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# PHYSIOCHEMICAL AND SENSORY PERSPECTIVES IN OBTAINING PROBIOTIC YOGHURT WITH WHITE MULBERRIES (*Morus alba*) AND BLACK MULBERRIES (*Morus nigra*)

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***Abstract:*** *Yoghurt is a food that supplies the body with essential nutrients and has benefits for human health. Plant products have attracted considerable interest in product development due to the secondary metabolites they contain. The aim of the present research is to develop a new dairy product with potential functional properties. The decision was taken to produce a yogurt from cow's milk, with the addition of white and black mulberry powder, which is rich in bioactive compounds. To assess the stability of the product during storage, the following parameters were determined: acidity, pH, water activity, dry matter, and syneresis. The product's acceptability was assessed by conducting a sensory evaluation. The impact of varying concentrations of fruit concentrates incorporated into the dairy product is discernible. The sensory characteristics of the yoghurt samples are found to be at their most intense on the first day of storage. The BMY2 sample obtained high scores for appearance, taste, and odor, while the WMY2 sample obtained high scores for appearance and odor. On days 8 and 16, a decline in the results obtained was observed for all samples evaluated. From a physicochemical perspective, the parameters analysed exhibited stability, thus indicating that mulberries are a promising ingredient for improving the product's quality. This study demonstrates the technological feasibility and physicochemical stability of the samples, which can be attributed to the effectiveness of mulberries in fermented dairy products. The results of the present study could act as a foundation for future research, with a view to the creation of other food products comprising additional bioactive ingredients.*

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***Key words:*** *white mulberry, black mulberry, probiotic yoghurt, bioactive compounds, physicochemical stability, sensory acceptability.*

## 1. Introduction

Recent years have seen a notable increase in the prevalence of chronic diseases such as obesity, diabetes and cardiovascular disease. This phenomenon has been attributed to a number of factors, including but not limited to: frequent antibiotic use, unhealthy eating habits, sedentary lifestyles and stress [14, 15, 20, 40]. Functional foods are defined as foods that, in addition to meeting the body's basic nutritional needs, offer additional benefits for human physiology and metabolic functions. These benefits include the prevention of disease and the maintenance of a healthy lifestyle [8]. This category encompasses supplements such as probiotics, prebiotics, and omega-3 fatty acids [19], along with essential nutrients such as vitamins, minerals, carbohydrates, proteins, and fibre [3]. Probiotics are defined as live microorganisms [30], and it is well-documented that they offer a number of benefits to the human body, including the regulation of intestinal flora, the prevention of diarrhea, the strengthening of the immune system, the regulation of cholesterol levels, the reduction of the risk of cancer, and the increase of mineral absorption [2, 17, 31].

Yoghurt is a food that supplies the body with vital nutrients and confers health benefits, including increased resistance to disease and the promotion of a healthier lifestyle [17]. The consumption of yoghurt has increased over time, and it is produced by the fermentation of milk with bacteria such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*, which forms lactic acid [4]. The bacteria present in starter cultures are unable to withstand the conditions of the gastrointestinal tract and lack the capacity to colonise the human intestine, thereby rendering it improbable that they can confer therapeutic benefits [9]. To forecast changes in the final products' physicochemical, rheological, and sensory profiles, more investigation is necessary on the microbiological interactions between starting cultures and probiotic bacteria during fermentation and storage [16].

