ± 2.07 (T2), and 51.56 ± 0.45 mg/L (T3).

By day 7, the betalains almost halved (p < 0.05), reaching 27.68 ± 0.58 (T1), 31.41 ± 0.37 (T2), and 33.64 ± 0.86 mg/L (T3). The decrease in betalains correlated with the decrease in betacyanins (r = 0.98): the betacyanins were reduced by 40–60%, reaching 17.83 ± 0.71 (T1),
18.93 ± 0.32 (T2), and 21.77 ± 0.58 mg/L (T3). The thermal treatment of the pigment and the formation of amino acids during fermentation impaired the stability of betacyanins [8]. Additionally, the H$_2$O$_2$ released during LAB growth could be involved in the degradation of betacyanins [11].

However, on day 14, the betalains increased by 40–20% compared with day 7. Although T1 had the highest regenerative capacity, the betalains varied between 38.78 ± 0.47 and 40.58 ± 0.19 mg/L in the treatments ($p > 0.05$). The betaxanthins increased by 86% (T1; 18.29 ± 0.27), 47% (T2; 18.32 ± 0.32), and 27% (T3; 15.05 ± 0.14) on day 14. In comparison, the betacyanins increased by 13–17% in T1, T2, and T3 to 20.49 ± 0.19, 21.40 ± 0.32, and 25.53 ± 0.32 mg/L, respectively. Betalains can be regenerated under acidic conditions developed via fermentation and other acids [12]. Moreover, betacyanins are degraded during storage to betalamic acid and cyclo-dopa-5-O-$\beta$-glucoside via a reversible reaction that allows the regeneration of betalains. However, this regeneration depends on the characteristics of environmental conditions: generally, betacyanins regenerate through Schiff-base condensation between the amino group of cyclodopa-5-O-glucoside and the aldehyde group of betalamic acid [19], while in the presence of amino acids, betalamic acid is involved in the regeneration of more betaxanthins than betacyanins [8].

The pattern of degradation and regeneration of betalains in the present study is consistent with the pattern of betalains from beetroot extract in yogurt [2]. However, betalains from red pitahaya in yogurt are reduced during cold storage (8.5–27.8%) [11]. Therefore, the composition of the pigment should be identified in further studies to gain more insight into the possible responses of the pigment in food matrices.

### 3.5.3. Antioxidant Activity

BSE increased the antioxidant activity of the treatments compared with the control ($p < 0.05$); however, BSE concentration did not affect the antioxidant activity of the treatments ($p > 0.05$) ($IC_{50}$ = 78.47 ± 3.27 (the control), 71.68 ± 1.30 (T1), 71.5 ± 1.29 (T2), and 69.18 ± 0.48 µL/mL (T3) (Figure 4F). The high antioxidant activity of the treatments correlated with betalains ($r = 0.97$), flavonoids ($r = 0.96$), and phenols ($r = 0.80$). Betalains are a potent antioxidant that could scavenge free radicals, particularly superoxide anions, more efficiently than vitamin C (~3-fold) and some anthocyanins, such as cyanidin-3-O-glucoside and cyanidin (~1.5–2-fold) [40]. Thus, the betalains from BSE prompted the scavenging activity of the treatments compared to the control. However, the lower concentration of BSE (1–5%) did not affect the antioxidant activity of the treatments on day 1. The obtained results are consistent with the previous literature: betalains from beetroot juice [35] and Alternanthera brasiliiana [12] boosted the antioxidant activity of treated yogurt compared to plain yogurt. In addition, BSE enriched the beverages with strong antioxidants, including rutin, catechin, chlorogenic acid, and ferulic acid, which effectively scavenged hydroxyl radicals and superoxide anions [37,38].

By day 7, the antioxidant activity of the beverages was increased ($p < 0.05$); $IC_{50}$ = 54.70 ± 0.91 (T3), 57.35 ± 1.29 (T2), 63.16 ± 0.50 (T1), and 67.27 ± 0.11 µL/mL (the control). The antioxidant activity of the beverages on day 7 correlated more strongly with flavonoids ($r = 0.99$) and betalains ($r = 0.92$) than with phenols ($r = 0.79$). Subsequently, the antioxidant activity of all yogurts on day 14 decreased to $IC_{50}$ values close to those of day 1 ($p > 0.05$). However, T3 (69.90 ± 1.48 µL/mL) and T2 (71.77 ± 1.43 µL/mL) had higher antioxidant activity than T1 (82.74 ± 2.77 µL/mL) and the control (83.85 ± 2.67 µL/mL) ($p < 0.05$). Our results are consistent with those of Muniandy et al. [51], who reported an increase and a subsequent decrease in the antioxidant activity of yogurt with tea during storage; this behavior could be related to the LAB activity, as well as the bioactive components of the additive. The viability of LAB during storage (14 days) correlated with the scavenging activity of the beverages ($r = 0.62$ (the control), 0.85 (T1), 1.00 (T2), and 0.99 (T3)). The high viability of LAB on day 7 (Table 4) contributed to the production of $\beta$-glucosidases, which catalyzed the release of bioactive peptides and flavonoids that boosted the antioxidant activity of the treatments [47], in addition to betalains, rutin, cate-
chin, chlorogenic acid, and ferulic acid from BSE. Subsequently, on day 14, the low viability of LAB and the degradation of bioactive components and betalains reduced the antioxidant activity of all beverages.

