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# THE EFFECT OF STORAGE TIME AND TEMPERATURE ON THE QUALITY OF NATURAL YOGHURT

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### **ABSTRACT**

Cooling temperature and storage time have a significant effect on the quality of natural yogurts and their durability in retail. The aim of the study was to perform a physicochemical, microbiological and organoleptic assessment of commercial yogurt, immediately after purchase and during 3 weeks of storage at 2°C and 8°C. The following physicochemical parameters were measured: pH, titratable acidity, color (CIEL a\*, b\*) and basic chemical composition. The organoleptic assessment included the general appearance and color, consistency, aroma and taste and desirability of yogurt. Microbiological tests have determined the amount of *Lactobacillus*, *Streptococcus*, *Enterobacteriaceae* bacteria, and checked the presence of mold and fungi. We have observed a significant effect of storage time on titratable and active acidity in the tested samples. No statistically significant effect of storage time on color of yogurt was observed. As the storage time increased, the number of yogurt microorganisms was decreasing. The minimum normative total amount of lactic acid bacteria was recorded on the day of purchase and after one week of storage at 8°C. As the storage time and the storage temperature increased, the taste and aroma of yogurt was decreasing significantly.

Key words: natural yoghurt,temperature, storage time, quality: physicochemical, sensory, microbiological

### INTRODUCTION

In the recent years, a dynamic increase in production of fermented milk drinks belonging to a "functional food" category has been observed. In Poland, the production of fermented milk drinks in 2017 reached 700 thousand tons, of which 380 thousand tons was yoghurt. Its annual consumption in a typical household since 2014 is around 6 kg per person [Brodziak et al. 2018]. The factors that caused the interest in consumption of yogurt include its organoleptic characteristics, nutritional, dietary and medical values, as well as its wide assortment at the market [Zaręba and Ziarno, 2014, Brodziak and Król 2016, Jakubowska and Matusevicius 2018].

Natural yogurt is a fermented dairy product that is obtained by acidifying milk with specific yogurt bacteria (*Lactobacillus delbruecckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*). In case of so-called new generation yoghurts, the products are also supplemented with *Lactobacillus acidophilus* and *Bifidobacterium* strains [Zaręba and Ziarno 2013].

An appropriate selection of raw materials and the method of production as well as storage conditions significantly affect physicochemical, sensory and microbiological characteristics of yogurt.

Yoghurt belongs to the group of dairy products with a short shelf life, which is normally is about 3 weeks or less [Mojka 2013]. Improper storage conditions can lead to a decrease in nutritional value and deterioration of sensory characteristics of yoghurts, as well as to a reduction in content of yogurt bacteria that carry out the process of lactic fermentation [Cais-Sokolińska and Pikul 2001, Zareba and Ziarno 2013, Wojtczak et al. 2018]. The storage temperature of dairy products also significantly determines their quality and durability. Improper storage temperature in yogurt coolers can lead to a change in the number and mutual proportions of lactic acid bacteria composition typical for the product, and in consequence, to its acidification, lowering pH and deterioration of its sensory quality [Cais-Sokolińska and Pikul 2001]. The recommended storage temperature of yoghurt ranges from 1°C to 8°C, but its durability may be enlarged by



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storing at 4°C or less during the whole shelf life [Pikul 2004]. The studies on the impact of storage time and conditions of fermented milk products are aimed at finding a method of handling yogurt that would maximally limit the changes in quality parameters (e.g. color, taste, aroma, pH, appropriate concentration of LAB bacteria) during retail storage. Recent reports underline the very good level of domestic fermented milk drinks, but still indicate their diversified quality [Molska et al. 2003, Zaręba and Ziarno 2013, Zaręba and Ziarno 2014, Wichrowska and Wojdyła 2014, Jakubowska and Matusevicius 2018].

Therefore, in our study we aimed at assessing the physicochemical, microbiological and organoleptic features of commercial natural yogurt, immediately after purchase and during 3 weeks of storage at various cooling temperatures: 2°C and 8°C.