It is evident that interest has been growing in the development of alternative products with improved functionalities, due to the secondary metabolites that are present in plants and vegetable products [1]. Mulberries (*Morus*) belong to the Moraceae family and are used as functional foods and for medicinal purposes [33]. White mulberry (*Morus alba* L.) is a plant that has been used traditionally to treat several health problems [26]. When the fruit is ripe, it changes colour from white to pink due to low concentrations of flavonoids and anthocyanins, and has a sweet taste [23]. *Morus alba* (L.) fruits are characterised by their high nutritional value, which has led to their extensive utilisation in various food products, including juices, jams, wines, and preserves. In addition to their application in food production, these fruits have found commercial value in the manufacturing of natural dyes, pharmaceuticals, and cosmetics [33]. The properties of the plant include purgative, diuretic, laxative and brain tonic effects, which are designed to enhance joint strength and visual acuity [1, 33]. As demonstrated in the extant literature, the pharmacological properties of white mulberries have been shown to encompass an array of effects, including antidiarrheal, antioxidant, anti-inflammatory, anxiolytic, immunomodulatory, antidiabetic, antithrombotic, and neuroprotective properties [12, 39]. The fruits of the black mulberry (*Morus nigra* L.) exhibit a transformation to a purple-black hue upon attaining full ripeness, a phenomenon attributable to their elevated flavonoid and anthocyanin concentrations [7]. These substances are consumed either in their solid state or in the form of syrups, jams, vinegars, or alcoholic beverages [18]. A plethora of studies have demonstrated the numerous beneficial properties of black mulberries, including antioxidant [25], anti-inflammatory, antimicrobial, antidiabetic [38], antimelanogenic, anticarcinogenic, antihyperlipidemic, and antiatherosclerotic properties [7]. It has been demonstrated that the substance in question exerts a protective effect on the central nervous system, gastrointestinal tract, and female reproductive system [13, 25, 38].

As demonstrated in the extant literature, a potential avenue for investigation lies in the analysis of the effectiveness of mulberry fruits in attributing various functional properties when incorporated into a range of food products [21]. This is of particular relevance given the significant contribution of their cultivation to the enhancement of labour and income in rural areas [5, 6, 28, 29]. The aim of the present research is to develop a new dairy product with potential functional properties. The decision was taken to produce a yogurt from cow's milk, with the addition of white and black mulberry powder, which is rich in bioactive compounds. To evaluate the stability of the finished product during storage, a number of analytical procedures were carried out, including the determination of acidity, pH, water activity, dry matter, and syneresis. The product's acceptability was assessed by conducting a sensory evaluation. This study constitutes an inaugural research study in which the physicochemical and sensory compounds are determined, and the functional potential of the product is subsequently investigated.

## 2. Materials and Methods

### 2.1. Experimental Design

The experimental design for obtaining yogurt samples with white and black mulberry powder is shown in Figure 1.

### 2.2. Obtaining Yoghurt Samples from Cow's Milk with Added White Mulberries and Black Mulberries

The samples were obtained and the tests were performed in the laboratory of the Research Centre for Biotechnology and Food Engineering (CCBIA) at Lucian Blaga University in Sibiu. The cow's milk yoghurt underwent processing in laboratory conditions in accordance with a technological recipe developed by the research team. The following materials were used: cow's milk from an authorized farm in Sibiu County (fat content – 3.5% and a protein content – 3.42%), Bio Joghurtferment LACTO PRO+ Inulin lactic acid bacteria culture purchased from distributor SC Globus Transport SRL (country of origin: Germany) containing a mixture of probiotic bacteria (*Lactobacillus acidophilus* La-5, *Bifidobacterium* BB-12, *Streptococcus thermophilus*, *Lactobacillus* *delbrueckii subsp. Bulgaricus*), organic dehydrated white mulberries and organic dehydrated black mulberries, produced by Obio. The dehydrated white and black mulberries were subsequently ground for 15 seconds with a grinder (Heinner, HCG-150P).

