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PHYSICOCHEMICAL ANALYSIS AND ANTIOXIDANT BENEFITS OF YOGURT ENRICHED WITH BETALAINS FROM RED BEET (*BETA VULGARIS* L.)

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ABSTRACT

Food color is a key purchasing factor for consumers and a quality indicator. Recently, there has been a shift towards natural food colors, leading to increased use of red beet as a colorant. Beet betalains are valued in the food industry for their natural coloring, high water solubility, and non-toxic nature. This study aimed to compare two types of yogurt: one with natural beetroot juice colorant at concentrations of 6 g/L and 12 g/L (chosen based on preliminary tests), and a control yogurt with no colorant.

Yogurts were analyzed for physicochemical properties (pH, acidity, ash content, moisture content, syneresis), nutritional content (protein and sugar), phytochemical properties (phenolic and betalain content, antioxidant activity), and sensory attributes over 21 days of refrigerated storage at 4°C. Statistical analysis was performed using Tukey-Kramer HSD test (Minitab software) with a significance level of 0.05.

pH decreased while acidity increased in betalain-enriched yogurt during storage, yet pH remained between 4.5 and 4.8. Significant differences in ash and moisture content were noted. Phytochemical parameters and antioxidant potential improved with betalain enrichment at both 6 g/L and 12 g/L. Sensory evaluation after 21 days showed that yogurt with beetroot juice at 6 g/L and 12 g/L was preferred for its pink color and sweet taste.

The study highlights the benefits of adding beetroot betalain to yogurt. This plant provides valuable polyphenols and betalains, which are beneficial for health. Betalain-enriched yogurt can thus enhance health and offer protection against free radical damage

1.Introduction

Yogurt is a widely consumed fermented dairy product, known for its nutritional benefits and rich in essential nutrients like protein, calcium, potassium, phosphorus, and vitamins (Yadav et al., 2015; Fisberg and Machado, 2015). Its color is a crucial sensory attribute that influences consumer attraction and product acceptance. However, synthetic colors, used as additives, pose serious health risks due to their carcinogenic effects (Alshehry, 2019), leading to a shift towards natural colors or 'bio-colors,' which are derived from vegetables, fruits, roots, and microorganisms (Ravichandran et al., 2011; Haddar, 2016).

Beetroot, known for its antimicrobial, antiviral properties, and its ability to inhibit tumor cell proliferation, contains bioactive compounds such as betalains (Shivangi et al., 2019; Débia et al., 2023). Betalains are water-soluble nitrogen pigments, classified into betacyanins (red-violet) and betaxanthins

(yellow) (Nirmal et al., 2021). Due to their glycosylation and acylation, betalains show significant structural diversity and are suitable for use in acidic foods like dairy products. However, they are temperature-sensitive and degrade at high temperatures, with their stability decreasing with increased temperature and heating duration (Herbach et al., 2006).

Betalains have garnered scientific interest due to their high antioxidant activity, which is seven times greater than that of vitamin C, making them valuable not only as natural dyes but also for their potential to enhance food packaging and provide additional benefits (Castro-Enríquez et al., 2020). Considering the nutritional value and seasonal availability of beetroot, this study aims to formulate stirred yogurts incorporating crude betalain extracts from red beetroot as a natural colorant and antioxidant at two concentrations. The study will evaluate their physicochemical, nutritional, and sensory characteristics, as well as their antioxidant activity during storage, comparing them to a control yogurt with no added colorant.

2. Materials and methods

2.1.Plant material

The plant material used was beetroot (Beta vulgaris L.), characterized by its round shape, reddish-purple color, and long, thin taproot. The beetroots were hand-sorted and washed to remove impurities and damaged specimens. This variety was selected due to its excellent antioxidant properties and high betalain content, which are less studied compared to other natural pigments.

2.2.Extraction of concentrated beetroot juice (betalains)

The washed beetroot was cut into small pieces and placed in an electric mixer for chopping. The chopped beetroot (5 g) was then shaken for 10 minutes in a beaker with 15 ml of distilled water. The resulting solution was filtered, centrifuged at 10,000 rpm for 20 minutes (HB110, Behsan Company, Iran), and subsequently concentrated using a Rotavapor (Laura et al., 2016).

2.3.Milk and lactic ferments

The raw materials for making the yogurt were purchased from a grocery store and underwent acceptance tests, including the boiling stability test and the methylene blue reduction test. The freeze-dried lactic acid bacteria culture used (Termophilic YoFlex Mild 1.0) was supplied by the COLAITAL group (Bir Khadem, Algeria). It contains a mixture of two bacterial species: Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus (200 U/1000 L).

2.4.Preparation of yoghurt

The enriched stirred yogurts were prepared separately following the recipe provided by the Colaittal Group, Algeria. A mixture of skimmed and whole cow's milk, adjusted for solids by adding milk powder (0% fat) and sugar, was heated to 95°C for 5 minutes and then cooled to 45°C. The mixture was inoculated with the freeze-dried lactic acid bacteria culture and beetroot betalain at different concentrations (6 g/L and 12 g/L) and incubated at 42°C. The resulting gel was vortexed, packed into jars, and rapidly cooled to a temperature between 4°C and 6°C. The negative control was prepared using the same steps, but without the addition of beetroot betalain.

2.5.Physico-chemical analyses

2.5.1.pH

The pH was measured using a pH meter (OHAUS, Germany). This measurement reflects the concentration of H+ ions resulting from the production of organic acids by lactic acid bacteria (Amirdivani and Baba, 2011).

2.5.2.Titratable acidity

Titratable acidity measures the amount of lactic acid in the yogurt. To determine it, an acid-base titration was performed using a sodium hydroxide (NaOH) solution. Ten milliliters of distilled water were added to 5 grams of each sample. Two to three drops of

phenolphthalein were added, and the mixture was titrated with NaOH (N/9) until the color changed to pink (Amirdivani, 2015). Phenolphthalein is colorless below pH 8 and turns pink at pH 8.

A permanent pink color indicates the end of the titration. Results were expressed using the following formula:

$$\textbf{Dornic°=V×10} \quad (1)$$

With :

V: the volume of sodium hydroxide solution (ml).

2.5.3.Syneresis

Yogurt syneresis (whey separation) was determined using the centrifugation method described by Özturk and Öner (1999). Briefly, 20 grams of yogurt were centrifuged at 6000 rpm for 20 minutes. The supernatant was then collected and weighed. Syneresis was calculated using the following equation (Martha et al., 2021):

$$\textbf{Syneresis (\%) = weight of supernatant (g)/ weight of yoghurt sample (g) X 100} \quad (2)$$

2.5.4.Brix degree

The Brix of the samples was determined by placing a drop of the sample on the prism plate of the instrument, facing the light. The Brix value was then read through the eyepiece of the instrument (Azzouzi et al., 2022).

