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Development of functional probiotic yogurt from buffalo milk supplemented with red beetroot (*Beta vulgaris* L.) as an antioxidant, natural colorant, and starter growth stimulant

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ABSTRACT

The physicochemical, sensorial, and probiotics viability properties of supplemented probiotic yogurt with beet juice (BJ) at various ratios (0–5)% in fresh and stored yogurts were determined. 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity (%) and minerals content of BJ-yogurts were increased. The color aspects (ΔE) were improved with the increasing of BJ. BJ-yogurts at 1 and 3 % were color-stable when stored in the refrigerator (5 ± 1 °C) for 9 days. The syneresis reached of 42.9, 43.0, 43.5, and 43.5 mL for control, 1 %, 3 %, and 5 % BJ, respectively in stored yogurts for 12 days. Moreover, the probiotic yogurt viability in BJ-yogurts treatments was found to be higher ($p < 0.05$) than control which recorded of 7.0 ± 0.08, 7.22±0.04, 7.34±0.002 log cfu/mL for streptococci, lactobacilli, and bifidobacteria counts respectively in 5 % BJ treatment, on the other hand, in control recorded of 6.76±0.07, 7.11±0.06, 6.66±0.030 log cfu/mL for streptococci, lactobacilli, and bifidobacteria counts respectively; indicating that BJ had a stimulating effect on the additive starter. Fresh yogurts with 1 % BJ had superior sensorial scores than other treatments, and generally maintained desirable organoleptic attributes for up to 9 days. This study provides a nutritious, low-cost, stable, and attractive colored yogurts from buffalo milk without negative impact on physiochemical qualities.

1. Introduction

Yogurt is the most widely accepted and consumed fermented milk product across all age groups in the world. Probiotics are live microorganisms that, when administered in adequate amount, offer a health benefit on the host, due to their exceptional antioxidant power (Hamad et al., 2022), and antimicrobial properties (Ahmed et al., 2023). The mostly used probiotic strains for fermenting the yogurt were lactic acid bacteria (LAB) i.e., *Lactobacillus delbrueckii* ssp. *bulgaricus* (L. *bulgaricus*) and *Streptococcus thermophilus* (*S. thermophilus*). These two strains are diversely and distinctly different in fermentation performance, acid formation capability, growth rate, and probiotic attributes (Ge et al., 2024). *S. thermophilus* provided several fermentation compounds e.g., formic acid, folic acid, CO_2, and fatty acids (FAs) in order to initiate the growth of *L. bulgaricus*, while *L. bulgaricus* is anticipated to generate a surplus of peptides and free amino acids (FAAs) to assemble the biosynthetic properties of *S. thermophilus* (Sieuwerts et al., 2010). On the other hands, previous studies have showed that efficient probiotic yogurt can also be produced using LAB species such as *Bifidobacterium animalis* subsp. *lactis* (Mysonhimer et al., 2024), *Lactiplantibacillus plantarum* (NUC08 and NUC101), *Lacticaseibacillus rhamnosus* (NUC55 and NUC201), *and Lacticaseibacillus paracasei* (NUC159, NUC216, and NUC351) (Cai et al., 2024), *Lacticaseibacillus rhamnosus, Lactobacillus acidophilus*, and *Bifidobacterium* spp (Sibanda et al., 2024), *Lactobacillus plantarum* B (LPB) (Yi et al., 2024), *Lactiplantibacillus plantarum* ZFM55 (Meng et al., 2024), and *Lactococcus lactis* R7 (Santos Pereira et al., 2024). Therefore, the implementation of various probiotics in yogurt has become a modern strategy and synergistic trend not only to expand human nutrition, beneficial metabolites, and health benefits but also to improve texture, flavor, and organoleptic properties of yogurt (Abbasi et al., 2022; Abdoli et al., 2022; da Cruz et al., 2022; Groves et al., 2020; Gupta et al., 2023; Savaiano & Hutkins, 2021; Sunmola et al., 2019).

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Furthermore, combining probiotic LABs with natural bioactive extracts is a promising approach to extend the health benefits and the market for functional foods (Shehata et al., 2023). In this regard, recent study of Ghasempour et al. (2020) has developed a probiotic yogurt containing red beet extract and basil seed gum using *L. paracasei* DVS culture, also, 0.1 and/or 0.2 % of red beet extract powder were utilized to study the effect of this incorporation on the technofunctional, and sensorial properties of the product. In other report (Flores-Mancha et al., 2021), an encapsulated beetroot extract in the probiotic yogurt didn't affect ($p > 0.05$) the rheological attributes of the product but reduced syneresis ($p < 0.05$) and increased the protein content as well antioxidant power significantly. More recently, beetroot extract was added to the yogurt to study the physicochemical, and sensorial characteristics by check-all-that-apply method (CATA- approach). The organoleptic scores i.e., acidic aroma, earthy flavor, bitter after taste, and the fluid texture were considered negative preference drivers at 20 % of the beet root, while the produced yogurt at 13 % was the most approved by the panelists (Soutelino et al., 2023). Several benefits of red beetroot are used in the medicinal system, such as lowering the risk of cancer in humans, being an antioxidant, and anti-inflammatory. Natural-food colorants i.e., betalains (Figure S1), anthocyanins, carotenoids, chlorophyll, and among others have been stand out as a promising substitute to the synthetic ones owing to their varied health concern (Boivin et al., 2009). Furthermore, natural colorants are a sustainable and healthy option for their pleasant organoleptic and technological properties when incorporated to food products (Mehany et al., 2023). In this regard, numerous natural colorants origins have been investigated to deliver the desired color required by consumers (Luzardo-Ocampo, Ramírez-Jiménez, Yañez, Mojica & Luna-Vital, 2021). Additionally, various studies have been conducted on use of natural pigments in food systems (Flores-Mancha et al., 2021). Consequently, the use of natural colorants instead of artificial ones in food industries limits several health issues. Moreover, adding natural colorants to food-stuffs led to enhancing the product's acceptance amongst consumers, along with utilizing the bioactive molecules that accompany plant pigments.

Many studies have been conducted to produce probiotic fermented milk based on different milk sources such as cow milk (Hussain et al., 2023; Fatima & Hekmat, 2020), sheep and goat milk (Balthazar et al., 2024; Ospanov et al., 2023; Sharma & Ramanathan, 2023; Sathya et al., 2019), and camel milk (Ayyash et al., 2018). Nevertheless, buffalo milk is richer in nutrient compounds e.g., protein and lipids (Vargas-Ramella et al., 2021, Yi et al., 2024), and is therefore hypothetically a better and promising raw material for producing healthy yogurt than cow milk which consider the common raw material for producing yogurt.

In the present research, we extend the concept of using the natural colorant to develop a stable colored-stirred yogurt which resulted in a highly nutritious product along with a sharp color, pungent flavor and greater appeal to customers, particularly children. Thus, the aim of the current research is (i) to develop functional and natural-colored probiotic yogurt from buffalo milk based on the classical culture along with *L. acidophilus* ATCC 4356 and *Bifidobacterium longum* and (ii) to study the impact of incorporation of red beetroot (*Beta vulgaris* L.) juice at various ratios as an antioxidant, natural colorant, and starter growth stimulant on the physicochemical qualities, sensorial acceptance and viability of probiotic yogurt model.

2. Materials and methods

2.1. Starter

The fresh milk (buffalo) used in the experiment was acquired from the Department of Animal Production, College of Agriculture, Al Azhar University (Egypt). Additionally, skim milk powder, beetroot, sugar and gelatin were obtained from the local market in Assiut City, Egypt. Commercial Yoflex culture, thermophilic yogurt strains (1:1 *L. bulgaricus* and *S. thermophilus*) was purchased from Christian Hansen (Copenhagen, Denmark). *L. acidophilus* ATCC 4356 and *Bifidobacterium longum* (ATCC 15,707) were obtained from the Cairo Microbiological Resource Center (MIRCEN), College of Agriculture, University of Ain Shams (ASU), Cairo, Egypt.

2.2. Preparation of beetroot juice

The red beetroot was sorted, washed, peeled off, and cut into small pieces, and then the juice was obtained by blending and straining. Red beetroot juice was heated at 80 °C for 10 min to inactivate the beet's enzymes.