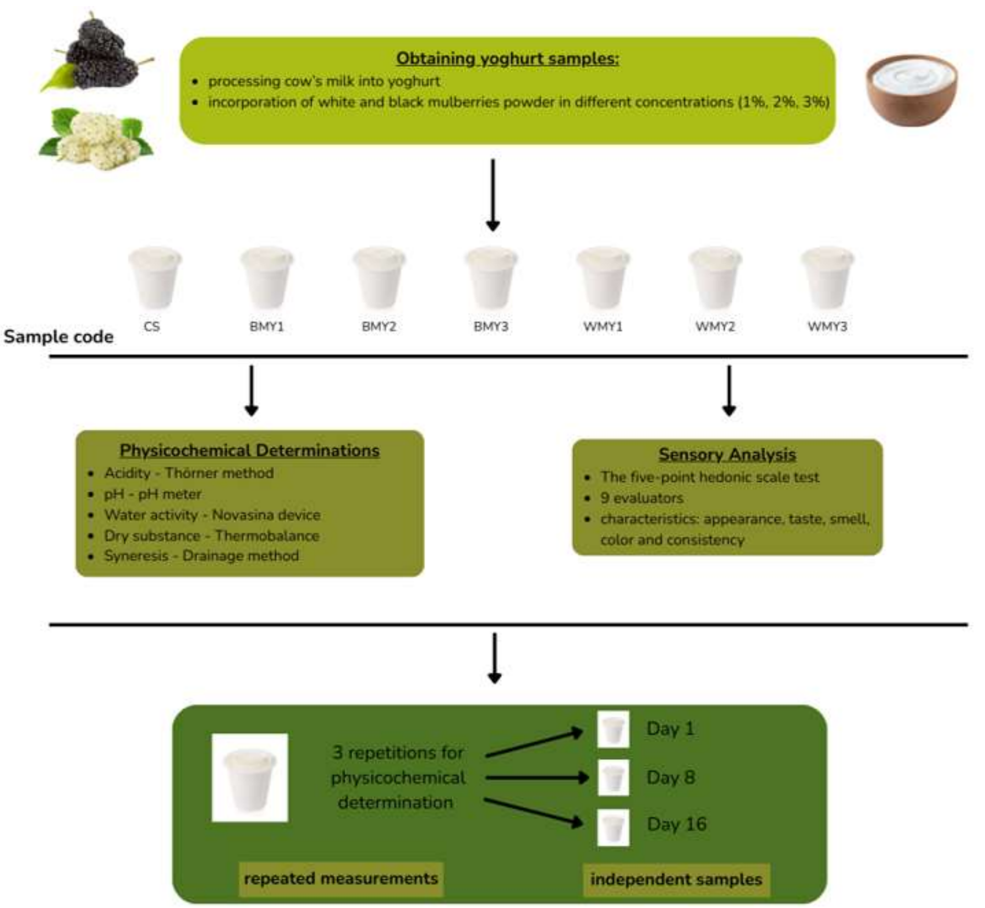


Fig. 1. *Experimental design for obtaining yogurt samples with white and black mulberry powder*

The raw cow's milk was subjected to pasteurisation for a duration of 20 minutes at a temperature ranging from 85-90°C, followed by cooling to a temperature of 48°C. The starter culture was added in accordance with the manufacturer's instructions, along with 100 g of partially skimmed milk powder, manufactured by Aristocrat (per 1 litre of seeded milk), with a view to obtaining a firmer curd. The distribution of the seeded milk was conducted into plastic cups, with a volume of 150 cm³. After a number of tests, the proportions of white mulberry and black mulberry powder were determined in accordance with the values presented in Table 1.

The samples were thermostated for a

period of three hours (until the pH of the samples reached 4.5) at a temperature of 43°C using a thermostat (Electronic April SRL). The yoghurt samples were subjected to a two-stage cooling process. Initially, the samples were subjected to a pre-cooling process for a duration of three hours at 20°C. Subsequent to this, the samples were stored at 4°C for a period of 16 days.

Table 1

*Yoghurt samples with added white and black mulberries powder: ingredients and proportions*

| Sample code | Cow's milk yoghurt [g] | Black mulberry powder [g] | White mulberry powder [g] |
|---|---|---|---|
| CS | 100 | 0 | 0 |
| BMY1 | 100 | 1 | 0 |
| BMY2 | 100 | 2 | 0 |
| BMY3 | 100 | 3 | 0 |
| WMY1 | 100 | 0 | 1 |
| WMY2 | 100 | 0 | 2 |
| WMY3 | 100 | 0 | 3 |

### 2.3. Physicochemical Determinations: Acidity, pH, Water Activity, Syneresis Index, Dry Substance

Acidity was determined using the Thörner method. The titration of 10 mL of sample, combined with 10 mL of distilled water, was conducted using 0.1 N NaOH in the presence of phenolphthalein. The titration was continued until the colour of the solution turned pale pink and remained unchanged for a duration of 30 seconds. The pH was monitored using a digital pH meter (Thermo Scientific, Orion 2-Star). The pH meter was calibrated, after which the pH of the samples was determined [36].

The Novasina device (LabMaster-aw) was utilised in order to ascertain the activity of water in the samples under investigation. The homogeneous sample was introduced into special plastic ampoules. The ampoules were then introduced into the apparatus, where the activity of the water in the product was determined at 25°C. The syneresis index of the samples was determined by placing 100 g of each sample in a funnel lined with filter paper. Following a six-hour process of draining, the volume of whey was measured [34]. The process of evaporating water from the sample was performed using a moisture analyser (AnD, ML-50) at a temperature of 150°C. A quantity of two grams of the sample was placed on the moisture analyser plate, whereafter the process of water evaporation was initiated [37]. Three repetitions were performed on each sample. On the first, eighth, and sixteenth days of storage, all physicochemical analyses were carried out.