Table 4. Lactic acid bacteria (LAB) viability of beverages during 1, 7, and 14 days of cold storage (4 °C) in cfu/mL.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
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<tr>
<td>LAB (10⁷)</td>
<td></td>
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<tr>
<td>Day 1</td>
<td>4.73 ± 0.25&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>0.90 ± 0.21&lt;sup&gt;bC&lt;/sup&gt;</td>
<td>0.72 ± 0.11&lt;sup&gt;bC&lt;/sup&gt;</td>
<td>0.70 ± 0.2&lt;sup&gt;bC&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 7</td>
<td>73.67 ± 3.21&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>55.33 ± 1.53&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>36.00 ± 3.46&lt;sup&gt;cA&lt;/sup&gt;</td>
<td>25.17 ± 0.29&lt;sup&gt;dA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 14</td>
<td>37.00 ± 1.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.13 ± 0.17&lt;sup&gt;bb&lt;/sup&gt;</td>
<td>2.60 ± 0.17&lt;sup&gt;bb&lt;/sup&gt;</td>
<td>3.13 ± 0.15&lt;sup&gt;bb&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as means ± SDs; means with different lowercase letters (a–d) in the same row indicate statistical differences between the different beverages on the same storage day (<i>p</i> < 0.05); means with different uppercase letters (A–C) in the same column indicate the statistical differences between each beverage during the storage days (<i>p</i> < 0.05).

3.6. LAB Viability

BSE successfully developed a probiotic stirred yogurt; BSE did not affect the fermentation process. Although the count of LAB in the treatments was reduced by increasing the BSE concentration (<i>p</i> < 0.05), the treatments contained LAB with a log of 6–8 (Table 4), which is an acceptable count of probiotics at the time of consumption [1], indicating the ability to use BSE in developing functional, probiotic stirred yogurt.

The control had the highest LAB count on the different days of storage (<i>p</i> < 0.05): on day 1, the control had 4.73 × 10⁷ CFU/mL, which was 6 to 7 times higher than the LAB counts of T1, T2, and T3. Moreover, an increase in the BSE concentration significantly prolonged the coagulation time from 3 ± 00 h (the control) to 3.16 ± 0.01 h (T1), 3.31 ± 0.02 h (T2), and 3.43 ± 0.01 h (T3), which may be due to the potent antimicrobial effect of BSE. It seems that anthocyanins from the raspberries positively influenced the growth of LAB, while the antimicrobial activity of BSE negatively affected their growth. Our result agrees with that of Wijesekara et al. [45], denoting that the high antimicrobial activity of turmeric and spinach extracts significantly hindered the growth of LAB in yogurt, while anthocyanins from hibiscus promoted their growth.

By day 7, a significant increase in LAB viability was observed in all samples. Although LAB grew rapidly in the control sample (16-fold), LAB also recorded remarkable growth in T1, T2, and T3; it was 62-, 50-, and 36-fold higher than on day 1, respectively. On the other hand, the count of LAB reduced by day 14 in all yogurt, but to a greater extent in the treatments than in control, which may be attributed to the post-acidification effect [45] and the cessation of the metabolic activity of the bacteria during storage [11]. However, the treatments contained an acceptable count of probiotics (log 6–8), reflecting the success of the developed product. Our results are consistent with the previous literature: Gengatharan et al. [11] and Mohammadi-Gouraji et al. [46] reported a decline in LAB in yogurt with red pitahaya and phycocyanin. On the other hand, Mousavi et al. [1] reported a gradual increase in the LAB count during storage of stirred yogurt containing 10% organic juices from beetroot, cassava, sweet potato, and corn. The discrepancy in the results could be due to the different effects of the additives and the adaptation pace of the LAB species to the surrounding environment.

3.7. Microbial Load of Yogurt

No coliform colonies were observed in the fresh products or during storage, indicating high hygiene practices [46]. On the other hand, yeasts and molds were detected in the control sample after 14 days of storage (5.3 × 10¹ ± 0.26 CFU/mL). Otherwise, the extract effectively inhibited the growth of yeasts and molds in the treatments until the end of the storage days. Here, BSE inhibited the growth of yeasts and molds and controlled the shelf life of the stirred yogurt. In contrast, phycocyanin [46] and beetroot peel extract [21] had
a slight effect on the fungal growth of yogurt and whey beverages, respectively, as fungi grew in the treatments, as in the control. Thus, BSE is a promising antimicrobial agent that needs further investigation.

4. Conclusions

The present study aimed to evaluate the biological activity of water extract from beetroot stalks and its potential application as a colorant for raspberry-flavored stirred yogurt. The findings of the investigation emphasized that the water extract of fresh stalks is a good source of a high phenolic content with a considerable betalain content, boosting the biological activity of the extract. The stability of betalains qualified the extract to be included in developing a functional stirred yogurt. The developed functional yogurt showed a high attractiveness in terms of color without a significant effect on the other sensory attributes. Although the pigment was subjected to degradation reactions during storage, the color change was acceptable compared to the control. Additionally, the extract enhanced the quality of the yogurt by increasing its nutritional value and extending its shelf life. Hence, beetroot stalks could be used as food colorants to develop various functional and nutraceutical foods with extended shelf lives. In conclusion, the current study highlights that the extract of beet stalks is a promising, natural, sustainable, cost-effective, and nutritious functional coloring agent for dairy products that had not been studied previously.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9100878/s1, Figure S1: HPLC chromatogram of beetroot stalks water extract; Figure S2: Color of raspberry-stirred yoghurt enriched with BSE at 0% (Control), 1% (T1), 2% (T2), and 5% (T3) on day 1, 7, and 14 of storage. (A) L*, (B) a*, and (C) b*. Different lowercase letters (a–d) indicate statistical differences of the different beverages on the same storage day (p < 0.05); different uppercase letters (A–C) indicate the statistical differences on each beverage during the storage days (p < 0.05); Figure S3: Change in the color of the treatments compared to control on day 1. Different lowercase letters (a–d) indicate statistical differences of the different beverages (p < 0.05).

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