2.3. Preparation of probiotic yogurt with red beetroot juice

Skim milk powder 1.5 % (w/w), gelatin 0.4 % (w/w) as a stabilizer in order to prevent the yogurt from the separation and to preserve the creamy texture throughout the shelf life, and sugar 4 % (w/w) were added to partly skimmed buffalo's milk (4 % fat) and separated into four equivalent parts, and each experimental unit has 12 liters of milk. The first portion was used to prepare plain probiotic yogurt (Control: the probiotic yogurt without any beetroot juice treatments), the control yoghurt was prepared from (*L. bulgaricus* and *S. thermophilus*), *L. acidophilus*, and *B. longum* and the other portions were used to prepare probiotic yogurt with 1% w/w (T1), 3% w/w (T2), and 5% w/w (T3) red beetroot juice. Moreover, the starter was standardized in the control and treatments in order to observe the potential statistical difference after addition of red beet juice. All parts were then heated at 90 °C/15 min, after, were cooled at 40 °C, then, inoculated with 6 % active probiotic yogurt culture, where culture strains of *L. bulgaricus, S. thermophilus, L. acidophilus,* and *B. longum* were activated in MRS broth and then activated in sterilized skim milk (triplicate), and finally incubated at 42 °C till coagulation process. All batches were stored overnight in the refrigerator, then, the products were stirred and bottled. The samples were held at 5 ± 1 °C for further analyses at fresh, 3, 6, 9, and 12 days of storage period. The prepared yogurt with different beetroot juice is presented in Figure S2.

2.4. Physicochemical analysis

Total soluble solids in the yogurt samples were analysed following the approach of Fekry, Rashad, Alaraidh and Mehany (2021), pH was determined by a pH-meter (HANNA Microprocessor model R-4–29, Hanna Instruments Ltd, Leighton Buzzard, United Kingdom). Total nitrogen contents, ash and fat contents were determined according to AOAC (2007). On the other hand, yogurt's syneresis was measured according to Farooq and Haque (1992) method. One hundred grams of yogurt sample were placed on a filter paper resting on top of a funnel. After 2 h of drainage at 7 °C, the quantity of whey collected in a 50-mL graduated cylinder and after that, it was used to calculate the index of syneresis.

2.5. Minerals content

The sample was wet-ashed with a combination of acid and a high-temperature oven to form a dissolvable product ready for analysis of potassium (K), sodium (Na), and calcium (Ca) concentrations via a flame photometer (BWB XP Flame Photometer Technologies, UK LTD, Berkshire, UK).

2.6. Antioxidant activity

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging power was analyzed following the assay of Hamad et al. (2022) and El Sohaimy et al. (2016). The absorbance was measured at 517 nm using a UV–VIS spectrophotometer (PG Instrument Ltd. UK), and DPPH percentage was estimated as follow (Eq. 1):

$$\% \text{ DPPH scavenging effect} = \frac{\text{Ab C} - - \text{Ab S}}{Ab\ C} \text{X } 100 \quad (1)$$

where, Ab C: the absorbance of the control; and Ab S: the absorbance of the sample.

2.7. Color properties

Colors were estimated by using a colorimeter PCE-CSM4 (PCE Instruments UK Ltd.). This device supports color space L* a * b*, which covers all visible colors attributes (i.e., white, black, yellow, red, blue, and green). When comparing two color samples, the difference is commonly stated as ΔE, which indicates how visually distinct the two samples are in the color sphere. The ΔE between a sample at fresh (having L*0, a*0 and b*0) and a sample at different storage times (having L*1, a*1 and b*1), then ΔE was calculated using the following equation (Eq. 2):

$$\Delta E = \sqrt{\left(L_1^* - L_0^*\right)^2 + \left(a_1^* - a_0^*\right)^2 + \left(b_1^* - b_0^*\right)^2} \quad (2)$$

2.8. Microbiological determinations

Total bacterial count (TBC) in the developed probiotic yogurt samples was measured following the assay of Marshal (1992). Lactobacilli count (LC) was measured on MRS media. For this purpose, the plates were incubated at 37 °C/48 h. Streptococci count (SC) was estimated on M_{17} agar (IDF, 1997), whilst bifidobacteria counts (BC) was enumerated by the adapted MRS agar following the approach of Dave and Shah (1996). Finally, coliform group were counted according to IDF (1985), and the yeasts and molds were evaluated by incubation the plates at 30 °C/18–72 h (FDA, 2002).

2.9. Sensory evaluation

The sensorial attributes of both fresh and stored stirred yogurt samples from different treatments were assessed according to Wood et al. 2021. Briefly, the organoleptic characteristics was carried out by 15 experienced panelists familiar with the consumption and evaluating of dairy products and fermented foods (Aged from 23 to 60 years) in College of Agriculture, Al Azhar University, Egypt. The samples were examined at room temperature and the panelists were asked to assess flavor, body and texture, appearance and color, acidity, and overall acceptability. The scale was carried out through 100-grade scale as follow; flavor (45), body and texture (30), appearance and color (15), acidity (10), overall acceptability scores (100).

2.10. Statistical analysis

A randomized two-level factorial experiment (without and with addition of beet root juice) at 3 various intervals from beet roots juice (1, 3, and 5% w/w) in fresh and stored yogurt samples was employed with three replications. The analysis of variance was carried out on the obtained data by the JMP®, software program, Version 16 pro. SAS Institute Inc., Cary, NC, USA. One-way analysis of variance (ANOVA) as well the Duncan test was used to analyze the obtained data at a significant level ($p < 0.05$).

3. Results and discussion

3.1. Chemical and microbiological analyses of red beetroot juice

The chemical composition of the prepared red beetroot juice, which is evident in Table S1, indicates that the mean moisture content is 87.56 ±0.15 % and the pH value appeared mild (6.47±0.01). Red beetroot juice is rich in ash content (13.96±0.23 %); in contrast it has a low content of protein (2.10±0.03 %).

Based on the mean antioxidant activity DPPH% (81.72±0.03 %) in red beetroot juice, it is considered a high antioxidant source. This makes the use of beet juice as an antioxidant much more effective and suitable as an additive in the dairy and food industry. Moreover, red beetroot juice has a moderate amount of calcium, potassium, and sodium (525.28 ±15.23, 430.41±9.66 and 351.60±28.62 mg/100 gm) respectively. Calcium (525.28±15.23 mg/100 gm) is the most prevalent mineral in red beetroot juice. The total bacterial count in red beet juice found to be low (2.63±0.03 log cfu/mL). That reduces the effect of the microbial content of beet juice on the microbial content of the resulting yogurt.

Moreover, red beetroot is considered a source of lycopene, betaine, α-carotene, folate, niacin, vitamin C, and iron (Ceclu & Nistor, 2020). In addition, beet contains phenols, tannins, flavonoids, saponins, phytosterols, and alkaloids (Olumese & Oboh, 2018).

3.2. Physicochemical and nutritional aspects of probiotic yogurt

3.2.1. Moisture content

The results in Table 1 illustrated that, addition of red beetroot juice led to a noteworthy growth ($p < 0.05$) of moisture content in the stirred probiotic yogurt affected by the high moisture content of red beetroot juice (87.56±0.20 %). In addition, the moisture content of stirred probiotic yogurt significantly decreased ($p < 0.05$) with the advance of storage time at cooled conditions up to twelve days in the all-developed treatments. Moreover, the moisture content was decreased but the decrease level was of minor significance where the moisture content reduced from 81.49±0.065 to 80.98±0.034 in the fresh and stored product at 12 days, respectively. The same trend was observed by Flores-Mancha et al. (2021). This decrease is due to evapotranspiration during storage because the bottle has a spiral cap that leaks the vapor.

3.2.2. Protein content

Treatments of stirred probiotic yogurt exhibited a weighty ($P < 0.05$) increase in the protein ratio during the storage period. The increase in protein concentration was related to the decrease in moisture ratio during storage time of stirred probiotic yogurt samples. On the other hand, the low content of protein in beet juice (2.10±0.20 %) led to a gradual decrease in the protein content of stirred probiotic yogurt samples with an increase of enrichment by beet juice. However, the protein content of stirred probiotic yogurt in the present research was higher than the findings confirmed by Flores-Mancha et al. (2021); Januário et al. (2017); Yadav, Masih and Sonkar (2016), and this is because the moisture content is decreased.