### 2.4. Sensory Analysis

The sensory analysis of the samples was then assessed using a five-point hedonic scale test (1 point = dislike very much; 2 points = dislike; 3 points= neither like nor dislike; 4 points = like; 5 points = like very much). The evaluation was conducted by nine tasters, comprising both female and male participants, aged between 18 to 64 years. The yoghurt samples were presented in plastic cups with a capacity of 150 $cm^3$. The characteristics evaluated included appearance, taste, smell, colour, and consistency. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Lucian Blaga University of Sibiu (no. 21 on 23/05/2025).

### 2.5. Statistical Analyses

The Minitab version 14 was used to process the data statistically. To facilitate a comparison of the mean values and an assessment of the results, a range of statistical procedures were employed. These comprised analysis of variance (ANOVA) and Tukey's Test at a significance level of 5% ($p < 0.05$). All experiments were performed in triplicate (with the exception of the sensory analysis), and the resulting data were presented as the mean and standard deviation of the three independent experiments (on days 1, 8, and 16).

## 3. Results and Discussion

### 3.1. Physicochemical Determinations: Acidity, pH, Water Activity, Syneresis Index, Dry Substance

The results of the physicochemical analyses of the yogurt samples during the storage period are shown in Table 2.

The acidity content of yoghurt samples is influenced by the addition of mulberries. It was demonstrated that an increase in fruit concentration was accompanied by an increase in acidity. The acidity levels of the control sample range from 92.667°T to 121.33°T, those of the samples with white mulberries vary between 93.833°T (WMY1) and 118.67°T (WMY3), and those of the samples with black mulberries between 95.167°T (BMY1) and 119.00°T (BMY3). Yoghurt is characterised by a slightly sour taste, which is attributable to the presence of organic acids, including lactic acid, within the composition of the substance. These organic acids have been demonstrated to facilitate the process of digestion and absorption of nutrients within the human body [11].

According to a study by Oh et al. 2016 [27], lactic acid, a common byproduct of lactic fermentation, is much higher in yoghurt samples containing white mulberries than in the control sample. Sigdel et al. [32] and Karaca et al. [22] have also confirmed that plain and white mulberry yoghurt samples became more acidic after storage. As the amount of fruit increases, so does the acidity content, as was confirmed by Sigdel et al. [32]. Thus, compared to the control sample, the increase in acidity of yoghurt samples containing mulberry is more constant, with values increasing slightly throughout storage.

During the storage period, the yoghurt samples' pH showed a decrease in acidity. It was observed that an increase in the quantity of mulberries present resulted in a decrease in the pH value of the sample. The pH values range between 3.9 and 4.51, values that are comparable to those reported in the study by Karaca et al. [22]. It has been shown that the inclusion of organic acids, phenolic acids, and flavonoids in plant extracts encourages the growth of lactobacilli and speeds up the pH drop. The addition of mulberry powder to yoghurt fermentation has been shown to result in a rapid decrease in pH [35]. Consistent findings have been documented in related studies [10, 32, 35]. During the initial phase of storage, the yoghurt samples containing mulberries exhibited a lower pH level compared to the control sample. The control sample's pH dropped to a level below the preset limits imposed by the current standards on the last day of storage.

Table 2

*Evolution of physicochemical characteristics (acidity, pH, dry matter, water activity, syneresis) of yogurt samples during storage*

