IMPACT OF THE ADDITION OF DIFFERENT FOREST BERRY FRUITS ON FUNCTIONAL, PHYSICOCHEMICAL AND SENSORY PROPERTIES OF YOGURT SHELF LIFE

Corina PREDESCU^{1*}, Georgeta ȘTEFAN¹, Camelia PAPUC²

¹University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Spl. Independentei, District 5, Bucharest, Romania ²Academy of Romanian Scientists (AOSR), 54 Splaiul Independentei, 050094, Bucharest, Romania

*Corresponding author, email: durduncorina@yahoo.com

Abstract

The present study aimed to report the effect of black mulberry -BM (Morus nigra), black chokeberry -BC (Aronia melanocarpa), and blackberry -BB (Rubus fruticosus) fruits on some functional and physicochemical parameters and sensorial properties of yoghurt during refrigeration, compared to untreated yoghurt.

Yoghurt was prepared from cow milk, provided from a farm near Bucharest. The fruits used for the experiment were bought from a local market. Fruit puree was added at 0% and 5% (w/v). Yoghurt samples were collected after 1, 5, 10, and 15 days of refrigeration for analysis of several parameters [total phenol content (TPC), total anthocyanins content, antioxidant activity (AA), TBARS value, protein carbonyl, pH, titratable acidity, water holding activity, syneresis, and sensory evaluation]. The highest TPC was found in samples treated with BC, and the highest AA was in the samples treated with BC also. For all samples treated with berries puree, protein carbonyl, and TBARS values were lower than untreated samples. The sensory evaluation results revealed no statistical differences (p>0.05) between the acceptability of the three types of yoghurts. The addition of black mulberry (Morus nigra), black chokeberry (Aronia melanocarpa), and blackberry (Rubus fruitosus) puree fruits in yoghurt enhanced the lipid oxidative stability, decreased syneresis, and modified its sensorial properties in the acceptability limits.

Key words: yoghurt, forest berry fruits, protein carbonyl, antioxidant activity, anthocyanins.

INTRODUCTION

Yoghurt is nutritious and fortified with fruits and can provide an important concentration of biologically active compounds, such as phenolic compounds with antioxidant activity. Consuming fruits and yoghurt have been identified in all diets as indicators of healthy patterns. Fruits are relatively low in energy and are an excellent source of antioxidants, fibres, and polyphenols, promoting health (Fernandez & Marette, 2017; Predescu et al., 2016). On the other hand, yoghurt is a nutritious food because is a good source of dairy protein, calcium, magnesium, vitamin B12, essential fatty acids, and other essential molecules. Furthermore, it contains beneficial bacterial cultures, making it a potential source of probiotics. Yoghurt's sources of fermentation are Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus makes a unique fermented food matrix that provides added health benefits by enhancing nutrient absorption and digestion (Zepeda-Hernández et al., 2021). Lactic acid bacteria commonly used as starter cultures are known to produce antimicrobial substances such as bacteriocins and have great potential as food bio-preservatives (Bamgbose et al., 2021; Mokoena, 2017). Combining voghurt and fruit provide probiotics, dietary could fibre (prebiotics), high-quality protein, important fatty acids, and a mixture of vitamins, phenolics and minerals that have the potential to exert synergistic effects on health. More recently, probiotics and prebiotics have been suggested to modulate the microbiota (Zepeda-Hernández et al., 2021; Tuohy et al., 2014). After more than 100 years, yoghurt is still the preferred dairy product with relatively high consumption, probably numerous health benefits. The acceptability by consumers of yoghurt as a functional dairy product remains very high and people of all ages have expressed their wishes to add it to their diet (Mokoena, 2017). study investigated This the supplementation of fermented dairy products

like yoghurt, with phenolic-rich products like some forest berry puree to optimize the benefits of probiotic products with prebiotic compound intake. Yoghurts are known to have unique characteristics that make them accepted by consumers. Therefore, it is important to clarify if the addition of berry puree may modify them positively or negatively. The addition of forest berry puree is expected to modify some specific aspects of yoghurt, for instance, total phenol content (TPC), total anthocyanin content, antioxidant activity (AA), titratable acidity, pH, water holding activity, syneresis, TBARS value, protein carbonyl and sensorial properties.