3.2.3. Fat content

According to the data in the same table (Table 1), and, during storage at cooled conditions for twelve days the fat content of stirred probiotic yogurt increased and showed significant differences ($p < 0.05$). The gradual increase in fat values is probably related to the decrease in moisture ratio during the storage process. However, as the concentration of beet juice was increased, the fat content of stirred probiotic yogurt decreased. The same results were found by Flores-Mancha et al. (2021). Furthermore, control samples had more fat than other treatments, which could be because beet juice contains no fat. As illustrated in Table 1., fat content was increased from 3.90±0.01 % to 4.30±0.01 % in the fresh control and the stored one for 12 days respectively, and the same trend occurred in the probiotic yogurt with beet root juice, and this is because the moisture content is decreased.

3.2.4. Ash concentration

A significant variance has been observed in ash ratios between treatments, in addition the ash ratio increased as beet juice concentration increased. Stirred probiotic yogurt samples containing 5 % beet juice had the highest ash values, whereas control had the lowest one. Furthermore, the high mineral content of red beet juice accounts for the increase in the ash levels of stirred probiotic yogurt as beet juice

Table 1
Chemical composition of stirred probiotic yogurt prepared with different ratios of beetroot juice.

Treatments	Storage periods	Moisture%	Protein/DM%	Fat/DM%	Ash/DM %
Control	Fresh	81.49 ±0.065 ^{BCDE}	6.72 ±0.049 ^{A}	3.90 ±0.01 ^{DE}	3.36 ±0.088 ^{F}
	3 days	81.41 ±0.075 ^{BCED}	6.73 ±0.058 ^{A}	3.98 ±0.017 ^{CD}	3.54 ±0.13 ^{EF}
	6 days	81.38 ±0.114 ^{BCDE}	6.74 ±0.147 ^{A}	4.27 ±0.017 ^{A}	3.86 ±0.289 ^{DE}
	9 days	81.31 ±0.044 ^{CDE}	6.84 ±0.073 ^{A}	4.30 ±0.033 ^{A}	3.95 ±0.040 ^{CDE}
	12 days	80.98 ±0.034 ^{F}	6.86 ±0.062 ^{A}	4.30 ±0.01 ^{A}	4.12 ±0.013 ^{BCD}
1 % red beet juice (T1)	Fresh	81.59 ±0.01 ^{BCD}	6.47 ±0.002 ^{AB}	3.82 ±0.033 ^{EF}	4.19 ±0.019 ^{ABCD}
	3 days	81.53 ±0.021 ^{BCDE}	6.52 ±0.209 ^{AB}	3.80 ±0.01 ^{EF}	4.20 ±0.106 ^{ABCD}
	6 days	81.41 ±0.078 ^{BCDE}	6.57 ±0.199 ^{A}	4.10 ±0.01 ^{BCE}	4.25 ±0.023 ^{ABCD}
	9 days	81.31 ±0.115 ^{DEF}	6.58 ±0.223 ^{A}	4.20 ±0.01 ^{AB}	4.33 ±0.09 ^{ABC}
	12 days	81.25 ±0.003 ^{EF}	6.76 ±0.078 ^{A}	4.20 ±0.01 ^{AB}	4.39 ±0.024 ^{AB}
3 % red beet juice (T2)	Fresh	81.66 ±0.024 ^{B}	6.42 ±0.075 ^{AB}	3.63 ±0.033 ^{G}	4.25 ±0.026 ^{ABCD}
	3 days	81.63 ±0.078 ^{BCD}	6.43 ±0.192 ^{AB}	3.70 ±0.01 ^{FG}	4.26 ±0.013 ^{ABCD}
	6 days	81.62 ±0.047 ^{BCD}	6.43 ±0.110 ^{AB}	4.10 ±0.01 ^{BCE}	4.27 ±0.03 ^{ABCD}
	9 days	81.44 ±0.002 ^{BCDE}	6.44 ±0.004 ^{AB}	4.18 ±0.017 ^{AB}	4.41 ±0.011 ^{AB}
	12 days	81.34 ±0.011 ^{BCDE}	6.48 ±0.19 ^{AB}	4.2 ± 0.01 ^{AB}	4.51 ±0.025 ^{AB}
5 % red beet juice (T3)	Fresh	82.24 ±0.106 ^{A}	5.34 ±0.161 ^{D}	3.35 ±0.017 ^{H}	4.26 ±0.011 ^{ABCD}
	3 days	82.15 ±0.006 ^{A}	5.55 ±0.241 ^{CD}	3.60 ±0.01 ^{G}	4.31 ±0.011 ^{ABC}
	6 days	82.01 ±0.040 ^{A}	5.70 ±0.222 ^{BCD}	4.00 ±0.01 ^{CD}	4.32 ±0.01 ^{ABC}
	9 days	81.64 ±0.090 ^{BCE}	5.71 ±0.295 ^{BCD}	4.03 ±0.033 ^{C}	4.46 ±0.010 ^{AB}
	12 days	81.50 ±0.002 ^{BCDE}	6.38 ±0.107 ^{ABC}	4.10 ±0.044 ^{BCE}	4.57 ±0.053 ^{A}

Data are the mean ± SE, $n = 3$. Means with the same letter are significantly different at $*p < 0.05$ between rows. DM: dry matter.

concentration rises. The same trend of ash content was observed by Hashem (2018); who reported ash content was increased with an increase of red beet juice addition.

3.2.5. pH values and syneresis

Data in Figure S3 presented substantial variances in pH values amongst stirred probiotic yogurt treatments and during storage time, and the pH values reached among (4.40±0.009 and 4.84±0.006). According to the data, pH values gradually reduced at the storage termination, recent investigations by Januário et al. (2017); Yadav et al. (2016), observed that, the determined pH values were decreased up to 14 days then increased up to 21 days, due to the formation of lactic acid (LA) formed by the LAB, the pH values of stirred yogurt samples steadily declined during the storage period of up to 12 days. As such, treatments 1 and 2, which contain 1 % and 3 % of beetroot juice, have higher pH values than those in control and treatment 3.

Data in Figure S4 showed that, in all tested treatments, the syneresis of stirred probiotic yogurt was meaningly augmented with an increase of red beetroot juice ratio and with development of storage time at cooled condition up to twelve days.

3.3. Radical scavenging activity (DPPH%)

The control samples had lower DPPH% radical scavenging activity than other treatments, while stirred probiotic yogurt DPPH free radical scavenging power augmented in a red beet juice ratio reliant on way (see Fig. 1). The results showed that adding red beet juice to the stirred probiotic yogurt samples increased the antioxidant activity as well as the bioactive components in the resulting stirred yogurt samples when compared to the control sample. Values of DPPH% free radical scavenging power displayed momentous alterations ($p < 0.05$) among all treatments as well as storage time. Data also presented a gradual decrease in the DPPH% radical scavenging activity throughout storage in all prepared treatments (for 12 days in refrigerator). Indeed, natural extracts and its accompanying bioactive substances e.g., phenolic compounds and flavonoids play an important role in scavenging of free radical with high antioxidant power (Shaukat et al., 2023).

3.4. Minerals content

From the findings in Table S2, we can notice that the calcium, potassium, and sodium contents of stirred probiotic yogurt were higher than those of control samples. Data also indicated that mineral content has significantly increased ($p < 0.05$) with increasing red beet juice addition, which is considered a rich source of calcium, potassium, and sodium minerals. In addition, all these elements of the developed yogurt were increased gradually throughout storage period for twelve days in the refrigerator. The increase in mineral content could be owing to the

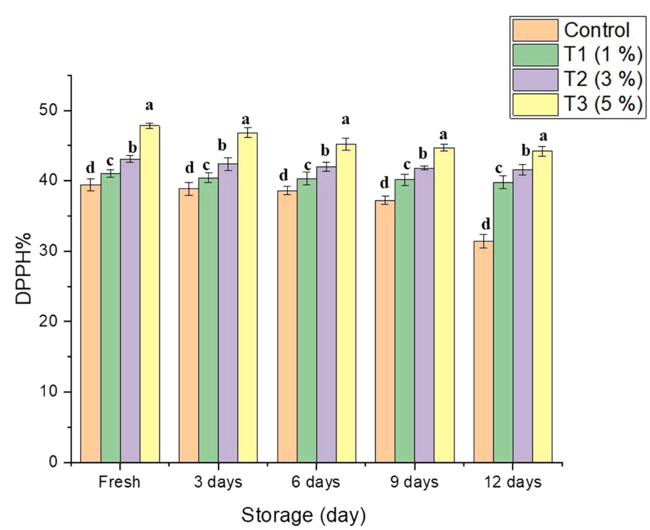

Fig. 1. Radical scavenging activity (DPPH%) values of stirred probiotic yogurt with different ratios of beetroot juice. Control: plain probiotic yogurt, T1: yogurt with 1 % (w/w) beet juice, T2: yogurt with 3 % (w/w) beet juice and T3: yogurt with 5 % (w/w) beet juice. [a, b, c, d, e] Mean values with different superscript letters in the same storage day (0–12 days) between the several treatments are significantly different ($p < 0.05$).

rise in total solids (TSs) through storage.