| Item | Sample | Storage time [days] | | |
|---|---|---|---|---|
| | | 1 | 8 | 16 |
| Acidity [°T] | CS | 92.667± 0.289$^{c,F}$ | 108.170± 0.289$^{b,F}$ | 121.330± 0.577$^{a,A}$ |
| | BMY1 | 95.167± 0.289$^{a,D}$ | 109.330± 0.289$^{a,E}$ | 116.170± 0.289$^{a,DEF}$ |
| | BMY2 | 98.167± 0.289$^{c,B}$ | 111.000±0.000$^{b,C}$ | 117.830± 0.289$^{a,D}$ |
| | BMY3 | 100.830± 0.289$^{b,A}$ | 114.330± 0.289$^{b,A}$ | 119.000±0.000$^{a,B}$ |
| | WMY1 | 93.833± 0.289$^{b,E}$ | 107.670± 0.289$^{ab,F}$ | 115.670± 0.289$^{a,DF}$ |
| | WMY2 | 97.000±0.000$^{c,C}$ | 109.830± 0.289$^{b,D}$ | 117.170± 0.289$^{a,DE}$ |
| | WMY3 | 99.333±0.289$^{b,AB}$ | 111.500± 0.500$^{ab,AB}$ | 118.670± 0.289$^{a,C}$ |
| pH | CS | 4.5167±0.0057$^{a,A}$ | 4.3867±0.0057$^{ab,A}$ | 3.9000±0.0000$^{c,D}$ |
| | BMY1 | 4.4667±0.0057$^{a,A}$ | 4.3467±0.0057$^{b,A}$ | 4.0733±0.0057$^{c,AB}$ |
| | BMY2 | 4.4533±0.0057$^{a,B}$ | 4.3200±0.0000$^{b,B}$ | 4.0533±0.0057$^{c,ABC}$ |
| | BMY3 | 4.4133±0.0057$^{a,C}$ | 4.3033±0.0057$^{ab,C}$ | 4.0333±0.0057$^{ac,BC}$ |
| | WMY1 | 4.4933±0.0057$^{a,A}$ | 4.3633±0.0057$^{b,A}$ | 4.0833±0.0057$^{b,A}$ |
| | WMY2 | 4.4567±0.0057$^{a,AB}$ | 4.3267±0.0057$^{b,A}$ | 4.0667±0.0057$^{b,AB}$ |
| | WMY3 | 4.4267±0.0057$^{a,BC}$ | 4.3133±0.0057$^{ab,C}$ | 4.0433±0.0057$^{b,BC}$ |
| Dry matter [%] | CS | 15.717±0.0289$^{c,D}$ | 18.100±0.0000$^{b,C}$ | 24.9170±0.0289$^{a,A}$ |
| | BMY1 | 16.367±0.0289$^{b,CD}$ | 18.267±0.0289$^{ab,B}$ | 22.133±0.0289$^{a,AC}$ |
| | BMY2 | 17.617±0.0289$^{b,C}$ | 19.067±0.0289$^{ab,A}$ | 22.817±0.0289$^{a,AB}$ |
| | BMY3 | 18.300±0.0000$^{b,A}$ | 20.517±0.0289$^{a,A}$ | 23.433±0.0289$^{a,A}$ |
| | WMY1 | 16.083±0.0289$^{ac,D}$ | 18.017±0.0289$^{ab,D}$ | 21.433±0.028$^{9a,AC}$ |
| | WMY2 | 17.433±0.0289$^{ac,C}$ | 18.833±0.0289$^{ab,AB}$ | 22.317±0.0289$^{a,ABC}$ |
| | WMY3 | 18.000±0.0000$^{c,B}$ | 19.433±0.0289$^{b,A}$ | 22.933±0.0289$^{a,AB}$ |
| Water activity | CS | 0.98300±0.00000$^{a,A}$ | 0.97767±0.00057$^{b,E}$ | 0.97100±0.00000$^{c,F}$ |
| | BMY1 | 0.98100±0.00000$^{a,B}$ | 0.98033±0.00057$^{b,A}$ | 0.97833±0.00057$^{c,A}$ |
| | BMY2 | 0.98000±0.00000$^{a,D}$ | 0.97800±0.00000$^{b,C}$ | 0.97667±0.00057$^{c,CD}$ |