The present study aimed to produce yoghurt with a 5% addition of forest berry puree and to evaluate the functional, physicochemical characteristics, and sensorial characteristics, during storage for up to 15 days.

MATERIALS AND METHODS

Source of raw materials used for voghurt preparation. Commercially packaged berries (no preservatives and no added sugars) from black mulberry - BM (Morus nigra), black chokeberry - BC (Aronia melanocarpa), and blackberry – BB (Rubus fruticosus): commercial pasteurized and homogenized cow's milk (Moara Domneasca Farm, Ilfov county, Romania); commercially pack starter culture for yoghurt production. This yoghurt starter consists of strains of Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus.

Puree berry fruits preparation. Berries were poured into a blender, and additional water was added (w:v, 1:1) and blended until smooth. With the help of a spoon, the puree was gently pushed through a sieve. The puree was simmered for about 15 minutes.

Yoghurt preparation. The pasteurized milk was heated to 42°C and then inoculated with a yoghurt starter culture. It was mixed lightly and the obtained mixture was transferred to the beakers with lids. The yoghurt was left to ferment in a yoghurt fermenter until the pH reached the value of 4.6, without stirring, to let the formation of the curd, and the pureed fruit was added to a concentration of 5%, by manually mixing for five minutes. The yoghurt cups were then stored in the refrigerator until

analysis was performed. Also, a control yoghurt (CY) was produced and was mixed also for five minutes without the addition of the fruit puree.

Preparation of hydroethanolic yoghurt extracts. Yoghurt samples (0.4 ml) were subjected to extraction with 9.6 ml ethanol (60%) for 2 h in a shaking water bath, at 50°C. Samples were then centrifuged at 5000 rpm for 15 min at 4°C and the supernatant was collected. The extracts were aliquoted and stored at -30°C until further analysis.

Total phenol content. Phenol content was measured using a modified Folin-Ciocalteau method (Deighton et al., 2000). 1000 ul of yoghurt extracts were mixed with 3 ml of water, and 250 µl of Folin-Ciocalteu reagent was added and incubated at room temperature for 1 min. Following the addition of 750 µl of 7.5% (w/v) sodium carbonate to the mixture, total polyphenols were determined after 1 h of incubation in the dark at room temperature. The absorbance of the reaction mixture was determined at 765 nm against a blank sample using a UV-VIS Jasco 670 spectrophotometer. Quantification was done concerning the standard curve of Gallic acid and results are expressed as mg/kg Gallic acid equivalents (GAE).

Total anthocyanin content. The total anthocyanin content was estimated by the pH differential absorbance method (Lee et al., 2005). Yoghurt extracts were diluted with pH 1.0 buffer (potassium chloride, 0.025 M) until the absorbance at 520 nm was around 0.5 when measured with the spectrophotometer, and the same dilution factor was used to prepare all samples for pH 4.5 buffer (sodium acetate, 0.4 M). If diluted samples were turbid, were therefore centrifuged before measuring absorbance at 520 and 700 nm (20-50 min after preparation). The diluted samples were read versus a blank filled with correspondent buffer. To calculate the total anthocyanin concentration, (expressed as mg/l cyanidin-3-glucoside equivalents), the following equation was used:

Total anthcyanins
$$\left(\frac{\text{mg}}{l}\right) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l}$$

Where, A = (A520 nm - A700 nm) pH 1.0 - (A520 nm - A700 nm) pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside

(cyd-3-glu); DF = dilution factor established in D; 1 = pathlength in cm; $\varepsilon = 26,900$ molar extinction coefficient for cyd-3-glu in 1 × mol⁻¹ × cm⁻¹ and 10³ = factor for conversion from g to mg.

Antioxidant activity. Antioxidant activity was determined using the free radical DPPH• (2,2 diphenyl-1-picrylhydrazyl) method (Chen et some modifications. al.. 2003). with Specifically, 1.0 ml diluted yoghurt ethanolic extract and 3.0 ml of DPPH• solution were added, vortexed, and allowed to stand at room temperature in darkness for 30 min. The absorbance of samples and blank (60% ethanol) was spectrophotometrically measured at 517 nm UV/VIS spectrophotometer Jasco 670 and quantified using Trolox as a standard.