The current findings are in parallel with those attained by Noureldin, Salman, Ali and Mansour (2020) who reported that the mineral content in novel molasses-yogurt was increased with an increase in molasses concentrations as well as with the progression of storage time. The abundance of whey amount (mL/100 gm) could be because of the acidity, which causes the protein matrix to shrink and the whey to separate (Kale, Kadam & Hashmi, 2011). A similar pattern was discovered by Katsiari, Voutsinas and Kondyli (2002); Noureldin et al. (2020).

3.5. Color attributes

One of the key factors influencing how food products appear is their color (Dey & Nagababu, 2022), especially when it comes for marketing to children. The addition of natural coloring to functional dairy foods makes them more enticing to children, which increases their consumption and becomes more advantageous for kids' health. The major pigment in beetroot is betanin. Because betanin degrades when exposed to light, heat, and oxygen, it is employed in products with a limited shelf life, like yogurt. The low pH value in yogurt decreases color changes in betanin because the alkaline condition leads to degradation of betanin (Singh & Kumar, 2014).

The color of stirred probiotic yogurt made with different levels of beetroot juice was analyzed. The L*, a*, b*, and DE measurements were used to estimate the color. The values for the stirred yogurts color properties throughout various storage times are shown in Table 2. The findings demonstrate that the colored yogurt sample has a color that is significantly brighter ($P < 0.05$) than the uncolored yogurt (control) and has lower "L*" values. The treatment containing 5 % red beet juice (T3) had a brighter color attribute compared with the other yogurt treatments. Red color increased in the colored yogurt sample with an increase in beet juice level and had more redness (high 'a*' value). While the uncolored yogurt (control) had a low 'a*' value, consequently, these values are within the green color space. The stirred probiotic yogurt samples showed a meaningfully dissimilar ($P < 0.05$) in 'b*' value. Positive values for b* could be attributed to the yellow pigments of betalains.

The changes in color among treatments and during the storage of probiotic yogurt were expressed by ΔE (Fig. 2). The change in red color among treatments was increased by the addition of red beet juice; therefore, the value of redness (a*) increased. The red color is corresponding to betacyanins which considered a major part of betalains (56 %) comparing with the yellow–orange betaxanthins (44 %), reflecting the red color of the developed probiotic yogurt (Calva-Estrada et al., 2022). All the white probiotic yogurts (control) displayed ΔE values that were under 3, which the human eye cannot detect. Red beet probiotic yogurt, on the other hand, displayed a higher ΔE value (which ranged from 2.74 to 10.70), demonstrating that the change in hue was apparent to a layperson. The changes of ΔE values were gradually increased with the addition of red beet juice. The change of color in the samples that had up to 3 % beet juice and were stored for 9 days could not be detected by a layperson. This indicated that the beet color of probiotic yogurt samples that contain 3 and 5 % beet juice was stable when stored in the refrigerator for 9 days.

3.6. Microbiological evaluation of probiotic yogurt prepared with red beetroot juice

Fig. 3 shows the microbiological examination of produced stirred probiotic yogurt (total bacterial, lactobacilli, streptococci, bifidobacteria, molds &yeasts, and coliform bacteria counts).

Total bacterial counts of stirred yogurt samples varied from 8.61 ±0.01to 10.78±0.004 log cfu/mL, with a noteworthy rise ($p < 0.05$) with increasing incorporation of red beet juice percentages, as shown in Fig. 3. The addition of red beet juice to stirred probiotic yogurt stimulated the growth of the yogurts microflora; these findings are consistent with pH variations throughout the fermentation (see Figure S3). The current findings are in parallel with the recent results obtained by Januário et al. (2017).

Table 2
Color attributes of probiotic yogurt prepared with different ratios of beetroot juice.

Treatments	Storage periods	L*	a*	b*	ΔE
Control	Fresh	92.40 ±0.26 A	−1.88 ±0.08 H	11.65 ±3.25 A	0.00
	3 days	95.03 ±0.37 A	−1.40 ±0.09 H	10.27 ±0.60 ABCD	1.32 GH
	6 days	93.51 ±0.59 A	−0.86 ±0.02 H	11.84 ±0.50 A	2.21 FG
	9 days	94.86 ±0.81 A	−2.03 ±0.11 H	10.11 ±0.15 ABCD	2.41 FG
	12 days	93.30 ±0.17 A	−1.56 ±0.03 H	11.09 ±0.10 AB	2.63 EFG
1 % red beet juice (T1)	Fresh	82.24 ±0.16 BCE	9.41 ±0.124 G	10.23 ±0.03 ABCD	0.00
	3 days	79.69 ±1.59 BCDE	10.82 ±0.74 G	11.26 ±0.75 A	3.72 DFE
	6 days	83.02 ±0.91 B	13.20 ±0.27 F	9.08 ±0.45 ABCD	4.25 DFE
	9 days	81.11 ±0.23 BCD	13.49 ±0.12 F	10.02 ±0.00 ABCD	4.31 DFE
	12 days	81.89 ±0.62 BCE	14.96 ±0.18 DE	6.726 ±0.25 CD	6.67 BCE
3 % red beet juice (T2)	Fresh	77.34 ±0.63 DEFG	13.12 ±0.05 F	11.41 ±0.04 A	0.00
	3 days	76.25 ±0.448 EFG	13.11 ±0.29 G	10.86 ±0.20 ABC	2.74 EFG
	6 days	78.17 ±0.86 CDEFG	15.83 ±0.52 CD	9.22 ±0.09 ABCD	3.90 DFE
	9 days	75.95 ±0.37 EFG	17.08 ±0.16 C	9.91 ±0.07 ABCD	4.65 B
	12 days	78.44 ±0.79 CDEF	19.08 ±0.19 B	6.98 ±0.07 BCD	7.69 B
5 % red beet juice (T3)	Fresh	74.13 ±1.02 GH	13.89 ±0.19 EF	11.61 ±0.36 A	0.00
	3 days	71.33 ±0.46 HI	16.11 ±0.02 CD	12.48 ±0.04 A	3.83 DEF
	6 days	74.67 ±1.72 FGH	19.00 ±0.38 B	9.99 ±0.19 ABCD	5.57 BCD
	9 days	68.26 ±0.78 I	22.22 ±0.19 A	10.66 ±0.18 ABCD	10.26 A
	12 days	69.63 ±0.72 I	22.02 ±0.25 A	6.60 ±0.16 D	10.70 A

Data are the mean ± SE, $n = 3$. Means with the same letter are significantly different at $*p < 0.05$between rows. L* is lightness; a*is redness; b* is yellowness.

There was a significant increase in the lactobacilli counts ($p < 0.05$) with the incorporation of beet juice additive, and it occurred in an equivalent way as the total viable bacteria (TVB). Also, the presence of red beet juice in stirred probiotic yogurt treatments might have enhanced lactobacilli group growth. Moreover, this concept is due to the increase of organic acids (OAs) formed in the developed stirred probiotic yogurt. Streptococci counts (SC) were listed in Fig. 3 and displayed substantial alterations ($p < 0.05$) between all experimented treatments as well as during the storage. The current findings clarified those treatments containing red beet juice had higher of streptococci group counts (SC) than control samples which had the lower counts at the late storage times (9 and 12 days).