2.5.5.Moisture content

Moisture content was determined as follows: 1 gram of each sample was weighed and then placed in an oven (Memmert, Germany) at 105°C until the weight stabilized (Mahaut et al., 2000).

$$\textbf{H\% = M2÷M1×100} \quad (3)$$

With :

H : Humidity(%) ;

M1 : Mass of sample before drying(g) ;

M2: Mass of sample after drying (g).

2.6.6.Ash content

Ash content is determined by calcining a test sample until the organic matter is completely burned off in a muffle furnace (Thermolyne, France) at 550°C ± 5°C (Fekata et al., 2022). The mineral content was then calculated using the following formula:

$$\textbf{C\% = [residue weight ÷ sample weight]×100} \quad (4)$$

2.5.7.Nutritional analysis

2.5.7.1.Sugar content

Total sugars were determined using the phenol-sulfuric acid method. One gram of the sample was placed in a test tube with 1 ml of 5% phenol. Five milliliters of sulfuric acid were added quickly without allowing it to run down the sides, and the mixture was shaken immediately. A yellow coloration developed, which remained stable for several hours. The tubes were then placed in a water bath at 25-30°C for 20 minutes and subsequently cooled under running water to 20°C. Absorbance was measured at 488 nm using a spectrophotometer (UV-Visible S-2150, UNICO, USA) (Feller et al., 1991). Sugar levels were determined by referencing a standard glucose curve.

2.5.7.2.Protein content

The Bradford protein assay is a colorimetric method that uses Coomassie Blue as its primary reagent. In its free cationic form, this reagent absorbs light at a wavelength of 465 nm. Upon binding to proteins and their aromatic groups, the absorption maximum shifts to 595 nm. Briefly, 5 ml of Bradford reagent was added to 100 µl of yogurt and allowed to react in the dark for 5 minutes. The absorbance was measured at 595 nm. Protein levels were determined by referencing a bovine serum albumin (BSA) standard series (Azerdo et al., 2003).

2.5.7.3.Preparation of the yoghurt supernatant

The yogurt sample (10 g) was mixed with 2.5 ml of distilled water, and the pH was

adjusted to 4.0 with 1 M HCl (Honeywell, Germany). The yogurt was then incubated at 45°C for 10 minutes and centrifuged at 6000 rpm for 20 minutes. The supernatant was collected and its pH adjusted to 7.0 with 1 N NaOH. The neutralized supernatant was centrifuged again at 6000 rpm for 20 minutes at 4°C, and the resulting supernatant was used for analysis (Shori, 2020).

2.5.7.4.Betalain content

Ten grams (10 g) of each yogurt sample were added to 50 ml of distilled water. The mixture was shaken for 30 minutes, then centrifuged at 3000 rpm for 10 minutes, and filtered through filter paper. The absorbance was measured at 532 nm for betacyanins and 482 nm for betaxanthins (Khatabi et al., 2013). Betacyanin content was expressed in mg/100 g and calculated using the following equation:

$$\boldsymbol{B}=\mathbf{A}\times\mathbf{FD}\times\mathbf{PM}\times\mathbf{1000}/\boldsymbol{\varepsilon}\times\mathbf{L} \quad (5)$$

With :

A: Absorbance at 532 nm (betacyanins) and 482 nm (indicaxanthins);

FD: dilution factor;

PM: molecular weight (550 and 380 g/mol for betacyanin and indicaxanthin, respectively);

ε: molar extinction coefficients (60000l/mol.cm for betacyanins and 48000l/mol.cm for indicaxanthins);

L: optical path (1 cm).

2.5.7.5.Total phenolic content

Total polyphenols were determined colorimetrically using the Folin-Ciocalteu reagent (Merck, Germany). The intensity of the blue color produced is proportional to the amount of polyphenols present in the samples. The reaction mixture was prepared by mixing 1 ml of yogurt extract with 1 ml of 95% ethanol (Merck, Germany) and 5 ml of distilled water. Then, 0.5 ml of 50% Folin-Ciocalteu reagent was added to each sample, which was then mixed thoroughly by vortexing. After incubating at room temperature and in the dark for 60 minutes, the absorbance was measured at 725 nm (Shori et al., 2018). Total polyphenol content was estimated from a calibration curve prepared with gallic acid and expressed as milligrams of gallic acid equivalents (mg EAG) per milliliter of yogurt supernatant.

2.5.7.6.Antioxidant activity

Total antioxidant capacity

0.3 ml of each yogurt extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). After incubation at 95°C for 90 minutes and subsequent cooling, the absorbance of the solution was measured at 695 nm. A blank control, containing 3 ml of reagent solution and 0.3 ml of distilled water, was incubated under the same conditions as the sample. The total antioxidant capacity was expressed as milligrams of ascorbic acid equivalents per milliliter of yogurt supernatant (mg EAA/ml supernatant) (Prieto et al., 1999).

2.5.7.7.Hydrogen peroxide reduction

The hydrogen peroxide reduction assay was performed according to the method of Taşkın and Bağdatlıoğlu (2020) with slight modifications. One milliliter of each yogurt extract was mixed with 2.4 ml of phosphate buffer (pH 7.4) and 0.6 ml of 4 mM H_2O_2. After 40 minutes, the absorbance was measured at 230 nm. Water was used in place of the H_2O_2 solution for the blank. Hydrogen peroxide uptake was determined using the following equation:

$$\mathbf{H_2O_2\% = [(AC-AE)/AC] \times 100} \quad (6)$$

With :

AC: control absorbance;

AE: sample absorbance.

2.5.7.8.DPPH free radical test

To evaluate the scavenging effect of each yogurt extract on the DPPH radical (Sigma-Aldrich, Germany), 500 µl of the sample was mixed with 60 mM methanolic DPPH solution. After incubating for one hour at 25°C, the absorbance was recorded at 517 nm. The free

radical scavenging activity was estimated using the following equation (Shori, 2020).

$$\% \text{ inhibition} = [(AC-AE)/AC] \times 100 \quad (7)$$

With :

AC: control absorbance

AE: extract absorbance.

2.5.7.9.Sensory analysis

Sensory analysis was conducted by a qualified panel of 20 individuals of varying ages, who completed a questionnaire. The evaluation used a nine-point hedonic scale to assess color, odor, sweetness, acidic flavor, and consistency attributes. According to the nine-point scale: 9 = very much liked, 8 = liked very much, 7 = liked moderately, 6 = liked slightly, 5 = neither liked nor disliked, 4 = disliked slightly, 3 = disliked moderately, 2 = disliked very much, and 1 = extremely disliked (Watts et al., 1991).