Determination of thiobarbituric acid reactive substances (TBARS). One gram of each yoghurt was transferred in a 25 ml test tube and 5 ml of 50% glacial acetic acid in water were added. BHT (0.01%) was used to prevent further oxidation of the samples (Zeb & Ullah, 2016). The samples were shaken for 1 h and filtered. The filtrate was centrifuged. The extract of each sample (1 ml) was mixed with 1 ml 4.0 mM TBA reagent. The standard stock solution of MDA (1 mM) was prepared in glacial acetic acid. The calibration curve was constructed in the concentration range of 0.1 to 1.0 mM. The TBARS was calculated using the formula µmol/g of the sample:

TBARS (
$$\mu$$
mol/g) = $\frac{Ac \times V}{W}$

Where, Ac is the amount determined from the calibration curve and W is the weight of the sample taken while V is the volume in ml or dilution factor of the total extract prepared.

Determination of protein carbonyl. An aliquot of aqueous yoghourt solution (corresponding to ca. 2 mg protein) was incubated with 10 mM DNPH in 2 N HCl (0.5 ml final volume) for 30 min at room temperature (Citta et al., 2017). Yoghourt proteins were then precipitated with 10% TCA (final concentration) and recovered by centrifugation for 5 min at 1677 × g. Protein pellets were washed three times, with 1 ml of ethanol/ethyl acetate (50:50; v/v) to remove unreacted free DNPH reagent, and redissolved in 0.6 ml of 6 M urea. The carbonyl content was calculated by UV spectrophotometry ($\lambda =$ 370 nm; $\varepsilon = 22,000 \text{ M}^{-1} \text{ cm}^{-1}$, Jasco 760 spectrophotometer (Levine et al., 1990). Protein concentrations were determined using the Bradford Protein Assay and results were expressed as nmol DNPH mg⁻¹ protein.

Titratable acidity and pH. Titratable acidity is the total amount of all the available hydrogen ions in a solution and was expressed as g lactic acid /100 g of yoghurt (Dimitrellou et al., 2020). 10 ml of yoghurt were transferred into a beaker and using the same pipette, 90 ml of distilled water were added. Lactic acid was titrated with 0.1 N NaOH using a digital titrator (TitroLine easy). The pH was measured with an electronic pH meter (Mettler Toledo pH Meters), and before each determination, the pH meter was first calibrated by 2 buffers 4 and 7.

Water Holding Capacity. A sample of about 10 g of yoghurt (Y) was centrifuged for 20 min at 5000 rpm and at 20°C (Sidira et al., 2017). The whey expelled (WE) was removed and weighed. The water holding capacity (WHC) was calculated using the next equation:

$$WHC(\%) = \frac{Y - WE}{Y} \times 100$$

Where, WE are whey g expelled and Y is the initial yoghurt sample in g.

Syneresis. Syneresis was determined using 50 g of unstirred yoghurt spread evenly on a filter paper in a funnel at 4°C. After 5 h of drainage, the volume of collected whey was measured, multiplied by 2, and expressed as syneresis (%) (Sidira et al., 2017).

Sensory evaluation of yoghurt samples. The sensory analysis of yoghurt samples was conducted by 10 panellists selected from graduated students in the Control and expertise of food products, using a 7-point hedonic scale: 1 – 'Strongly disliked'; 2 – 'Moderately disliked'; 3 - 'Slightly disliked'; 4 -'Indifferent'; 5 – 'Slightly liked'; 6 – 'Moderately liked', and 7 - 'Strongly liked'. Yoghurt samples were coded with numbers and randomly tested for appearance, colour, consistency, taste and smell. Mineral water and cracker biscuits were available as neutralizers between samples to avoid carryover effects. The test was performed in 3 replications, on the seventh day of yoghurt refrigeration time (Varedesara et al., 2021).