### **MATERIAL AND METHODS**

Our research material included the samples of natural yoghurt produced by one of the Dairy Cooperatives in Podlaskie, available in retail chains in West Pomerania.

105 unit packages of yogurt were purchased at the local dairy wholesaler's. From the whole purchased batch, 10 packages were randomly selected for physicochemical and organoleptic tests, and this group was set as the control. Additionally, 5 randomly selected yoghurts were subjected to microbiological tests. The rest of the yoghurts was divided into two groups and cooled in refrigerators at 8°C and 2°C. The analyzed shelf life of yogurt was 3 weeks and included the proper shelf life and the week after the expiration date. The plan of our experiment included testing of yogurt immediately after purchase and after each week of storage.

After each week of storage, from each of the experimental group, we have randomly selected 10 yoghurts for physicochemical and organoleptic examinations and 5 yoghurts for microbiological tests.

The yogurt composition, according to the manufacturer's data was as follows: pasteurized milk, milk proteins, sugar, live yogurt bacteria cultures and bifidobacteria. Nutritional value in 100 g of product: energy proteins 275 kJ/61 kcal, proteins 3.6 g, carbohydrates 6.1 g, fat 2.5 g.

### Tests and measures

The following physiochemical parameters were analyzed in our yogurt samples: pH, titratable acidity and color. The color assessment was carried out with the MiniScan XE Plus 45/0 camera, with a 31.8 mm diam. measuring port. We used the CIE L\* a\* b\* scale, D 65 illuminate and used the standard 10°C observer [CIE 1976].

The pH measurement was taken using a combined glass electrode and a Cyber Scan pH meter. Evaluation

of the total acidity in Soxhlet-Henkel grades was performed according to the standard method [PN-75 1975]. The acidity of tested products was measured at room temperature, and the final result was the arithmetic mean of the two parallel measurements.

We have also analyzed the content of sugars, total protein and fat [Krełowska-Kułas 1993]. The Kjeldahl method was used to determine the total protein content, and the fat content was measured with the Röse-Gottlieb method. The Bertrand method was used to determine the content of sugars (lactose).

Our study included also microbiological testing of the natural yoghurts. For the microbiological tests, 1 g of yogurt was transferred to 9 ml of sterile saline. The suspension was stirred and then the decimal dilutions were made. One ml of suspension from each of the dilutions was inoculated into the following media: MacConkey Agar, XLD Medium (to identify the Gram-negative Enterobacteriaceae bacteria), Baird-Parker Agar (to identify Staphylococcus bacteria), Edwards Medium (to identify streptococci) and MRS Agar (to isolate Lactobacillus spp.). The cultures were incubated under aerobic conditions at 37°C for 24 hours. After that time the colonies were counted with a consideration to the previously made decimal dilutions [PN-93 1993b, PN-93 1993c]. The number of yeast and mold cells was determined in reference to the Polish Standard [PN-93 1993a]. The results of all microbiological determinations were expressed as CFU/g.

Sensory analysis was performed with a five-point scale according to Pieczonka [1995]: 1 point – disqualifying rate, 5 points – very good rate. The appearance, color, consistency, aroma and taste of natural yoghurts were evaluated. Sensory analysis was carried out by a five-person team verified in terms of sensory sensitivity, according to PN-98 [1998].

Statistical analysis of the results was performed with the Statistica 10 software. We calculated the mean values and standard deviations, and carried out a one-way analysis of variance, with regard to the effect of storage at 2°C and 8°C on the quality features of examined samples. The Duncan test was used to estimate the differences between the experimental groups.