| | | | | |
|---|---|---|---|---|
| | BMY3 | 0.97967±0.00057$^{a,E}$ | 0.97767±0.00057$^{ab,EF}$ | 0.97633±0.00057$^{ab,DE}$ |
| | WMY1 | 0.98033±0.00057$^{a,C}$ | 0.97867±0.00057$^{b,B}$ | 0.97800±0.00000$^{c,B}$ |
| | WMY2 | 0.97900±0.00000$^{a,F}$ | 0.97800±0.00000$^{b,D}$ | 0.97733±0.00057$^{c,C}$ |
| | WMY3 | 0.97833±0.00057$^{a,G}$ | 0.97733±0.00057$^{ab,F}$ | 0.97633±0.00057$^{b,DE}$ |
| Syneresis [%] | CS | 3.5333±0.1155$^{ac,CD}$ | 4.4333±0.1155$^{ab,B}$ | 5.2000±0.1000$^{a,A}$ |
| | BMY1 | 3.4667±0.0577$^{c,DE}$ | 4.2667±0.1155$^{b,DE}$ | 4.7333±0.0577$^{a,CDE}$ |
| | BMY2 | 3.6333±0.0577$^{c,C}$ | 4.4000±0.0000$^{b,C}$ | 4.7667±0.0577$^{a,C}$ |
| | BMY3 | 3.8667±0.0577$^{ab,A}$ | 4.5333±0.0577$^{ab,A}$ | 4.8333±0.0577$^{a,A}$ |
| | WMY1 | 3.4667±0.0577$^{c,E}$ | 4.2667±0.0577$^{b,DE}$ | 4.6667±0.0577$^{a,DE}$ |
| | WMY2 | 3.5667±0.0577$^{b,C}$ | 4.3667±0.0577$^{b,D}$ | 4.7667±0.0577$^{a,CD}$ |
| | WMY3 | 3.8000±0.0000$^{c,B}$ | 4.4667±0.0577$^{b,B}$ | 4.8000±0.0000$^{a,B}$ |

Note: Results are presented in the form of mean ± standard deviation (n = 3). In each column and row, values with different lowercase and uppercase letters, respectively, are significantly different ($p < 0.05$). The results in the same row followed by the same lowercase letters are not significantly different ($p > 0.05$). The results in the same column followed by the same uppercase letters are not significantly different ($p > 0.05$).

The dry matter content exhibited a range from 15.717 to 24.917%, with an increase observed during the 18-day storage period. This occurrence can be attributed to the dry matter content of the fruit, which is approximately 90% [24, 34]. The dry matter content values obtained in this study are similar to those reported by Sigdel et al., 2018 (range: 16.33-23.16%) [32].

The amount of free water in food that microorganisms can use to proliferate is known as water activity [22]. The control sample exhibited a distinct water activity value in comparison to the samples containing mulberries. It can be posited that the reduced water activity in dehydrated residues, attributable to the addition of mulberry powder, may underpin the observed decrease in water activity values in the samples.

A significant challenge confronting the yoghurt industry pertains to the phenomenon of whey separation. The expulsion of whey from three-dimensional networks, appearing on the surface, is known as syneresis [32]. The syneresis index values range from 3.46 (BMY1) to 5.20% (CS). As the storage period progresses, over an 18-day duration, the syneresis index demonstrates an upward trend in all yoghurt samples examined. The findings of the present study are consistent with those reported in studies conducted by Du et al. [10] and Sigdel et al. [32]. Consequently, the polysaccharide present in mulberry pomace exerts a significant influence on syneresis, thereby enhancing the rheological performance of yoghurt [10].

### 3.2. Sensory Analysis

Determining the characteristics of a product that are important in determining consumer acceptance is made possible through sensory evaluation. The addition of flavours to yoghurt has been demonstrated to influence sensory properties (e.g. aroma, taste, and appearance) and overall consumer acceptance [4]. In this study, the impact of white mulberry and black mulberry incorporation, along with storage duration, on the sensory characteristics of yoghurt is illustrated in Figure 2.