Statistical analysis. The statistical analysis was done using the Statistical Analysis System

Program, version 20.0 (SPSS Inc., Chicago, IL, USA) at a significance level of p < 0.05. The measurements were performed in triplicate for each sample and results were expressed as mean value \pm standard deviation.

RESULTS AND DISCUSSIONS

Total phenol content. Black mulberry (Morus chokeberry nigra). black (Aronia blackberry melanocarpa), and (Rubus fruticosus) fruits are good sources of phenolic compounds. especially anthocvanins. Therefore, the total anthocyanin and total phenol content of voghurt samples containing fruit purees were analysed during their shelflife period. As presented in Table 1, total phenol contents in yoghurt containing BM, BC and BB were both higher than the control yoghurt (CY). This effect was significant (p < 0.05) for the first seven days of storage. The difference in total phenol content of fortified yoghurt with fruits purees (BC > BM > BB).

Table 1. Total phenol content, total anthocyanin and antioxidant activity of tested yoghurts

| Yogurt | Control | Black | Black | Blackberry |
|----------------|---|------------|------------|------------|
| sample | yoghurt | mulberry | chokeberry | ыаскретту |
| Fruit | 0% | 5% | 5% | 5% |
| puree | 0/0 | 5/0 | 5/0 | 370 |
| Time | Total phenol content (mg/kg) | | | |
| (days) | | • | | |
| 1 | 15.24±1.24 | 39.14±4.15 | 41.56±5.40 | 26.17±2.31 |
| 5 | 15.21±3.01 | 40.25±3.07 | 43.20±3.14 | 29.02±1.92 |
| 10 | 17.21±1.20 | 42.21±1.32 | 47.81±1.25 | 31.74±2.11 |
| 15 | 18.90±1.98 | 39.10±3.21 | 43.31±1.67 | 29.31±1.67 |
| Time (days) | Total anthocyanin (mg C3GE/I) | | | |
| 1 | 0.14±0.09 | 3.58±0.65 | 4.54±0.42 | 3.24±0.56 |
| 5 | 0.11±0.08 | 3.25±0.22 | 4.31±0.52 | 3.28±0.36 |
| 10 | 0.14±0.06 | 3.15±0.20 | 4.88±0.54 | 3.15±0.54 |
| 15 | 0.12±1.98 | 3.10±0.21 | 4.25±0.65 | 3.04±0.31 |
| Time (days) | Antioxidant activity (μ mol TE 100 g ⁻¹) | | | |
| 1 | 19.14±1.54 | 37.25±4.22 | 42.31±3.52 | 27.28±2.36 |
| 5 | 14.51±3.21 | 25.21±1.65 | 37.54±2.12 | 24.24±1.56 |
| 10 | 9.24±1.26 | 18.45±1.20 | 34.88±1.54 | 18.85±1.54 |
| 15 | 5.90±1.98 | 17.10±1.21 | 28.25±2.65 | 17.04±1.11 |

Results are presented as means $\pm SD$

An increase in total phenols was observed for all yoghurts during the first 10 days of storage. A similar evolution in the phenolic content during refrigerated storage has been presented by (Raikos et al., 2019) and was attributed to the formation of compounds that react with the Folin-Ciocalteu reagent. For instance. proteolysis of milk proteins may release amino acids with phenolic side chains, such as phenylalanine and tyrosine, which could contribute to the increase in total phenol content. In addition, the phenolic compounds added to voghurt may suffer glycoside hydrolysis or C-ring cleavage and release of simple phenolics such as phenolic acids (Raikos et al., 2019). After the first ten days of storage, the total phenol content in the voghurt sample had a small reduction, which was similar to other research on yoghurts containing different fruit extracts. Polyphenols interact with milk proteins forming insoluble complexes, and this is reflected by reducing the total free polyphenol content in the yoghurt sample (Raikos et al., 2019). However, significant levels of polyphenols were detected even after fifteen days of storage especially in followed by BM and BB. BC, A11 concentrations of total phenolic content were higher than CY even after 15 days of refrigeration.