2.6.Statistical analysis

Results were expressed as mean ± standard deviation (from three replicates), and data were compared based on these means. Differences between means were assessed using the Tukey-Kramer HSD test (Minitab software) at a significance level of 0.05.

3.Results and discussions

3.1.Physico-chemical analysis of prepared stirred yoghurts

3.1.1.pH values

The pH values (Figure 01) did not decrease significantly ($p > 0.05$) for the negative control yogurt (from 4.66 ± 0.06 to 4.54 ± 0.015) and the betalain-enriched yogurt at 12 g/L (from 4.83 ± 0.119 to 4.64 ± 0.04). In contrast, a non-significant increase ($p > 0.05$) was observed from day 1 to day 7 for the betalain-enriched yogurt at 6 g/L (from 4.64 ± 0.04 to 4.72 ± 0.06). From day 7 to day 14, there was a non-significant increase ($p > 0.05$) in pH values for the betalain-enriched yogurt at 12 g/L (from 4.64 ± 0.045 to 4.75 ± 0.006), the betalain-enriched yogurt at 6 g/L (from 4.72 ± 0.065 to 4.76 ± 0.005), and the negative control yogurt (from 4.54 ± 0.015 to 4.82 ± 0.006). From day 14 to day 21, a highly significant decrease ($p < 0.001$) was observed for all yogurts. The results indicated that yogurt enriched with betalain at both concentrations had a higher pH than the negative control during days 1-7, whereas the opposite was observed during days 14-21.

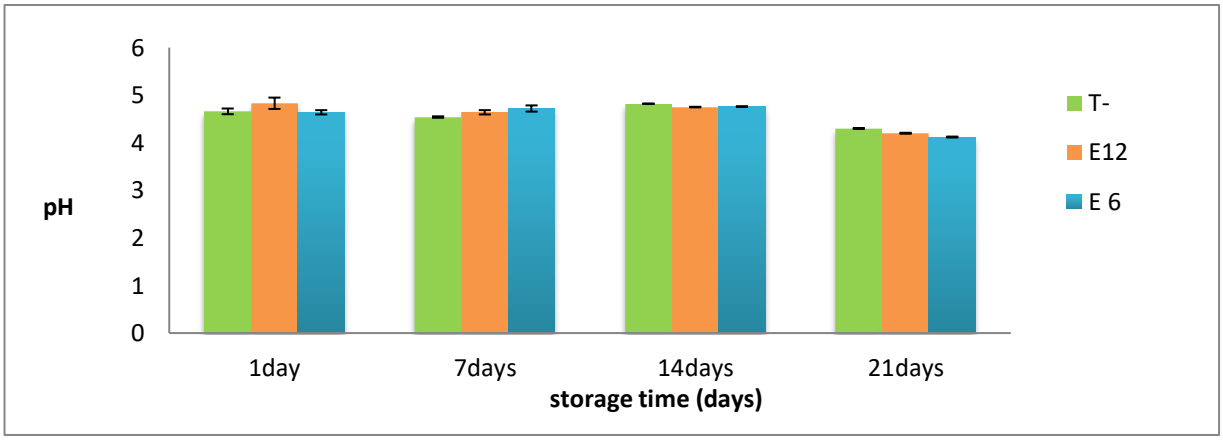

Figure 1. Changes in pH of formulated yoghurts during 21 days' storage at 4°C. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.1.2.Acidity

Analysis of Table 1 shows a non-significant decrease ($p > 0.05$) in the acidity of the negative control yogurt (from 0.99 ± 0.0057 Dornic to 0.76 ± 0.252 Dornic) and the betalain-enriched yogurt at 12 g/L (from 1.66 ± 0.577 Dornic to

0.83 ± 0.289 Dornic) during the period from day 1 to day 7. The period from day 7 to day 14 shows a non-significant increase ($p > 0.05$) in acidity for the negative control yogurt, the yogurt enriched with betalain at 12 g/L, and the yogurt enriched with betalain at 6 g/L. The period from day 14 to day 21 showed a non-significant decrease ($p > 0.05$) in acidity for the negative control yogurt and the yogurt enriched with betalain at 6 g/L. However, a non-significant increase ($p > 0.05$) was observed for the yogurt fortified with betalain at 12 g/L.

Table 1. Changes in acidity of formulated yoghurts during 21 days of storage at 4°C.

Types of yoghurt	1day (D°)	7days (D°)	14days (D°)	21days (D°)
Negative control	0.99± 0.005	0.76± 0.252	0.96± 0.057	0.93± 0.057
Betalain-enriched yoghurt at 12g/l	1.66± 0.577	0.83± 0.289	1.33± 0.577	1.83± 0.289
Betalain-enriched yoghurt at 6g/l	0.99± 0.005	1± 0.500	1.33± 0.577	0.96± 0.057

3.1.3.Brix level

The results of the Brix determination for the five prepared yogurts are summarized in Table 2.

The data indicate a non-significant decrease ($p > 0.05$) in Brix values for the negative control yogurt and a significant decrease ($p < 0.05$) for the yogurt fortified with betalain at 6 g/L during the period from day 1 to day 7. The yogurt fortified with betalain at 12 g/L remained stable during this period.

From day 7 to day 14, a non-significant increase ($p > 0.05$) was observed for the yogurt fortified with betalain at 12 g/L, while a significant increase ($p < 0.05$) was noted for the yogurt fortified with betalain at 6 g/L.

During the period from day 14 to day 21, all types of yogurt showed a decrease: significant ($p < 0.05$) for the negative control yogurt, and highly significant ($p \leq 0.01$) for both the yogurt enriched with betalain at 12 g/L and the yogurt enriched with betalain at 6 g/L.

Table 2. Brix evolution of formulated yoghurts during 21 days of storage at 4°C.

Types of yoghurt	1day (B°)	7 days (B°)	14 days (B°)	21 days (B°)
Negative control	14.66± 0.577	14.33± 0.577	14.33± 0.577	12.33± 0.577
Betalain-enriched yoghurt at 12g/l	14.33± 0.577	14.33± 0.577	14.66± 0.577	12.33± 0.577
Betalain-enriched yoghurt at 6g/l	14.99± 0.005	13.33± 0.577	14.66 ±0.577	12.33 ±0.577

3.1.4.Moisture content

The results of determining the moisture content of the five prepared yogurts are shown in Figure 02. Figure 02 indicates a highly significant decrease ($p \leq 0.01$) in moisture content for yogurts fortified with betalain at 12 g/L and 6 g/L, and a very highly significant decrease ($p \leq 0.001$) for the negative control yogurt during the period from day 1 to day 7.