The addition of beet juice appeared to significantly increase ($p <$

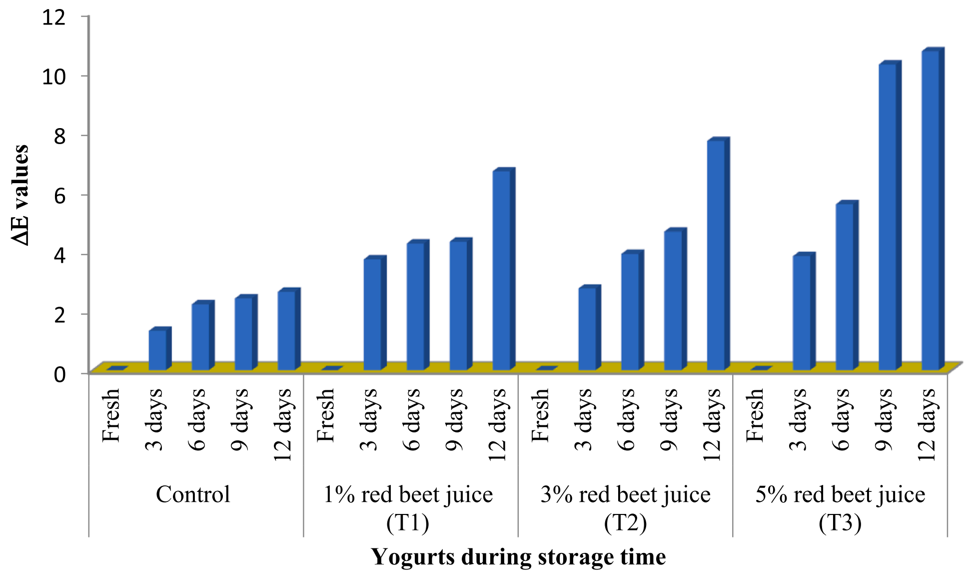

Fig. 2. ΔE values of stirred probiotic yogurt with different ratio of beetroot juice. Control: plain probiotic yogurt, T1: yogurt with 1 % (w/w) beet juice, T2: yogurt with 3 % beet juice and T3: yogurt with 5 % beet juice.

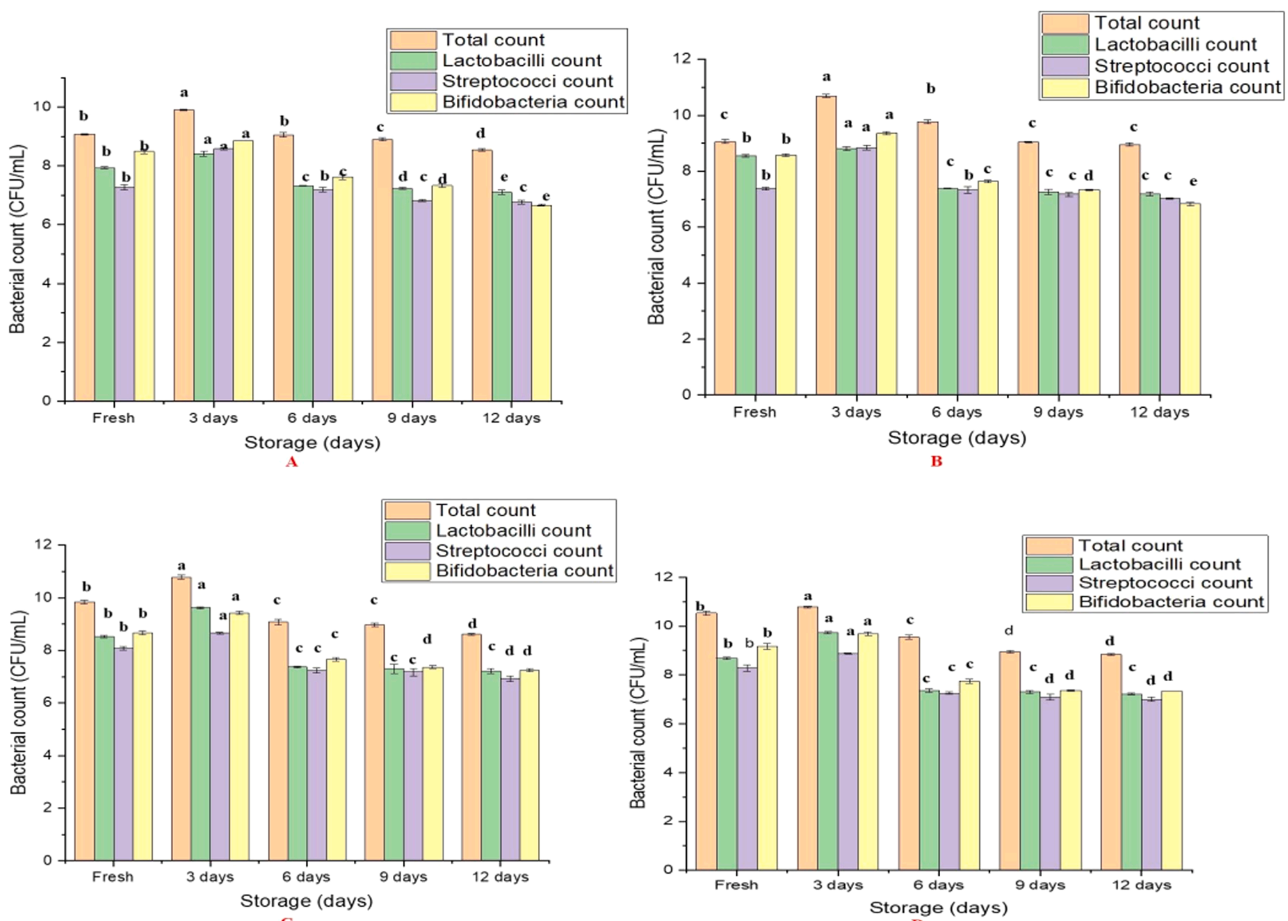

Fig. 3. Microbiological analysis of probiotic yogurt prepared with different ratios of red beetroot juice. The control was plain probiotic yogurt (A); the treatments were produced yogurt with 1 % (w/w) beet juice (B), 3 % (w/w) beet juice (C), and 5 % (w/w) beet juice (D). [a, b, c, d, e] Mean values with different superscript letters in the same bacterial count throughout the storage (0–12 days) for each treatment (A, B, C, and D) are significantly different ($p < 0.05$).

0.05) bifidobacteria counts. The viable cell counts of bifidobacteria during storage time were found to decrease, but the viable bifidobacteria cells at the end of storage time was increased by an increase in beet juice percentage (from 6.66±0.030 to 7.34±0.002 log cfu/mL). This indicates that beet juice may enhance the viability of bacteria (Yoon, Woodams & Hang, 2005). In addition, the viability of bifidobacteria still remained at

10^6 - 10^7 at 12th day (the end of storage time), which is higher than the recommended number (10^6 cfu/mL) to be considered probiotic products by Donkor, Nilmini, Stolic, Vasiljevic and Shah (2007), Gengatharan, Dykes and Choo (2017), and Wijesekara et al. (2022) who reported that the yogurt contained (log 6–8) of probiotics count is consider an acceptable developed probiotic product. Moreover, yeasts and molds and coliform bacteria as well as coliform bacteria are not detected in any yogurt treatments.

3.7. Sensorial properties of probiotic yogurt

The sensorial properties for each treatment were examined by the organoleptic panelists, and scores were granted as follow; flavor (45), texture and body (30), appearance and color (15), and acidity (10 points) Table 3. The flavor data obtained revealed clear substantial variations ($p < 0.05$) between all tested treatments and throughout the cooled-storage as well. The samples of treatment 1 and 2, which contained 1 and 3 % red beet juice, had higher flavor scores than those of control and treatment 3 at any storage time up to 12 days. In addition, freshly stirred yogurt samples with 1 % red beet juice scoured the higher flavor. Regarding body and texture, the data showed no substantial variances ($p < 0.05$) amongst the treatments and the cooled-storage periods and gained variable scores. The control samples, which were stored for 12 days, had minor values than those in other samples, while the highest score (28.83±3.728) of body and texture was gained by the samples containing 3 % red beet juice, which were stored for 9 days.