Total anthocyanin content. Anthocyanins are important to the food industry because of their attractive and stable pink-red colour in acid foods like yoghourts and their antioxidant power. The total anthocyanin content of different types of yoghurts was analysed. As shown in Table 1, the addition of BM, BB and BC purees increased the total anthocyanin content in yoghurt compared to the control yoghurt.

Antioxidant activity. Generally, the DPPH free radical activity test is used to measure the antioxidant ability of a sample. DPPH radical solution has maximum absorption at 517 nm, but in the presence of an antioxidant, it reduces the absorption measurement by DPPH free radical scavenging. Therefore, the antioxidant property of a compound is expressed as DPPH• ability to scavenge free radicals (Varedesara et al., 2021). The results of the present study showed (Table 1) that all yoghurts had a higher potential for DPPH free radical scavenging. The bioactive fruit purees exert their antioxidant activity through various mechanisms such as inhibition of lipid

peroxidation, inhibition of protein oxidation, and free radicals scavenging.

Determination of thiobarbituric acid reactive substances (TBARS). To quantify the spontaneous lipide peroxidation of yoghurt occurring during the shelf life, a test based on TBARS production was used. First of all, adding berries puree to yoghurts show lower levels of TBARS, especially in the presence of BC (Table 2). In addition, it was observed a slight increase in lipoperoxidation for all treated yoghurts, and especially for control voghurt. However, the levels of lipid peroxidation were relatively low reaching a maximum of about 21.21±2.11 umol/g (Table 2). In the presence of berries, the TBARS level was even lower in the presence of CB, BM and BB. In addition, during the shelf life, a constant increase in TBARS formation until the 15th day was observed. This behaviour was found both in the presence and in the absence of berries. However, in the absence of berries puree, a lower TBARS production was observed for control yoghurt alone. Similarly, the presence of berries determines a decrease in lipid peroxidation of yoghurt compared to yoghurt without berries.

Determination of protein carbonyl content. During the fermentation process of yoghurt, proteins are partially hydrolysed in compounds susceptible to oxidation (Citta et al., 2017).

Table 2. TBARS value and protein carbonyl content of tested yoghurts

| Yogurt sample | Control yoghurt | Black mulberry | Black chokeberry | Blackberry |
|------------------|--------------------------|-------------------|---------------------|-------------|
| Fruit puree | 0% | 5% | 5% | 5% |
| Time (days) | TBARS value (μmol/g) | | | |
| 1 | 14.11±0.87 | 14.78±1.62 | 14.03±0.32 | 15.02±1.33 |
| 5 | 16.21±1.54 | 16.05±1.87 | 15.02±1.21 | 15.31±1.64 |
| 10 | 19.45±2.11 | 17.87±1.51 | 17.91±1.61 | 17.41±1.95 |
| 15 | 21.21±2.11 | 20.25±2.14 | 19.41±1.21 | 20.51±2.25 |
| Time | Protein carbonyl content | | | |
| (days) | (nmol DNPH/mg protein) | | | |
| 1 | 3.38 ± 0.32 | 3.28 ± 0.37 | 3.48 ± 0.51 | 3.21 ± 0.32 |
| 5 | 5.37 ± 0.97 | 4.3 ± 0.39 | 3.47 ± 0.48 | 5.44 ± 0.42 |
| 10 | 6.39 ± 1.30 | 5.38 ± 0.40 | 5.49 ± 0.35 | 6.45 ± 0.31 |
| 15 | 9.38 ± 1.36 | 7.34 ± 0.38 | 6.46 ± 0.51 | 8.43 ± 0.39 |

Results are presented as means \pm *SD*

The yoghurt samples (control, and puree berries treatments) were also tested for protein

carbonyl formation during the shelf life, on the same days on which lipid peroxidation was measured. The resulting amount of protein carbonyl (Table 2) was very similar in the presence of puree berries, indicating that berries were able to prevent protein oxidation.

A higher protein carbonyl value was shown for control yoghurt. This result is quite the same as that obtained for the TBARS value (Table 2) indicating that the antioxidants of yoghurt are capable of protecting against lipid and protein oxidation. During the shelf life of 15 days, the proteolytic activity of bacteria in yoghurt proceeds (Germani et al., 2014) and determines fragmentation and oxidation, particularly in control yoghurt. Protein oxidation is higher for BB compared with BC and BM at the end of the treatment (Table 2).