From day 7 to day 14, there was a significant decrease ($p \leq 0.05$) in moisture content for the yogurt enriched with betalain at 12 g/L and a highly significant decrease ($p \leq 0.01$) for the yogurt enriched with betalain at 6 g/L. Conversely, a non-significant increase was observed in the negative control yogurt.

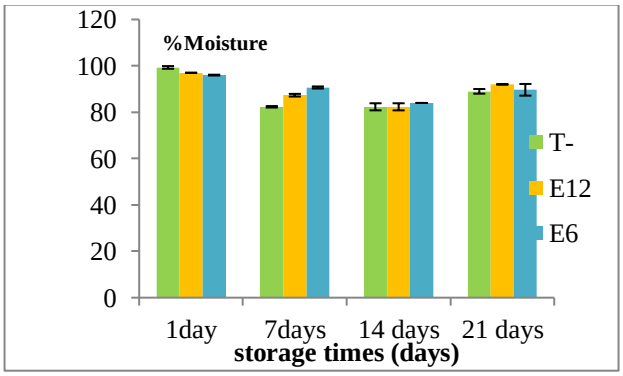

Figure 2. Moisture evolution of formulated yoghurts stored at 4°C for 21 days. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

From day 14 to day 21, a highly significant increase ($p \leq 0.01$) in moisture content was observed for the negative control yogurt and the yogurt fortified with betalain at 12 g/L, while a non-significant increase ($p > 0.05$) was noted for the yogurt with betalain at 6 g/L.

3.1.5.Ash content

The results for ash content are shown in the table below:

The data indicate a significant decrease ($p < 0.05$) in ash content for the negative control yogurt, while a non-significant decrease ($p > 0.05$) was observed for the other yogurts during the period from day 1 to day 7.

During the period from day 7 to day 14, there was a non-significant increase ($p > 0.05$) in ash content for the yogurts enriched with betalain at 12 g/L and 6 g/L, while the negative control yogurt showed a non-significant decrease ($p > 0.05$).

From day 14 to day 21, a highly significant decrease ($p \leq 0.01$) in ash content was observed for the negative control yogurt. The ash content of yogurt fortified with betalain at 12 g/L showed a highly significant increase ($p \leq 0.01$), while the ash content of yogurt fortified with betalain at 6 g/L increased significantly ($p < 0.05$).

Table 3. Change in ash content of formulated yoghurts stored at 4°C for 21 days.

Types of yoghurt	1day (%)	7 days (%)	14 days (%)	21 days (%)
Negative control	14.33 ±1.53	10.67 ±1.15	8.33 ±1.15	2 ±1.00
Betalain-enriched yoghurt at 12g/l	5 ±1.73	2.33 ±1.53	4 ±1.00	9 ±1.00
Betalain-enriched yoghurt at 6g/l	3±1.73	1.99±0.005	4.67± 1.15	10.67± 1.53

3.1.6.Syneresis

The results of the syneresis measurement are shown in Figure 3.

During the period from day 14 to day 21, a highly significant increase ($p \leq 0.01$) in syneresis was observed for the negative control yogurt (from 62.71 ± 0.247% to 68.06 ± 0.050%). A non-significant increase ($p > 0.05$) was noted for the yogurt enriched with betalain at 12 g/L (from 62.91 ± 0.843% to 62.93 ± 0.161%). Conversely, a highly significant decrease ($p \leq 0.01$) was observed for the yogurt enriched with betalain at 6 g/L (from 63.86 ± 0.473% to 60.45 ± 0.050%).

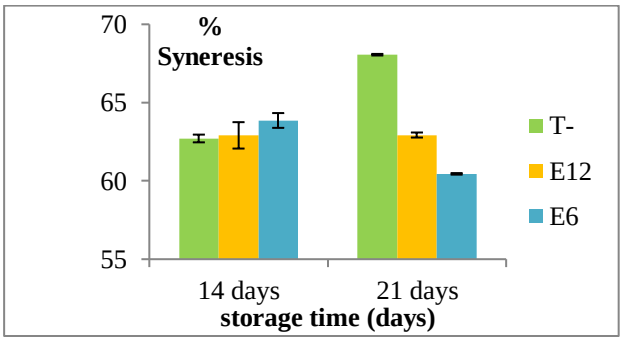

Figure 3. Syneresis of formulated yoghurt. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.2.Nutritional analysis of prepared stirred yoghurts

3.2.1.Sugar content

The sugar content measurements are shown in Figure 4.

From day 1 to day 7, a highly significant decrease ($p < 0.001$) in sugar content was observed for all types of yogurt.

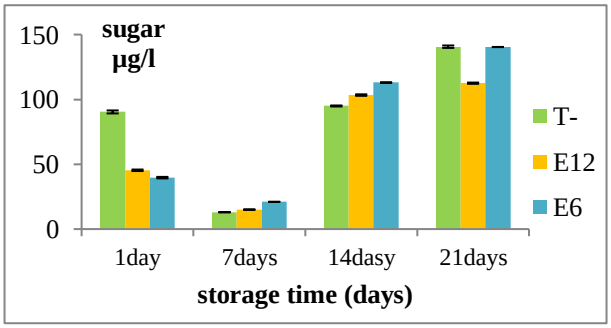

Figure 4. Changes in sugar content of formulated yoghurts during 21 days storage at 4°C.negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

During the period from day 7 to day 14, there was a highly significant increase ($p < 0.001$) in sugar content for all types of yogurt.

From day 14 to day 21, a highly significant increase ($p < 0.001$) in sugar content was again observed for all yogurts.

3.1.7.Protein content

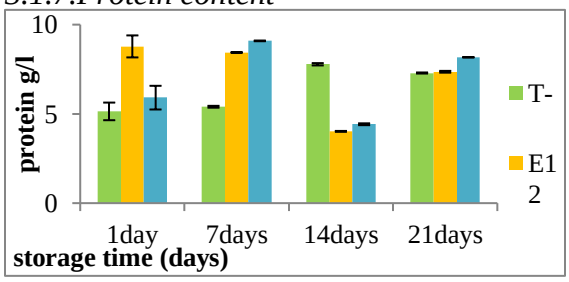

Figure 5. Changes in protein content of formulated yoghurts during 21 days storage at 4°C. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6)

The results of the protein analysis of the yogurts are shown in Figure 5.

The analysis reveals a non-significant decrease ($p > 0.05$) in protein content for the negative control yogurt and the yogurt enriched with betalain at 12 g/L during the period from day 1 to day 7. However, a significant increase ($p < 0.05$) was observed for the yogurt enriched with betalain at 6 g/L.