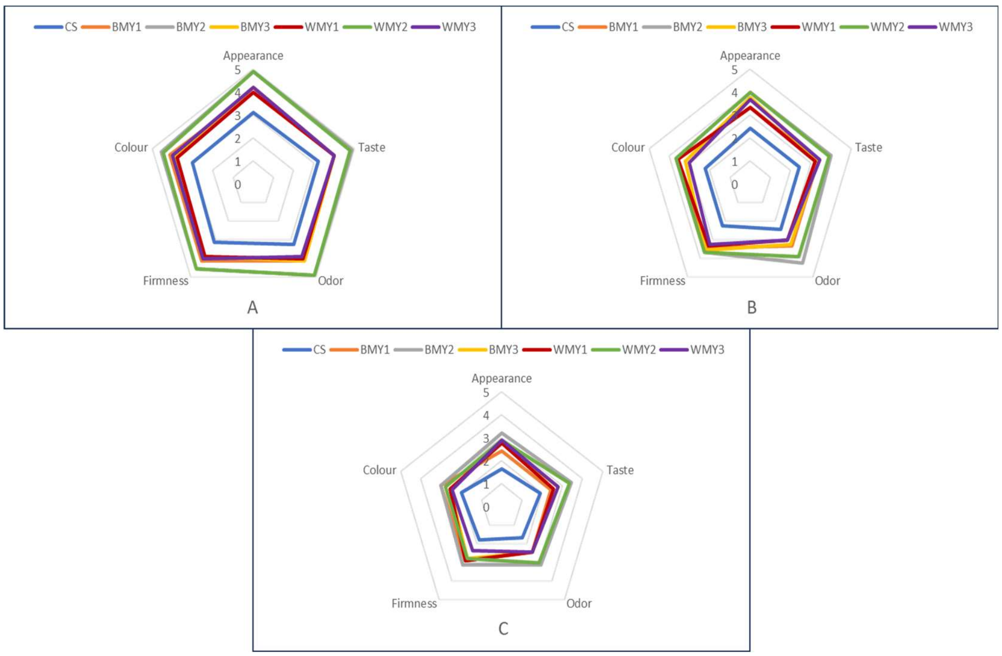


Fig. 2. *The evolution of the sensory properties of yoghurt samples with added white mulberries and black mulberries and the control sample during storage: CS – control sample; BMY1 – yoghurt with 1% black mulberry ; BMY2 – yoghurt with 2% black mulberry; BMY3 – yoghurt with 3% black mulberry; WMY1 – yoghurt with 1% white mulberry ; WMY2 – yoghurt with 2% white mulberry; WMY3 – yoghurt with 3% white mulberry; A – day 1, B – day 8, C – day 16*

The sensory characteristics of the yoghurt samples are found to be at their most intense on the first day of storage. The BMY2 sample obtained high scores for appearance (4.889), taste (4.889), and smell (4.889), while the WMY2 sample obtained high scores for appearance (4.889) and smell (4.889). On days 8 and 16, a decline in the results obtained was observed for all samples evaluated. The BMY2 sample obtained the highest result for smell on day 8 (4.222) and day 16 (3.444). As demonstrated in the study conducted by Karaca et al. [22], it is evident that the duration of storage has a detrimental effect on the scores, with a decline observed in all cases during this period. During the three-day tasting period, the 2% concentration was consistently rated as the most favourable by the tasters, irrespective of whether the samples contained black or white mulberries. The samples of yoghurt containing 3% were obtained and the results were the lowest (on day 1, sample WMY3 for odor – 3.889; on day 8, sample WMY3 for odor and colour – 3.000; on day 16, sample WMY3 for consistency – 2.333). In the study conducted by Tang et al. [35], it was determined that an increase in the quantity of mulberry leaf extract resulted in a substantial enhancement of the acidity and astringency of the yoghurt sample. It can thus be concluded that the addition of a flavouring agent to yoghurt has a significant impact on the sensory characteristics of the product. However, it is important to consider the quantity of the agent used in order to achieve the desired effect.

## 4. Conclusions

In conclusion, this study demonstrates the positive effect of adding white and black mulberries to yoghurt. The addition of these ingredients to yogurt samples has been shown to maintain an equilibrium between physicochemical and sensory properties over the storage period. The sensory analysis revealed that the addition of mulberry powder to the product had a significant impact on the results obtained. The characteristics of the yoghurt samples are found to be at their most intense on the first day of storage. The BMY2 sample obtained high scores for appearance, taste, and odor, while the WMY2 sample obtained high scores for appearance and odor. On days 8 and 16, a decline in the results obtained was observed for all samples evaluated. From a physicochemical perspective, the parameters analysed exhibited stability, thus indicating that mulberries are a promising ingredient for improving the quality of the product. This study demonstrates the technological feasibility and physicochemical stability of the samples due to the effectiveness of mulberries in fermented dairy products. Proposed future research directions include antioxidant and antimicrobial analyses, as well as probiotic viability investigations, with the objective of substantiating the potential classification of the product as a functional dairy product. These results could serve as a starting point for further research into the creation of other food products with additional bioactive ingredients.

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