Titratable acidity and pH. pH is the negative logarithmic measure of the ionic strength of hydrogen in aqueous solutions and reflects the degree of acidity or alkalinity of aqueous solutions.

Table 3. pH and titratable acidity of tested yoghurts

| Yogurt sample | Control yoghurt | Black mulberry | Black chokeberry | Blackberry |
|------------------|--------------------|-------------------|---------------------|-------------|
| Fruit puree | 0% | 5% | 5% | 5% |
| Time (days) | рН | | | |
| 1 | 4.65 ±0.38 | 4.58 ± 0.47 | 4.66 ± 0.44 | 4.59 ± 0.53 |
| 5 | 4.53 ±0.16 | 4.41 ± 0.33 | 4.38 ± 0.33 | 4.45 ±0.34 |
| 10 | 4.37 ±0.29 | 4.41 ± 0.41 | 4.39 ± 0.52 | 4.37 ±0.42 |
| 15 | 4.19±0.34 | 4.47 ±0.54 | 4.32 ± 0.45 | 4.25 ± 0.63 |
| Time (days) | Total acidity (%) | | | |
| 1 | 0.91±0.11 | 1.04±0.18 | 0.90±0.08 | 1.10±0.01 |
| 5 | 1.05±0.11 | 1.11±0.11 | 1.01±0.08 | 1.14±0.05 |
| 10 | 1.02±0.17 | 1.14±0.16 | 1.11±00.11 | 1.14±0.04 |
| 15 | 1.01±0.11 | 1.12±0.14 | 1.16±0.09 | 1.15±0.05 |

Results are presented as means $\pm SD$

In particular, for yoghurt production standards, pH and titratable acidity are the most important tests for increasing yoghurt shelf life and better acceptance by the consumer. Acidity is the consequence of lactic acid production, by the fermentation of yoghurt carbohydrates. pH and titratable acidity are considered the most important tests and also acceptable indicators for yoghurt storage (Varedesara et al., 2021). The results of changes in pH and acidity (Table 3) during the maintenance process showed that pH values decreased over time in all treatments and acidity increased. During the production

and storage of yoghurt, the starter bacteria cause the production of lactic acid and consequently, increase the titratable acidity and decrease the pH. The decreasing pH and increasing acidity during storage have been reported in most related studies (Varedesara et al., 2021; Dimitrellou et all., 2020). The results related to the pH and acidity of yoghurt values showed that the addition of fruit purees didn't have a remarkable effect on the pH and acidity, in all storage periods. On the last day of treatment, the highest pH and lowest acidity values were observed in the treatment containing BM.

Water Holding Capacity. Measuring the value of water holding capacity is one of the most important physical tests to measure the quality of yoghurt. WHC represent the gel capacity to prevent the separation of the aqueous phase from the continuous phase of the casein network. As it is shown in table 4, WHC decrease during the 15 days of storage for all tested yoghurts.

Table 4. Syneresis and water holding capacity of tested yoghurts

| Yogurt sample | Control yoghurt | Black mulberry | Black chokeberry | Blackberry |
|------------------|--------------------|-------------------|---------------------|------------|
| Fruit puree | 0% | 5% | 5% | 5% |
| Time (days) | Syneresis (%) | | | |
| 1 | 31.10±3.45 | 34.41±3.12 | 33.31±3.38 | 35.21±3.24 |
| 5 | 32.48±3.14 | 36.51±3.11 | 35.99±3.98 | 36.82±3.03 |
| 10 | 35.14±3.13 | 37.21±3.21 | 36.36±3.89 | 38.57±3.02 |
| 15 | 37.94±3.68 | 37.94±3.68 | 37.81±3.51 | 39.11±3.31 |
| Time (days) | WHC (%) | | | |
| 1 | 71.10±5.45 | 69.41±5.12 | 70.81±4.51 | 63.57±5.02 |
| 5 | 67.48±5.14 | 65.51±5.11 | 69.31±4.38 | 61.21±5.24 |
| 10 | 66.14±5.13 | 62.21±5.21 | 65.99±5.98 | 59.82±5.03 |
| 15 | 60.94±4.68 | 61.94±4.68 | 62.36±6.89 | 58.11±5.31 |