Furthermore, regarding both the color and appearance, the samples with a 3 % of red beet juice, which were left for 6 days, had a higher scores of appearance and color than those in the other treatments. While the lowest score was gained by the samples containing 5 % red beet juice, which were stored for 9 days. Moreover, a slight degradation of red color occurred during storage time and this action is compatible with Guneser (2021); Tobolková, Polovka, Daško, Belajová and Durec (2020). Despite color degradation, no significant differences ($p < 0.05$) were obtained from data among storage periods and that did not affect appearance and color scores.

The data obtained show momentous changes ($p < 0.05$) in acidity values. Also, data revealed that the scores of fresh stirred yogurts with 1 % red beet juice had higher ratios than those in other treatments, whereas, 5 % of beet juice sample that stored for 12 days had the lowest ratios. Furthermore, overall acceptance of the prepared fermented yogurt results revealed noteworthy variances ($p < 0.05$) among treatments and storage periods (Table 3). Fresh stirred probiotic yogurt samples with 1 % beet juice had excellent sensorial properties, followed by 1 % beet juice stored for three days. Whereas control samples of stirred yogurt which were stored for twelve days had the lowest values of overall acceptability of the end product.

Finally, according to the data obtained in the present research; further studies are highly recommended in order to develop a natural-colored probiotic yogurt on the large scale. Indeed, these colored functional fermented products will improve the health status of the humans and prevent several diseases, thanks to the high polyphenolic and antioxidant compounds.

4. Conclusions

In the present research, incorporation of red beetroot juice as natural colorants and antioxidant to probiotic yogurt led to improve the product acceptance among panelists, along with utilizing the bioactive compounds that accompany beetroot. Moreover, the current findings demonstrated that addition of red beetroot juice not only modified the physicochemical characteristics of developed yogurt but also significantly incremented mineral content and antioxidant power. The red beet probiotic yogurt samples that contain 1 and 3 % beet juice were stabled when stored in the refrigerator for 9 days. On the other hands, the syneresis of developed stirred probiotic yogurt was significantly increased with an increase of red beetroot juice ratio and with development of storage time at cooled temperature for twelve days, thus, further investigations to address the synereses issue is highly recommended by utilize of several natural hydrocolloids. In sum, fresh stirred probiotic yogurt samples with 1 % beet juice had superior sensorial scores than other treatments and control sample, and generally maintained desirable organoleptic attributes for up to 9 days. Thus, the current study confirm that beetroot should be useful candidate for improving the nutritional value of fermented dairy products on large scale to reduce the artificial colorants.

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CRediT authorship contribution statement

Khaled H. Salman: Conceptualization, Methodology, Investigation,

Table 3
Organoleptic properties of probiotic yogurt prepared with different ratios of red beetroot juice.

Treatments	Storage periods	Flavor (45)	Body and texture (30)	Appearance and color (15)	Acidity (10)	Overall scores (100)
Control	Fresh	41.00±0.856 ABC	27.17±0.547 A	13.50±0.563 A	8.33±0.333 AB	90.00±1.592 A
	3 days	40.17±0.703 ABC	27.17±0.601 A	13.33±0.760 A	7.50±0.224 ABC	88.17±1.558 AB
	6 days	39.50±1.996 ABC	28.33±0.333 A	12.67±0.558 A	7.33±0.333 ABC	87.83±2.561 AB
	9 days	38.50±1.648 ABC	27.83±3.525 A	13.17±0.703 A	8.17±0.543 AB	87.67±3.748 AB
	12 days	31.67±3.073 BCE	20.83±1.537 A	10.83±1.327 A	8.00±1.065 AB	71.33±4.890 B
1 % red beet juice (T1)	Fresh	44.00±0.258 A	27.83±0.307 A	13.17±0.477 A	9.00±0.00 A	94.00±0.730 A
	3 days	43.17±0.307 A	28.33±0.494 A	13.33±0.422 A	8.83±0.187 AB	93.67±0.760 A
	6 days	41.33±0.615 ABC	26.83±1.014 A	13.17±0.477 A	7.67±0.882 ABC	89.00±2.145 AB
	9 days	39.67±1.838 ABC	28.83±2.88 A	13.17±0.833 A	8.67±0.211 AB	90.33±2.565 A
	12 days	38.17±2.007 ABC	24.17±3.005 A	12.17±0.980 A	7.83±0.91 ABC	82.33±5.083 AB
3 % red beet juice (T2)	Fresh	42.83±0.749 AB	28.33±0.558 A	13.00±0.258 A	8.67±0.333 AB	92.83±0.401 A
	3 days	42.83±0.543 AB	28.5 ± 0.563 A	13.00±0.258 A	8.50±0.224 AB	92.83±0.654 A
	6 days	40.33±3.148 ABC	27.83±0.477 A	14.17±0.307 A	8.50±0.224 AB	90.83±3.628 A
	9 days	40.67±2.275 ABC	28.83±3.728 A	12.50±1.118 A	8.33±0.667 AB	90.33±2.565 A
	12 days	34.0 ± 3.559 ABC	25.83±2.701 A	12.17±0.654 A	6.00±1.033 BCE	78.00±4.858 AB
5 % red beet juice (T3)	Fresh	37.29±2.317 ABC	26.71±0.865 A	13.71±0.474 A	7.29±0.286 ABC	85.00±2.828 AB
	3 days	33.83±4.157 ABC	28.00±0.683 A	12.67±0.803 A	7.00±0.516 ABC	81.50±4.752 AB
	6 days	35.20±4.488 ABC	27.60±0.812 A	12.20±0.860 A	7.00±0.548 ABC	82.00±4.764 AB
	9 days	36.83±2.522 ABC	26.00±2.805 A	10.67±0.955 A	8.17±0.307 AB	90.33±7.347 A
	12 days	30.00±0.01 C	25.83±2.386 A	11.17±0.833 A	5.00±0.931C	72.00±2.352 B

Data are the mean ± SE, $n = 10$. Means with the same letter are significantly different at *$p < 0.05$between rows.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All the data are in the paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2024.100776.

References

Abbasi, A., Rad, A. H., Ghasempour, Z., Sabahi, S., Kafil, H. S., Hasannezhad, P., et al. (2022). The biological activities of postbiotics in gastrointestinal disorders. *Critical Reviews in Food Science and Nutrition, 62*(22), 5983–6004. https://doi.org/10.1080/10408398.2021.1895061

Abdoli, M., Mohammadi, G., Mansouri, K., Khaledian, S., Taran, M., & Martinez, F. (2022). A review on anticancer, antibacterial and photo catalytic activity of various nanoparticles synthesized by probiotics. *Journal of Biotechnology, 354*, 63–71. https://doi.org/10.1016/j.jbiotec.2022.06.005

Ahmed, W. I., Kamar, A. M., Hamad, G. M., Mehany, T., El-Desoki, W. I., Ali, E., et al. (2023). Biocontrol of *Bacillus cereus* by *Lactobacillus planetarium* in Kareish cheese and yogurt. *LWT, 183*, Article 114946. https://doi.org/10.1016/j.lwt.2023.114946

AOAC. (2007). Official methods of analysis. In *Association of official agricultural chemists* (18th ed,, *34* pp. 72–80). Washington D.C., USA: CH.