During the period from day 7 to day 14, there was a highly significant decrease ($p < 0.001$) in protein content for the yogurts enriched with betalain at 6 g/L and 12 g/L. The negative control yogurt showed a very highly significant increase ($p < 0.001$).

From day 14 to day 21, a highly significant decrease ($p < 0.01$) in protein content was observed for the negative control yogurt. In contrast, a very highly significant increase ($p < 0.001$) was noted for the yogurts enriched with betalain at 12 g/L and 6 g/L.

3.3.Phytochemical analyses

3.3.1.Betalain content

The results of the betalain determination in the yogurts are shown in Figure 6.

During the period from day 1 to day 7, a very highly significant decrease ($p < 0.001$) in betalain content was observed for the negative control yogurt and the yogurt fortified with betalain at 6 g/L. In contrast, a very highly significant increase ($p < 0.001$) was noted for the yogurt fortified with betalain at 12 g/L.

From day 7 to day 14, a very highly significant decrease ($p < 0.001$) in betalain content was observed for all types of yogurt. However, during the period from day 14 to day 21, a very highly significant increase ($p < 0.001$) in betalain content was observed for all yogurts.

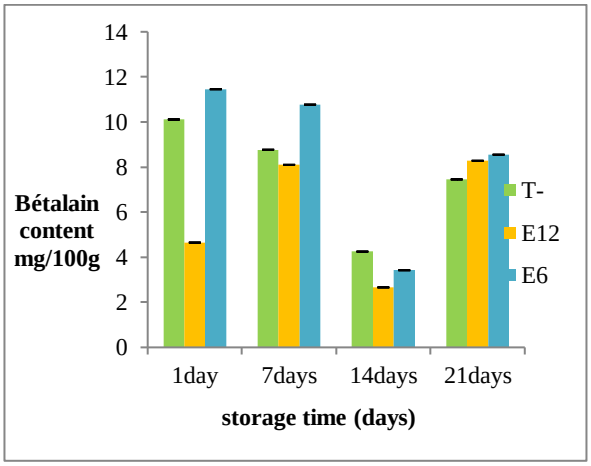

Figure 6. Evolution of betalain content in prepared yoghurts during 21 days of storage at 4°C. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.3.2.Total phenolic content

The test identified total polyphenols in the aqueous extracts of the prepared yogurts.

The results presented in Figure 7 show a non-significant decrease ($p > 0.05$) in polyphenol content for the supernatant of the yogurt enriched with betalain at 6 g/L. Conversely, a non-significant increase ($p > 0.05$) was observed in the supernatants of the negative control yogurt and the yogurt fortified with

betalain at 12 g/L during the period from day 1 to day 7.

From day 7 to day 14, a significant decrease ($p < 0.05$) in phenolic compound content was observed for the supernatants of the negative control yogurt, and the yogurts fortified with betalain at 12 g/L and 6 g/L.

During the period from day 14 to day 21, a very highly significant increase ($p < 0.001$) in phenolic compound content was observed for the supernatants of the yogurts enriched with betalain at 6 g/L and 12 g/L. In contrast, a significant decrease ($p < 0.05$) was noted for the supernatant of the negative control yogurt.

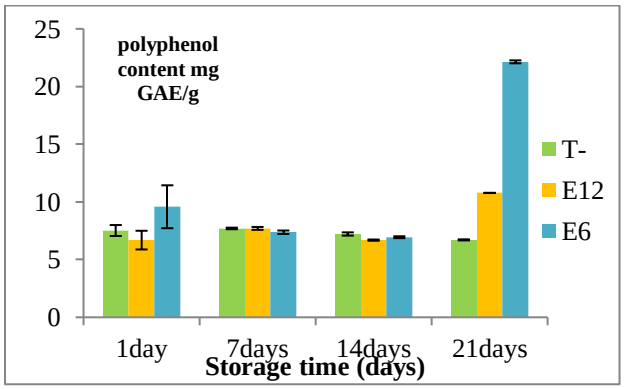

Figure 7. Changes in total phenolic compound content in the supernatants of yoghurts prepared during 21 days storage at 4°C.negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.3.Antioxidant activity

3.3.1.Total antioxidant capacity

The results obtained are presented in Figure 8.

During the period from day 1 to day 7, there was a significant increase ($p < 0.05$) in total antioxidant capacity for the supernatant of the negative control yogurt. Additionally, a highly significant increase ($p < 0.01$) was observed for the supernatants of the yogurts enriched with betalain at 6 g/L and 12 g/L.

From day 7 to day 14, a significant decrease ($p < 0.05$) in total antioxidant capacity was noted for the supernatant of the negative control yogurt. A highly significant decrease ($p \leq 0.01$) was observed for the supernatant of the yogurt enriched with betalain at 12 g/L. In contrast, a significant increase ($p < 0.05$) was found for the supernatant of the yogurt enriched with betalain at 6 g/L during this period. This could be attributed to syneresis or betalain instability.

During the period from day 14 to day 21, there was a significant decrease ($p < 0.05$) in total antioxidant capacity for the supernatant of the negative control yogurt and the supernatant of the yogurt enriched with betalain at 6 g/L. However, a very highly significant increase ($p < 0.001$) was observed for the supernatant of the yogurt enriched with betalain at 12 g/L.

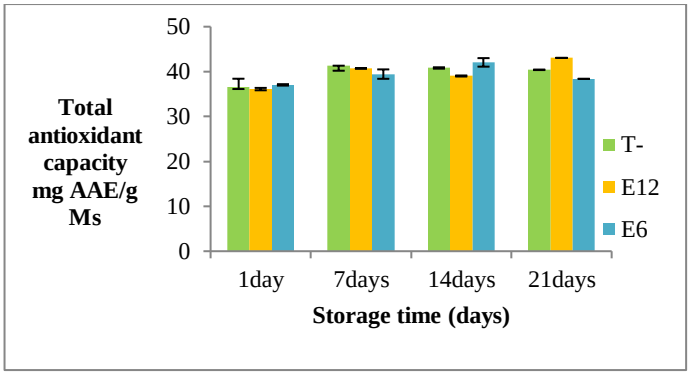

Figure 8. Changes in total antioxidant capacity in the supernatants of yoghurts prepared during 21 days' storage at 4°C.negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6)

3.3.2.Anti-free radical activity against the free radical DPPH·

The results are presented in Figure 9.