Results are presented as means $\pm SD$

Syneresis. Syneresis represent the separation of whey and is one of the most common defects in fermented milk products like yoghurts (Amatayakul et al., 2006). This defect may have a negative impact on its acceptability by the consumers due to the undesirable appearance, and limit the shelf life of the product. Overall, the consumers correlate syneresis with the potential yoghurt microbial infection (Dimitrellou et al., 2019; 2020). Moreover, previous studies have correlated the addition of fruit with reduced viscosity and increased syneresis of yoghurts. In order to reduce syneresis, the addition of milk solids or stabilizers can be added to yoghourts. In the present study, the syneresis increased during the yoghurt refrigeration for 15 days, and BB treatment showed the highest syneresis at the end of the treatment (Table 4).

Sensory evaluation of yoghurt samples. The most important factors in accepting or rejecting products and taking satisfaction from their consumption are sensory characteristics (Shori et al., 2018). In the present study, sensory analysis of yoghurt samples was determined based on colour, aroma, softness, taste, and overall liking.



Figure 1. Sensory evaluation of yoghurt samples (CY = control yogurt; BM = yogurt with black mulberry; BC = yogurt with black chokeberry; BB = yogurt with blackberry)

According to the statistical analysis results, the sensory attributes were increased for yoghurt samples added with fruit puree (Figure 1). Overall, the three puree treatments were appreciated by evaluators. The colour and the softness of the yoghurts sample treated with BC and BB were appreciated more than the BM treatment. The aroma results showed that all three added puree fruits were preferred to CY and BB was appreciated as the most fragrant yoghurt of all samples. The taste evaluation showed probably a personal preference for the fruits added to the yoghurt, BB>BM>BC>CY. For all evaluated attributes, a higher sensory score was observed for BB yoghurt.

CONCLUSIONS

Significant levels of phenols were detected even after fifteen days of storage especially in

BC, followed by BM and BB. The bioactive fruit purees exert their antioxidant activity through various mechanisms such as inhibition of lipid peroxidation, inhibition of protein oxidation, free radicals scavenging. The addition of BM, BB and BC purees increased the total anthocyanin content in yoghurt. The syneresis increased during the yoghurt refrigeration for 15 days, and BB treatment showed the highest syneresis at the end of the treatment. WHC decrease during the 15 days of storage for all tested yoghurts. The decreasing pH and increasing titratable acidity during storage were observed for all voghurts. In the presence of CB, BM and BB berries, the TBARS level was lower than control yoghurt (CY). Protein oxidation is higher for BB compared with BC and BM at the end of the treatment. A higher sensory score was observed for BB yoghurts, for all evaluated attributes.

REFERENCES

- Amatayakul, T., Sherkat, F., & Shah, N.P. (2006). Syneresis in set yogurt as affected by EPS starter cultures and levels of solids. Int. J. Dairy Technol. 59, 216–221.
- Bamgbose, T., Iliyasu, A. H., & Anvikar A. R. (2021). Bacteriocins of Lactic Acid Bacteria and Their Industrial Application. Curr. Top. Lact. Acid Bact. Probiotics;7(1):1-13.
- Chen, J., Lindmark-Mansson, H., Gorton, L., & Akesson, B. (2003). Antioxidant capacity of bovine milk as assayed by spectrophotometric and amperometric methods. Int Dairy J.;13:927–35.
- Citta, A., Folda, A., & Scalcon, V. (2017). Oxidative changes in lipids, proteins, and antioxidants in yogurt during the shelf life. Food Sci Nutr. 5:1079–1087. https://doi.org/10.1002/fsn3.49
- Deighton, N., Brennan, R., Finn, C., & Davies, H. V. (2000). Antioxidant properties of domesticated and wild Rubus species. Journal of the science of food and agriculture, 80(9), 1307-1313.
- Dimitrellou, D., Solomakou, N., Kokkinomagoulos, E., &Kandylis, P. (2020). Yogurts Supplemented with Juices from Grapes and Berries. Foods. 9(9):1158. https://doi.org/10.3390/foods9091158
- Dimitrellou, D., Kandylis, P., & Kourkoutas, Y. (2019). Assessment of freeze-dried immobilized Lactobacillus casei as probiotic adjunct culture in yogurts. Foods, 8, 374.
- Fernandez, M. A., & Marette, A. (2017). Potential Health Benefits of Combining Yogurt and Fruits Based on Their Probiotic and Prebiotic Properties. Advances in nutrition (Bethesda, Md.), 8(1), 155S–164S. <u>https://doi.org/10.3945/an.115.011114</u>
- Germani, A., Luneia, R., Nigro, F., Vitiello, V., Donini, L. M., & del Balzo, V. (2014). The yogurt amino acid