Ayyash, M., Al-Nuaimi, A. K., Al-Mahadin, S., & Liu, S. Q. (2018). In vitro investigation of anticancer and ACE-inhibiting activity, α-amylase and α-glucosidase inhibition, and antioxidant activity of camel milk fermented with camel milk probiotic: A comparative study with fermented bovine milk. *Food Chemistry, 239*, 588–597. https://doi.org/10.1016/j.foodchem.2017.06.149

Balthazar, C. F., Teixeira, S., Bertolo, M. R., Ranadheera, C. S., Raices, R. S., Russo, P., et al. (2024). Functional benefits of probiotic fermented dairy drink elaborated with sheep milk processed by ohmic heating. *Food Bioscience, 59*, Article 103781. https://doi.org/10.1016/j.fbio.2024.103781

Boivin, D., Lamy, S., Lord-Dufour, S., Jackson, J., Beaulieu, E., Côté, M., et al. (2009). Antiproliferative and antioxidant activities of common vegetables: A comparative study. *Food Chemistry, 112*(2), 374–380. https://doi.org/10.1016/j.foodchem.2008.05.084

Cai, Z., Guo, Y., Zheng, Q., Liu, Z., Zhong, G., Zeng, L., et al. (2024). Screening of a potential probiotic *Lactiplantibacillus plantarum* NUC08 and its synergistic effects with yogurt starter. *Journal of Dairy Science, 107*(5), 2760–2773. https://doi.org/10.3168/jds.2023-24113

Calva-Estrada, S. J., Jiménez-Fernández, M., & Lugo-Cervantes, E. (2022). Betalains and their applications in food: The current state of processing, stability and future opportunities in the industry. *Food Chemistry: Molecular Sciences, 4*, Article 100089. https://doi.org/10.1016/j.fochms.2022.100089

Ceclu, L., & Nistor, O. V. (2020). Red beetroot: Composition and health effects—A review. *Journal of Nutritional Medicine and Diet Care, 6*(1), 1–9. https://doi.org/10.23937/2572-3278.1510043

da Cruz, M. F., Magno, M. B., Jural, L. A., Pimentel, T. C., Masterson, D., Esmerino, E. A., et al. (2022). Probiotics and dairy products in dentistry: A bibliometric and critical review of randomized clinical trials. *Food Research International, 157*, Article 111228. https://doi.org/10.1016/j.foodres.2022.111228

Dave, R. I., & Shah, N. P. (1996). Evaluation of media for selective enumeration of *Streptococcus thermophilus, Lactobacillus delbrueckii* ssp. *bulgaricus, Lactobacillus acidophilus*, and bifidobacteria. *Journal of Dairy Science, 79*(9), 1529–1536. https://doi.org/10.3168/jds.S0022-0302(96)76513-X

Dey, S., & Nagababu, B. H. (2022). Applications of food colour and bio-preservatives in the food and its effect on the human health. *Food Chemistry Advances, 1*, Article 100019. https://doi.org/10.1016/j.focha.2022.100019

Donkor, O. N., Nilmini, S. L. I., Stolic, P., Vasiljevic, T., & Shah, N. P. (2007). Survival and activity of selected probiotic organisms in set-type yogurt during cold storage. *International Dairy Journal, 17*(6), 657–665. https://doi.org/10.1016/j.idairyj.2006.08.006

El Sohaimy, A. S., El-Sheikh, H. M., Refaay, M. T., & Zaytoun, A. M. (2016). Effect of harvesting in different ripening stages on olive (*Olea europea*) oil quality. *Am. J. Food Technol, 11*(1–2), 1–11. https://doi.org/10.3923/ajft.2016.1.11

Farooq, K., & Haque, Z. U. (1992). Effect of sugar esters on the textural properties of nonfat low calorie yogurt. *Journal of Dairy Science, 75*(10), 2676–2680. https://doi.org/10.3168/jds.S0022-0302(92)78029-1

Fatima, S. M., & Hekmat, S. (2020). Microbial and sensory analysis of soy and cow milk-based yogurt as a probiotic matrix for *Lactobacillus rhamnosus* GR-1. *Fermentation, 6*(3), 74. https://doi.org/10.3390/fermentation6030074

FDA. (2002). *Bacteriological analytical manual* (9th Ed.). Arlington, VA, USA: AOAC International.

Fekry, W. M., Rashad, Y. M., Alaraidh, I. A., & Mehany, T. (2021). Exogenous application of melatonin and methyl jasmonate as a pre-harvest treatment enhances growth of Barhi date palm trees, prolongs storability, and maintains quality of their fruits under storage conditions. *Plants, 11*(1), 96. https://doi.org/10.3390/plants11010096

Flores-Mancha, M. A., Ruíz-Gutiérrez, M. G., Rentería-Monterrubio, A. L., Sánchez-Vega, R., Juárez-Moya, J., Santellano-Estrada, E., et al. (2021). Stirred yogurt added with beetroot extracts as an antioxidant source: Rheological, sensory, and physicochemical characteristics. *Journal of Food Processing and Preservation, 45*(7), e15628. https://doi.org/10.1111/jfpp.15628

Ge, Y., Yu, X., Zhao, X., Liu, C., Li, T., Mu, S., et al. (2024). Fermentation characteristics and postacidification of yogurt by *Streptococcus thermophilus* CICC 6038 and *Lactobacillus delbrueckii* ssp. *bulgaricus* CICC 6047 at optimal inoculum ratio. *Journal of Dairy Science, 107*(1), 123–140. https://doi.org/10.3168/jds.2023-23817

Gengatharan, A., Dykes, G. A., & Choo, W. S. (2017). The effect of pH treatment and refrigerated storage on natural colourant preparations (betacyanins) from red pitahaya and their potential application in yoghurt. *LWT, 80*, 437–445. https://doi.org/10.1016/j.lwt.2017.03.014

Ghasempour, Z., Javanmard, N., Langroodi, A. M., Alizadeh-Sani, M., Ehsani, A., & Kia, E. M. (2020). Development of probiotic yogurt containing red beet extract and basil seed gum; techno-functional, microbial and sensorial characterization. *Biocatalysis and Agricultural Biotechnology, 29*, Article 101785. https://doi.org/10.1016/j.bcab.2020.101785

Groves, H. T., Higham, S. L., Moffatt, M. F., Cox, M. J., & Tregoning, J. S. (2020). Respiratory viral infection alters the gut microbiota by inducing inappetence. *mBio, 11*(1). https://doi.org/10.1128/mBio.03236-19. e03236–19.

Gupta, A., Sanwal, N., Bareen, M. A., Barua, S., Sharma, N., Olatunji, O. J., et al. (2023). Trends in functional beverages: Functional ingredients, processing technologies, stability, health benefits, and consumer perspective. *Food Research International, 170*, Article 113046. https://doi.org/10.1016/j.foodres.2023.113046

Guneser, O. (2021). Kinetic modelling of Betalain stability and color changes in yogurt during storage. *Polish Journal of Food and Nutrition Sciences*, (2), 71. http://journal.pan.olsztyn.pl/.

Hamad, G., Ombarak, R. A., Eskander, M., Mehany, T., Anees, F. R., Elfayoumy, R. A., et al. (2022). Detection and inhibition of *Clostridium botulinum* in some Egyptian fish products by probiotics cell-free supernatants as bio-preservation agents. *LWT, 163*(6), Article 113603. https://doi.org/10.1016/j.lwt.2022.113603

Hashem, M. I. (2018). Supplementation of buttermilk with red beet root for producing fermented milk beverage. *Egyptian Journal of Agricultural Research, 96*(3), 1111–1125. https://doi.org/10.21608/ejar.2018.140400

Hussain, M., Akhtar, S., Khalid, N., Azam, M., Iqbal, M. W., Ismail, T., et al. (2023). Hydrolysis, microstructural profiling and utilization of *Cyamopsis tetragonoloba* in yoghurt. *Fermentation, 9*(1), 45. https://doi.org/10.3390/fermentation9010045

IDF. (1985). Milk and milk products. enumeration of coliforms- colony counts technique and most probable number technique at 30°C. fil-idf 73A international dairy federation brussels, Belgium.

IDF (1997). Yogurt. enumeration of characteristic microorganisms. colony count technique at 37°C. fil-idf 117B international dairy federation brussels, Belgium.

Januário, J. G. B., da Silva, I. C. F., De Oliveira, A. S., De Oliveira, J. F., Dionísio, J. N., Klososki, S. J., et al. (2017). Probiotic yogurt flavored with organic beet with carrot, cassava, sweet potato or corn juice: Physicochemical and texture evaluation, probiotic viability and acceptance. *International Food Research Journal*, (1), 24.

Kale, R. V., Kadam, S. S., & Hashmi, S. I. (2011). Studies on effect of different varieties of date palm paste incorporation on quality characteristics of yogurt. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 10*(6), 2371–2381.

Katsiari, M. C., Voutsinas, L. P., & Kondyli, E. (2002). Manufacture of yogurt from stored frozen sheep's milk. *Food Chemistry, 77*(4), 413–420. https://doi.org/10.1016/S0308-8146(01)00367-3

Luzardo-Ocampo, I., Ramírez-Jiménez, A. K., Yañez, J., Mojica, L., & Luna-Vital, D. A. (2021). Technological applications of natural colorants in food systems: A review. *Foods, 10*(3), 634. https://doi.org/10.3390/foods10030634

Marshall, R. T. (1992). *Standard methods for the examination of dairy products* (16th ed.). Washington, DC: Am. Publ. Health Assoc.