### RESULTS AND DISCUSSION

### Effect of storage on physicochemical features

The tests showed a significant effect (P  $\leq$  0.05) of storage time at both tested temperature ranges (2°C and 8°C), on the active acidity of examined samples. The acidity of yoghurts on the day of purchase was 4.17. During 14 days of storage of yoghurts at 2°C, the pH dropped to 4.10, and later a slight increase was noted on the 21st day of storage to the initial value of 4.17 that was measured on the day of purchase. A similar relationship was observed in

Table 1. Effect of storage on changes in active and potential acidity of yoghurts

Tabela 1. Wpływ przechowywania na zmiany kwasowości jogurtów

| Days<br>Dni |                   | p     | Н                 | Acidity, °SH<br>Kwasowość, °SH |                    |       |                    |      |
|-------------|-------------------|-------|-------------------|--------------------------------|--------------------|-------|--------------------|------|
|             | Temp              | . 2°C | Temp              | o. 8°C                         | Temp               | . 2°C | Temp. 8°C          |      |
|             | x                 | S     | x                 | S                              | x                  | s     | x                  | s    |
| 0           | 4.17ª             | 0.03  | 4.17a             | 0.03                           | 37.76a             | 0.01  | 37.76a             | 0.01 |
| 7           | 4.20a             | 0.02  | $4.13^{b}$        | 0.01                           | 37.60a             | 3.61  | 40.76a             | 0.96 |
| 14          | $4.10^{b}$        | 0.01  | 4.13 <sup>b</sup> | 0.01                           | 46.08 <sup>b</sup> | 0.95  | 46.80 <sup>b</sup> | 1.94 |
| 21          | 4.17 <sup>a</sup> | 0.01  | 4.16 <sup>a</sup> | 0.01                           | 42.92 <sup>b</sup> | 1.15  | 45.60 <sup>b</sup> | 1.11 |

a, b, c – Means marked by different lower-case letters differ significantly ( $P \le 0.05$ ).

**Table 2.** Effect of storage on changes in yoghurt color

Tabela 2. Wpływ przechowywania na zmiany barwy jogurtów

| Days<br>Dni | L*        |      |           |      |           | ;    | a*                 |      | b*        |      |           |      |
|-------------|-----------|------|-----------|------|-----------|------|--------------------|------|-----------|------|-----------|------|
|             | Temp. 2°C |      | Temp. 8°C |      | Temp. 2°C |      | Temp. 8°C          |      | Temp. 2°C |      | Temp. 8°C |      |
|             |           | s    | x         | s    | x         | s    | x                  | s    | x         | s    | x         | s    |
| 0           | 93.78     | 0.13 | 93.78     | 0.13 | -2.07     | 0.02 | -2.07 <sup>b</sup> | 0.02 | 11.24     | 0.13 | 11.24     | 0.12 |
| 7           | 93.39     | 0.10 | 93.76     | 0.41 | -2.10     | 0.02 | $-2.05^{b}$        | 0.02 | 11.03     | 0.12 | 11.16     | 0.05 |
| 14          | 93.68     | 0.47 | 93.59     | 0.10 | -1.88     | 0.03 | $-1.98^{b}$        | 0.05 | 10.77     | 0.07 | 11.27     | 0.05 |
| 21          | 91.11     | 4.82 | 92.56     | 1.47 | -2.29     | 0.95 | $-1.85^{a}$        | 0.05 | 11.81     | 2.07 | 11.00     | 0.43 |

a, b – Means marked by different lower-case letters differ significantly ( $P \le 0.05$ ).

**Table 3.** Effect of storage time on microbiological changes in yoghurts

Tabela 3. Wpływ czasu przechowywania na zmiany mikrobiologiczne jogurtów

| Dni  | Lactob                                | acillus                | Streptococcus       |                        |  |  |
|------|---------------------------------------|------------------------|---------------------|------------------------|--|--|
| Days | Temp. 2°C                             | Temp. 8°C              | Temp. 2°C           | Temp. 8°C              |  |  |
| 0    | 9.50 · 10 <sup>5</sup>                | 9.50 · 10 <sup>5</sup> | $5.17 \cdot 10^{3}$ | 5.17 · 10 <sup>3</sup> |  |  |
| 7    | $1.67 \cdot 10^{3}$                   | $1.34\cdot 10^4$       | $3.21 \cdot 10^{3}$ | $2.59 \cdot 10^{3}$    |  |  |
| 14   | $3.17\cdot 10^2$                      | $4.10\cdot 10^3$       | $2.93 \cdot 10^{2}$ | $1.14 \cdot 10^{2}$    |  |  |
| 21   | $4.67\cdot 10^{\scriptscriptstyle 0}$ | $1.27\cdot 10^2$       | 0                   | $2.67 \cdot 10^{1}$    |  |  |