During the period from day 1 to day 7, there was a highly significant decrease ($p < 0.001$) in anti-free radical activity for the supernatants of the negative control yogurt and the yogurt enriched with betalain at 12 g/L. A highly significant decrease ($p \leq 0.01$) was also observed for the supernatant of the yogurt enriched with betalain at 6 g/L.

From day 7 to day 14, a highly significant decrease ($p \leq 0.01$) in anti-free radical activity was noted for the supernatant of the negative control yogurt, with a highly significant

decrease ($p < 0.001$) for the supernatants of the other yogurt samples.

During the period from day 14 to day 21, a highly significant increase ($p < 0.001$) in anti-free radical activity was observed for all yogurt supernatants.

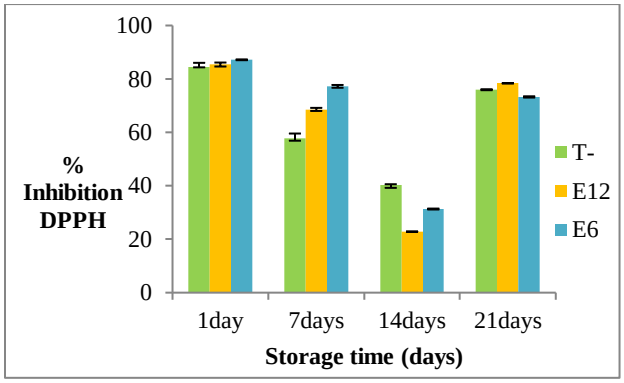

Figure 9. Changes in anti-free radical activity of supernatants prepared from yoghurts stored at 4°C for 21 days. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.3.3.Antioxidant activity by reduction of hydrogen peroxide

The results are presented in the table 4.The data show a highly significant decrease ($p < 0.001$) in the percentage reduction of hydrogen peroxide for all supernatants during the period from day 1 to day 7.

From day 7 to day 14, there was a significant increase ($p < 0.05$) in the percentage reduction of hydrogen peroxide for the negative control yogurt supernatant and the yogurt enriched with betalain at 12 g/L. A non-significant increase ($p > 0.05$) was observed for the yogurt enriched with betalain at 6 g/L.

From day 14 to day 21, a highly significant decrease ($p \leq 0.01$) in the percentage reduction of hydrogen peroxide was observed for the negative control yogurt supernatant, while a significant increase ($p < 0.05$) was noted for the supernatants of the other yogurts.

Table 4. Antioxidant activity measured by reduction of hydrogen peroxide in the supernatants of formulated yoghurts.

Percentage reduction in hydrogen peroxide (%)				
Types of yoghurt	1Day	7 Days	14 Dyas	21 Days
Negative control	78.59± 0.005	29.51±0.658	35.04±1.81	20.17±0.30
Betalain-enriched yoghurt at 12g/l	93.36± 0.05	33.13±0.520	35.74±0.250	46.58±1.21
Betalain-enriched yoghurt at 6g/l	92.06± 0.060	36.34±0.340	36.54±0.450	48.69±0.173

3.4.Sensory analysis

3.4.1.Colour

The color profile analysis of the formulated yogurts is shown in Figure 10. The results indicate the following:

-Days 1-7:Tasters reported a pink color for the yogurts fortified with betalain. Among these, the yogurt fortified with 6 g/L betalain was preferred over the others in sensory evaluations.

- Day 21:Five tasters noted that the color of the yogurt fortified with 6 g/L betalain had changed to beige.

3.4.2.Odour

The odor profile analysis of the formulated yogurts is presented in Figure 11. The sensory evaluation revealed distinct differences over the period:

-Days 1-14: The odor profiles varied significantly among the yogurts.

-Day 21: All tasters reported a strong odor in the formulated yogurts, except for the control yogurt, which was described as having a medium odor by four tasters.

3.4.3.Sweetness

The sweetness profile analysis of the formulated yogurts is presented in Figure 12. - Day 1 : Twelve tasters reported the control yogurt as having low sweetness, while eight found it to be medium.

- Day 7 : One taster perceived no sweetness, five noted low sweetness, and fourteen described it as medium.

- Day 14 : Twenty tasters rated the sweetness of the control yogurt as medium.

- Day 21: The sweetness was also rated as medium by twenty tasters.

For yogurts fortified with betalain at concentrations of 6 g/l and 12 g/l, the majority of tasters consistently rated the sweetness as medium throughout the period from Day 1 to Day 21.

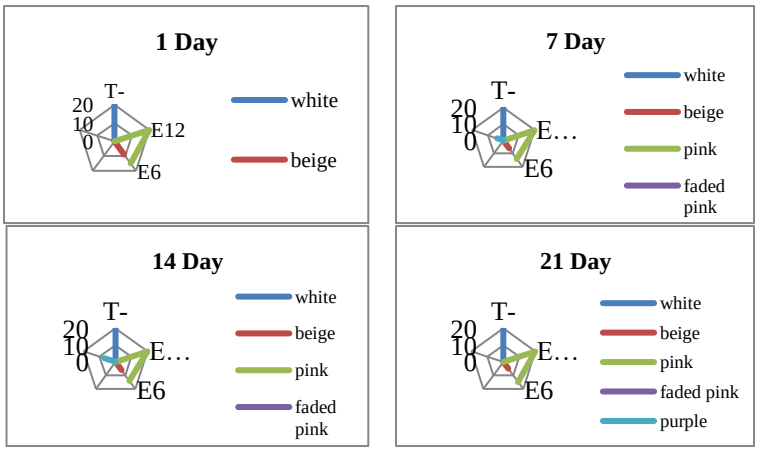

Figure 10. Colour profile of formulated yoghurts during storage. negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6)

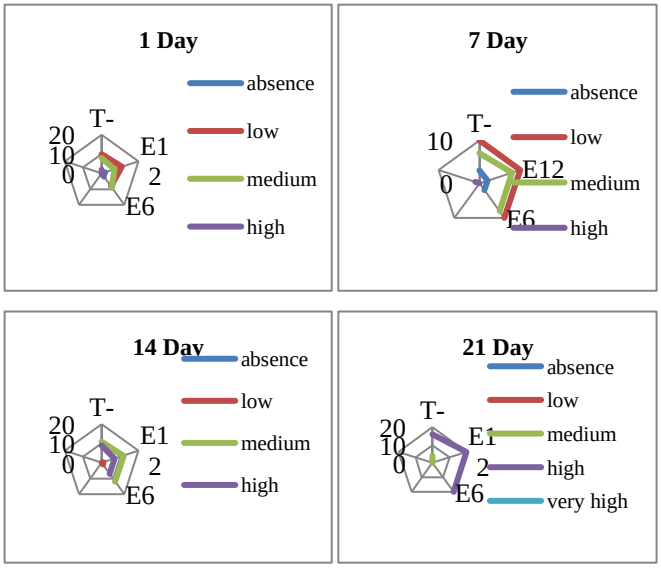

Figure 11. Odour profile of formulated yoghurts during storage.
negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