Mehany, T., Taha, A., Olawoye, B., Korma, S. A., Popoola, O. O., Esua, O. J., et al. (2023). Pigmented Pseudocereals: Chemistry, Functionality, and Technological Aspects in Food Systems. *Pigmented cereals and millets: Bioactive profile and food applications* (pp. 144–180). UK: The Royal Society of Chemistry. https://10.1039/9781837670291-00144.

Meng, X., Huang, Y., Xiong, J., Cheng, Z., Yang, T., Li, Z., et al. (2024). *Lactiplantibacillus plantarum* ZFM55 improves texture and flavor of yogurt, increases beneficial metabolites, and the co-fermented yogurt promotes human gut microbiota health. *LWT, 198*, Article 115929. https://doi.org/10.1016/j.lwt.2024.115929

Mysonhimer, A. R., Brown, M. D., Alvarado, D. A., Cornman, E., Esmail, M., Abdiel, T., et al. (2024). Honey added to yogurt with *Bifidobacterium animalis* subsp. *lactis* DN-173 010/CNCM I-2494 supports probiotic enrichment but does not reduce intestinal transit time in healthy adults: A randomized, controlled, crossover trial. *The Journal of Nutrition*. https://doi.org/10.1016/j.tjnut.2024.05.028. In press.

Noureldin, H. A., Salman, K. H., Ali, H. M., & Mansour, A. I. A. (2020). Using of sugarcane molasses on novel-yogurt making. *Archives of Agriculture Sciences Journal, 3*(2), 156–167. https://doi.org/10.21608/aasj.2020.37510.1027

Olumese, F. E., & Oboh, H. A. (2018). Hepatoprotective effect of beetroot juice on liver injury in male Sprague–Dawley rats. *Annals of Tropical Pathology, 9*(1), 83–88. https://www.atpjournal.org/text.asp?2018/9/1/83/234152.

Ospanov, A., Velyamov, S., Tlevlessova, D., Schetinina, E., Kairbayeva, A., Makeeva, R., et al. (2023). Survival of lactic acid bacteria when using the developed yogurt from the milk of small cattle under in-vitro conditions. *Food Science and Technology, 43*, Article e117722. https://doi.org/10.1590/fst.117722

Santos Pereira, E. D., de Oliveira Raphaelli, C., Massaut, K. B., Camargo, T. M., Radünz, M., Hoffmann, J. F., et al. (2024). Probiotic yogurt supplemented with *Lactococcus lactis* R7 and red guava extract: Bioaccessibility of phenolic compounds and influence in antioxidant activity and action of alpha-amylase and alpha-glucosidase enzymes. *Plant Foods for Human Nutrition, 79*, 219–224. https://link.springer.com/article/10.1007/s11130-024-01149-y.

Sathya, P., Radha, K., Sathian, C. T., Ramnath, V., Jayavardhanan, J., & Venkatachalapathy, R. T. (2019). Isolation and characterisation of angiotensin converting enzyme (ACE) inhibitory peptides in fermented milk of Malabari goat. *Indian Journal of Small Ruminants (The), 25*(2), 217–221.

Savaiano, D. A., & Hutkins, R. W. (2021). Yogurt, cultured fermented milk, and health: A systematic review. *Nutrition Reviews, 79*(5), 599–614. https://doi.org/10.1093/nutrit/nuaa013

Sharma, H., & Ramanathan, R. (2023). Differences and correlation among various fatty acids of cow milk and goat milk probiotic yoghurt: Gas chromatography, PCA and network based analysis. *Food Chemistry Advances, 3*, Article 100430. https://doi.org/10.1016/j.focha.2023.100430

Shaukat, H., Ali, A., Zhang, Y., Ahmad, A., Riaz, S., Khan, A., et al. (2023). Tea polyphenols: Extraction techniques and its potency as a nutraceutical. *Frontiers in Sustainable Food Systems, 7*, 281. https://doi.org/10.3389/fsufs.2023.1175893

Shehata, M. G., Abd El-Aziz, N. M., Mehany, T., & Simal-Gandara, J (2023). Taro leaves extract and probiotic lactic acid bacteria: A synergistic approach to improve antioxidant capacity and bioaccessibility in fermented milk beverages. *LWT, 187*, Article 115280. https://doi.org/10.1016/j.lwt.2023.115280

Sibanda, T., Marole, T. A., Thomashoff, U. L., Thantsha, M. S., & Buys, E. M. (2024). *Bifidobacterium* species viability in dairy-based probiotic foods: Challenges and innovative approaches for accurate viability determination and monitoring of probiotic functionality. *Frontiers in Microbiology, 15*, Article 1327010. https://doi.org/10.3389/fmicb.2024.1327010

Sieuwerts, S., Molenaar, D., van Hijum, S. A., Beerthuyzen, M., Stevens, M. J., Janssen, P. W., et al. (2010). Mixed-culture transcriptome analysis reveals the molecular basis of mixed-culture growth in *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. *Applied and Environmental Microbiology, 76*(23), 7775–7784. https://doi.org/10.1128/AEM.01122-10

Singh, H.B., and Kumar, A.B. (2014). Handbook of natural dyes and pigments. Woodhead Publishing India Pvt Limited.

Soutelino, M. E. M., da Silva, D. B., da Silva Rocha, R., de Oliveira, B. C. R., Esmerino, E. A., da Cruz, A. G., et al. (2023). Yogurt added with beetroot extract: Physicochemical parameters, biological activities and sensory evaluation by check-all-that-apply method. *International Journal of Food Science & Technology, 58*(6), 3303–3309, 10.1111/ijfs.16214.

Sunmola, A. A., Ogbole, O. O., Faleye, T. O., Adetoye, A., Adeniji, J. A., & Ayeni, F. A. (2019). Antiviral potentials of *Lactobacillus plantarum, Lactobacillus amylovorus*, and *Enterococcus hirae* against selected Enterovirus. *Folia Microbiologica, 64*(2), 257–264, 10.1007/s12223-018-0648-6.

Tobolková, B., Polovka, M., Daško, Ľ., Belajová, E., & Durec, J. (2020). Evaluation of qualitative changes of apple-beetroot juice during long-term storage at different temperatures. *Journal of Food Measurement and Characterization, 14*(6), 3381–3388. https://doi.org/10.1007/s11694-020-00592-0

Vargas-Ramella, M., Pateiro, M., Maggiolino, A., Faccia, M., Franco, D., De Palo, P., & Lorenzo, J. M. (2021). Buffalo milk as a source of probiotic functional products. *Microorganisms, 9*(11), 2303. https://doi.org/10.3390/microorganisms9112303

Wijesekara, A., Weerasingha, V., Jayarathna, S., & Priyashantha, H. (2022). Quality parameters of natural phenolics and its impact on physicochemical, microbiological, and sensory quality attributes of probiotic stirred yogurt during the storage. *Food Chemistry: X, 14*, Article 100332. https://doi.org/10.1016/j.fochx.2022.100332

Wood, J. E., Gill, B. D., Longstaff, W. M., Crawford, R. A., Indyk, H. E., Kissling, R. C., et al. (2021). Dairy product quality using screening of aroma compounds by selected ion flow tube−mass spectrometry: A chemometric approach. *International Dairy Journal, 121*, Article 105107. https://doi.org/10.1016/j.idairyj.2021.105107

Yadav, M., Masih, D., & Sonkar, C. (2016). Development and quality evaluation of beetroot powder incorporated yogurt. *International Journal of Science, Engineering and Technology, 4*(4), 582–586.

Yi, L., Min, J. T., Jun, C. L., Long, H. X., Khoo, H. E., Ying, Z. J., et al. (2024). Buffalo yogurt fermented with commercial starter and *Lactobacillus plantarum* originating from breast milk lowered blood pressure in pregnant hypertensive rats. *Journal of Dairy Science, 107*(1), 62–73. https://doi.org/10.3168/jds.2023-23566

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2005). Fermentation of beet juice by beneficial lactic acid bacteria. *LWT, 38*(1), 73–75. https://doi.org/10.1016/j.lwt.2004.04.008