case of storage at 8°C; on days the 7<sup>th</sup> and 14<sup>th</sup>, the pH dropped to 4.13 and then slightly increased to 4.16 on the 21<sup>st</sup> day of storage (Table 1). The results obtained by other researchers differ from the above. Cais-Sokolińska and Pikul [2001] noted a regular increase in curd acidity (from 0.1 to 0.2 units) after each week of storage of natural yogurt. They have also showed that the decrease in active acidity is significantly affected by storage temperature. At higher temperatures, pH of dairy products decreases faster than in the same products, but stored at lower temperatures. Also Kneifel et al. [1993], Imhof et al. [1994], Sady et al. [2007] and Eissa et al. [2010] reported in their publications a steady decrease in pH associated with a prolonged storage time.

An increase in the acidity of yogurt during storage is a result of the fermentation activity of microorganisms that

make up the yogurt vaccine, and that even under refrigerated conditions are still able to digest lactose, although the process is slower [Jankowska and Reps 2013].

The pH values measured in this study turned out to be lower than those recorded by Wichrowska and Wojdyła [2014] and Jakubowska and Matusevicius [2018] in yoghurts obtained from the retail chain (4.38 and 4.48, respectively) after a week of storage, while similar to those recorded by Sady et al. [2007] in natural yogurts after 14 days of storage (4.15). The reported pH values confirm the correct acidity of the tested samples; according to Jurczak [2003], the active acidity of yoghurts should range from 4.0 to 4.5. However, basing on the observations by Jankowska and Reps [2013], the acidity of yoghurts that responds to the consumer requirements is narrower and remains in the range of 4.2–4.5.

a, b, c – Średnie oznaczone różnymi małymi literami różnią się od siebie istotnie ( $P \le 0.05$ ).

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On the day of purchase, we have demonstrated that the titration acidity of natural yogurt was 37.76°SH (Table 1). During 14 days of storage, there was a slight increase in titratable acidity in both analyzed temperature ranges (up to 46.08°SH at 2°C and up to 46.80°SH at 8°C). On the other hand, on the 21<sup>st</sup> day of storage there was a slight decrease in titratable acidity, compared to the 14<sup>th</sup> day of storage. A statistically significant effect of storage on the titratable acidity was recorded only on days 14<sup>th</sup> and 21<sup>st</sup>. At the same time, we have observed that changes in titratable acidity of the samples proceeded more slowly during 14 days of storage for products stored at 2°C than at 8°C (Table 1).

Kneifel et al. [1993], Imhof et al. [1994], Sady et al. [2007] and Eissa et al. [2010] observed an increase in titratable acidity with longer storage time. In studies of Cais-Sokolińska and Pikula [2001], the titratable acidity of yogurt at the end of storage period compared to the acidity measured immediately after its production was on average about 32% higher. These authors also report that changes in titratable acidity occurred more slowly in yoghurts stored at  $6\pm1^{\circ}$ C than at  $10\pm1^{\circ}$ C.

A decrease in acidity of yogurt is affected by its microflora, which is diversified in its activity in acidifying, proteolytic and lipolytic processes. During the maturation and storage of milk drinks, the bacteria use milk ingredients at various rates and ranges. First, the lactose is converted into lactic acid, which causes a increase of yogurt acidity [Cais-Sokolińska et al. 2009].

We have demonstrated that the L\* parameter in the tested samples ranged from 93.78 to 91.11. There was no significant effect of storage time on the brightness of yogurt samples (Table 2). As the storage time increased, a slight decrease in yogurt brightness was observed only on the 21st day of storage at 2°C and 8°C (Table 2). However, Cais-Sokolińska and Pikul [2008] noted a significant deterioration in brightness of color during the subsequent weeks of storage. Teichert et al. [2015] noted a significant increase in brightness of color in goat yoghurts on the 21st day of cool storage.