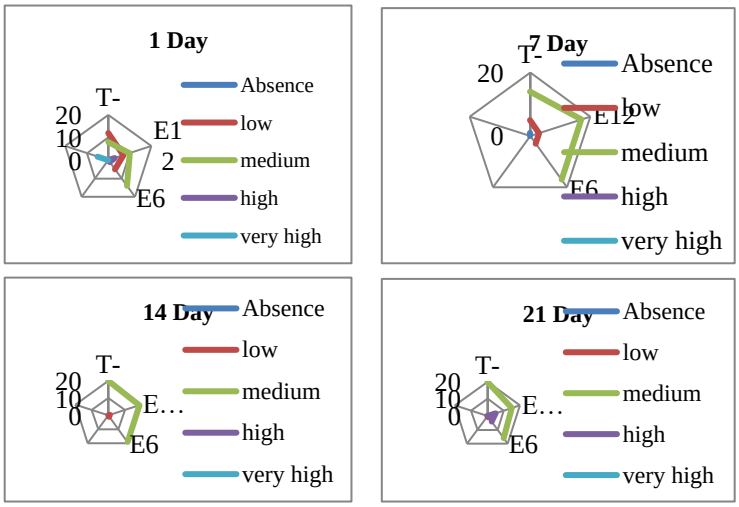

Figure12. Sweetness profile of formulated yoghurts during storage.
negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

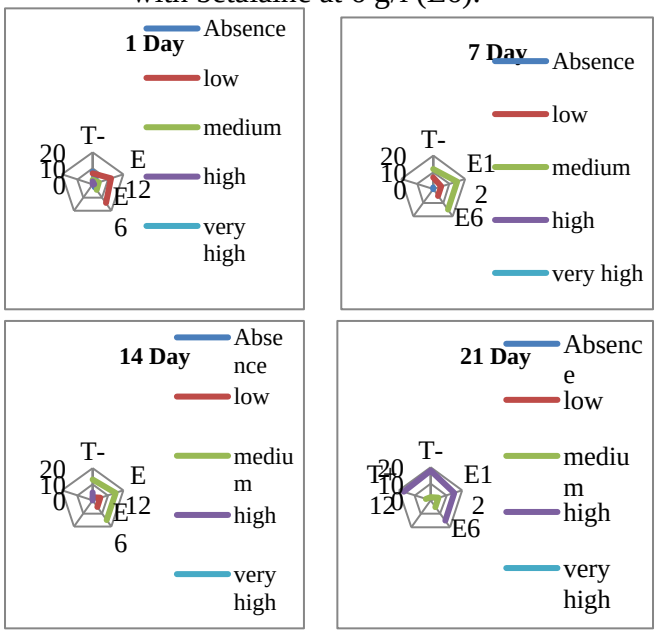

Figure 13. Acid flavour profile of formulated yoghurts during storage.
negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.4.4.Acidic flavour

The analysis of the acid flavor profile for the formulated yogurts is presented in Figure 13.

- Day 1: Most tasters perceived a low acid flavor in both the control yogurt and the yogurts fortified with betalain at 6 g/l and 12 g/l.
- Day 7: The majority of tasters noted a medium acid flavor in the yogurts fortified with betalain at both concentrations.
- Day 21: Most tasters reported a high acid flavor in the yogurts fortified with betalain at 6 g/l and 12 g/l.

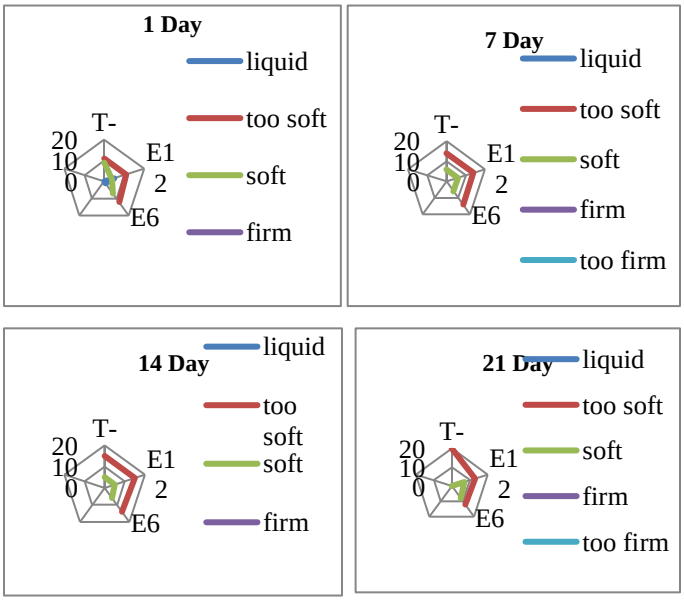

Figure 14. Consistency profile of formulated yoghurts during storage.
negative control yoghurt (T-), yoghurt enriched with betalaine at 12 g/l (E12), yoghurt enriched with betalaine at 6 g/l (E6).

3.4.5.Consistency

Analysis of the consistency profile of the formulated yoghurts is presented in Figure 14.

For the control yoghurt and according to the results in the figure above, we can see that for :

D 1: Eleven tasters found the consistency too soft, compared to nine tasters who found it moderately soft.

D 7: Most tasters found the consistency too soft.

D 14: Fifteen tasters found the consistency too soft and five said it was soft.

D 21: Twenty tasters found the consistency to be too soft.

For the yoghurt fortified with betalaine at 6g/l and 12g/l, most of the tasters noted a too soft consistency on days D1, D7, D14 and D21.

3.5.Discussion

The color analysis of the samples reveals that the color remains consistent with pH changes because betalains are relatively stable within a pH range of 3-7. Betacyanins, the natural pigments in question, are effective for coloring low-acid foods red to purple. Under anaerobic conditions, betanin is stable at a pH of 5.5–5.8, but this stability shifts to a lower pH range (4.0–5.0) in the presence of oxygen (Izabela Sadowska & Grzegorz, 2021).

The observed decrease in yogurt pH is attributed to the growth of lactic bacteria and continued production of lactic acid. Cold storage slows but does not entirely halt bacterial metabolic activity (Kaur et al., 2017). These findings are consistent with Jimoh and Kolapo (2007), who reported pH values between 3.39 and 5.68. However, the pH values for our enriched yogurt samples consistently ranged from 4.1 to 4.7, which helps maintain the product's functionality and flavor. Consequently, betalain enrichment helps stabilize pH during storage.