profile's variation during the shelf-life. Annali di Igiene, 26, 205–212.

- Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. Journal of AOAC International, 88(5), 1269–1278.
- Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., Ahn, B. W., Shaltiel, S., & Stadtman, E. R. (1990). Determination of carbonyl content in oxidatively modified proteins. Methods in enzymology, 186, 464–478. https://doi.org/10.1016/0076-6879(90)86141-h
- Mokoena M. P. (2017). Lactic Acid Bacteria and Their Bacteriocins: Classification, Biosynthesis and Applications against Uropathogens: A Mini-Review. Molecules (Basel, Switzerland), 22(8), 1255.
- Predescu, N.C., Nicorescu V., Petcu C., Stefan G., & Papuc C. (2016). Antioxidant activity of polyphenols extracted from Hawthorn and Dog-rose fruits on linoleic acid emulsion model system compared to BHA synthetic antioxidant. *Sci Works Series C Vet Med.* 62, 32-35.
- Raikos, V, Ni H, Hayes, H, & Ranawana V. (2019). Antioxidant Properties of a Yogurt Beverage Enriched with Salal (Gaultheria shallon) Berries and Blackcurrant (Ribes nigrum) Pomace during Cold Storage. Beverages.; 5(1):2.
- Shori, A. B., Rashid, F., & Baba, A.S. (2018). Effect of the addition of phytomix-3+ mangosteen on antioxidant activity, the viability of lactic acid bacteria, type 2 diabetes key-enzymes, and sensory evaluation of yogurt. LWT, 94, 33–39
- Sidira, M., Santarmaki, V., Kiourtzidis, M., Argyri, A.A., Papadopoulou, O.S., Chorianopoulos, N., Tassou, C., Kaloutsas, S., Galanis, A., & Kourkoutas, Y. (2017). Evaluation of immobilized Lactobacillus plantarum 2035 on whey protein as adjunct probiotic culture in yoghurt production. LWT-Food Sci. Technol., 75, 137–146
- Tuohy, K., Fava, F., & Viola, R. (2014). 'The way to a man's heart is through his gut microbiota' – dietary pro-and prebiotics for the management of cardiovascular risk. *Proceedings of the Nutrition Society*, 73(2), 172-185. doi:10.1017/S0029665113003911.
- Varedesara, M.S., Ariaii, P., & Hesari J. (2021). The effect of grape seed protein hydrolysate on the properties of stirred yogurt and viability of Lactobacillus casei in it. Food Sci Nutr.; 9:2180– 2190. https://doi.org/10.1002/fsn3.2188.
- Zeb, A., & Ullah, F. (2016). A Simple Spectrophotometric Method for the Determination of Thiobarbituric Acid Reactive Substances in Fried Fast Foods. Journal of analytical methods in chemistry, 2016, 9412767. https://doi.org/10.1155/2016/9412767
- Zepeda-Hernández, A., García-Amezquita, L. E., Requena, T., & García-Cayuela, T. (2021). Probiotics, prebiotics, and synbiotics added to dairy products: Uses and applications to manage type 2 diabetes. Food research international (Ottawa, Ont.), 142, 110208. https://doi.org/10.1016/j.foodres.2021. 110208