Our titratable acidity results are similar to those reported by Shalaby and Hassenin (2020), who found values between 1.09 D° and 1.11 D°. The decrease in titratable acidity may be due to the decomposition of fermentable substrates and sugars by microorganisms, particularly lactobacilli, which ferment carbohydrates to produce energy and lactic acid (Amirdivani, 2015). The increase in titratable acidity results from the accumulation of lactic acid produced by lactic acid bacteria (Kim et al., 2019).

Brix degrees in our study ranged from 0.55 to 1.30 °B, which is lower than the 7.64 to 10.36 °B reported by Madora et al. (2016) in their study on yogurt enriched with carrot powder. This discrepancy could be attributed to variations in sugar additions, climatic conditions, geographic location, soil type, and the vegetable varieties used. The decrease in Brix level is related to protein hydrolysis by lactic acid bacteria and the depletion of organic components (Won et al., 2018). It is worth noting that literature on Brix levels in yogurt enriched with betalains is limited, making comparisons challenging.

Betalain stability decreases exponentially with increasing moisture, likely due to the susceptibility of pigments to aldimine bond cleavage. The values found in our study fall within the range reported by Dhineshkumar and Ramasamy (2016) for beetroot juice-based yogurt, which ranged from 62.09 ± 0.4% to 91.08 ± 1.6%. Bourlioux et al. (2011) reported water content in yogurts and fermented milks between 80% and 90%, which is similar to the values observed for the negative control yogurt in this study.

Ash content reflects the mineral content in the food. Its decrease may be due to the activity of lactic ferments depleting mineral elements in yogurt (Oguneyemi et al., 2021). Limited studies on ash content in yogurt enriched with betalains make comparisons difficult. Our results differ from those of Martha et al. (2021), who reported syneresis ranging from 18.01 ± 0.7% to 32 ± 0.97%.

Syneresis in yogurt is influenced by the microstructure of the protein network ; insufficient water binding results in whey expulsion during storage (Oguneyemi et al., 2021). Total solids in yogurt help prevent or reduce syneresis, and high fat and protein content are associated with lower whey separation (Martha et al., 2021).

The enrichment of yogurt with plant proteins might improve protein content (Oguneyemi et al., 2021). Decreases in protein content could be due to the degradation of milk proteins, especially whey proteins (Oliveria et al., 2009).

The reduction in betalain content may be due to its hydrolysis to betalamic acid (Laura et al., 2016). Fermentation of red beets lowers betalain content, and lactic fermentation can cause isomerization and dehydrogenation of betanin. Additionally, glycosylation in betacyanins usually reduces antioxidant activity (Izabela Sadowska & Grzegorz, 2021).

The decrease in total polyphenol content could be attributed to the action of lactic acid bacteria during cold storage, which degrades polymerized phenolic compounds (Muniandy et al., 2017). High levels of phenolic compounds in the control yogurt supernatant reflect milk protein degradation. Tyrosine, with its phenolic side chains, can be used to increase polyphenol content in yogurt (Amirdivani, 2015). This increase can be explained by the gradual release of phenolic compounds associated with milk proteins (Muniandy et al., 2017).

Betalains' antioxidant activity increases when a hydroxyl group is present at the C-5 position of the aglycone. Betanin has been shown to scavenge various free radicals dose-dependently (Izabela Sadowska & Grzegorz, 2021). Our results are consistent with Atmani et al. (2009), who reported hydrogen peroxide reduction percentages between 22.5% and 75.11%. Taşkın and Bağdatlıoğlu (2020) found variable percentages between 1.07% and 4%, while EL-Haci (2016) reported a range of 4.25% to 39.50%.

Some lactic acid bacteria and bifidobacteria produce NADH oxidase, which forms H2O2 by oxidizing NADH (Taşkın & Bağdatlıoğlu, 2020). Fermented dairy products, including yogurt, may not effectively reduce H2O2 due to enzymatic activity (Taşkın & Bağdatlıoğlu, 2020). Decreased antioxidant activity during refrigerated storage is likely due to the degradation of phenolic compounds with antioxidant properties and/or increased interactions between milk proteins and polyphenols. Consuming yogurt within 7 days of manufacture is recommended to maximize

live bacterial content and antioxidant activity beneficial for cardiovascular health (Amirdivani, 2015).

The observed decrease in antioxidant activity could be due to increased degradation of phenolic compounds or enhanced interactions between milk proteins and polyphenols (Shori, 2020). An increase might be attributed to the formation of bioactive peptides with enhanced antioxidant activity following milk protein degradation (Virtanen, 2007).

Thus, a concentration of 12 g/l of betalain (from concentrated beetroot juice) is optimal for maintaining the color of betalain-enriched yogurt during refrigerated storage.

Odor is a subjective parameter influenced by individual perception and sensitivity. The fermentation of sugars, which starts near the end of the product's shelf life, could contribute to changes in odor.

Taste evaluations showed that the sweetness was medium, indicating that the amount of added sugar was appropriate. Beet sugar content did not negatively affect the taste, suggesting that the yogurt produced was well-received, as most consumers prefer dairy products that are not excessively sweet.

The results indicate that concentrated beetroot juice (betalain) acts as a preservative, helping to maintain pH levels, prevent the development of an acidic taste, and inhibit sugar fermentation compared to the control.

4. Conclusions

The aim of our study was to evaluate the impact of enriching yogurt with beetroot betalain on various physicochemical parameters, nutrient and polyphenol composition, sensory profile, and antioxidant activity.

Regarding physicochemical criteria, the addition of betalain extract resulted in a decrease in pH during refrigerated storage, while the acidity of the betalain-enriched yogurt increased compared to the control yogurt. However, over the same storage period, there were no significant differences in syneresis, Brix, moisture content, and ash content between the enriched yogurts and the control yogurt.

Our results also indicated that the protein content was not significantly affected by the addition of betalain, as the protein levels in all yogurts were comparable. Conversely, there was an increase in both sugar and phenolic compound levels in the fortified yogurt compared to the control yogurt during the period from day 1 to day 21.

Additionally, the fortification of yogurt with betalain positively impacted the percentage of DPPH free radical inhibition and hydrogen peroxide reduction.

Sensory analysis revealed that yogurts fortified with 6 g/l and 12 g/l of betalain were well-received by tasters, who appreciated their pink color and mild taste.

Overall, our findings support the beneficial effects of adding beetroot betalain to yogurt. Betalain is a valuable source of polyphenols and betalain, which contribute significantly to human health. Consuming betalain-enriched yogurt can thus enhance health and offer protection against the harmful effects of free radicals.

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