The a\* parameter of yoghurts (change of color in range from green to red) was negative for all the tested samples in range from -2.29 to -1.85 (Table 2). In yoghurts stored at 2°C, on the day of purchase and on the 7<sup>th</sup> day of storage, the values of a\* were from -2.07 to -2.10, and on the 14<sup>th</sup> day there was a decrease in a\* to -1.88, and then in turn an increase on the  $21^{st}$  day to -2.29 occured. In yoghurts stored at 8°C, a significantly (P  $\leq 0.05$ ) lower share of green color (a\*) along with the extension of the storage time (from -2.07 on the day of purchase to -1.85 on the  $21^{st}$  day of storage) was noted.

On the other hand, the parameter b\* (change of color in range from blue to yellow) was positive for all the tested yogurt samples in range from 11.24 (on the day

of purchase) to 11.81–11.00 (on the 21<sup>st</sup> day of storage). There was no statistically significant effect of storage time on this feature in any of the tested temperature ranges (Table 2).

The use of instrumental measurement of yogurt color may reflect the changes that occur in yogurt as a result of activity of microflora that is intentionally introduced during the production process [Cais Sokolińska and Pikul 2008]. As a result of the tests, no significant color changes in the stored yogurt were found, while only a slight lightening was observed on the 21st day of storage. The measured color parameters were stable and did not change over time except for the change of the a\* parameter in samples stored at 8°C. Meanwhile, studies conducted by Sofu and Ekinci [2007] revealed some changes in yogurt color during storage. Gravish-greenish-yellow tones predominated in yoghurts at the end of their shelf life. According to Cais Sokolińska and Pikula [2008], the microbiological activity of yogurt microflora leads to the changes in potential acidity of the clot, and thus cause physical changes in the clot, resulting in a difference in the color brightness and the degree of color saturation. According to the research by Cais-Sokolińska and Majcher [2009] and after Teichert et al. [2015], the source of changes in color parameters during storage lays in the fact that the casein complex passes from the micelar state into a dispersed state. The colloidal casein-calcium complex disperses light and make the milk look white.

## Effect of storage time on changes in yogurt microflora

On the day of purchase, the concentration of *Lactobacillus* bacteria in yoghurts was  $9.5 \cdot 10^5$  CFU  $\cdot$  g<sup>-1</sup>. On the  $14^{th}$  day of their shelf life and storage at 2°C, their quantity was  $3.17 \cdot 10^2$  and at  $8^{\circ}\text{C} - 4.10 \cdot 10^3$ . These results show a decrease in concentration of *Lactobacillus* bacteria over the entire storage period. Faster (from  $10^5$  to  $10^0$  CFU  $\cdot$  g<sup>-1</sup>), the number of bacteria decreased in yoghurts stored at  $2^{\circ}\text{C}$  than when stored at  $8^{\circ}\text{C}$  ( $10^2$  CFU  $\cdot$  g<sup>-1</sup>) (Table 3). The growth of these bacteria depends primarily on the storage temperature and the type of strains included in the sourdough [Sady et al. 2007]. *Lactobacillus* bacteria grow better at warmer temperatures

The number of *Streptococcus* bacteria was also examined in the tested samples. Also in the case of these bacteria, their number was decreasing along with the storage time. The number of bacteria found in yoghurts immediately after purchase was  $5.17 \cdot 10^3$  CFU  $\cdot$  g<sup>-1</sup>, while on the  $14^{th}$  day of the study, their number fluctuated regardless of the temperature from  $2.93 \cdot 10^2$  to  $1.14 \cdot 10^2$ . However, on the  $21^{st}$  day of the study after the shelf-life, in yoghurts stored at  $2^{\circ}$ C, the number of *Streptococcus* bacteria dropped to zero, and in yoghurts stored at  $8^{\circ}$ C to  $10^1$  CFU  $\cdot$  g<sup>-1</sup> (Table 3).

**Table 4.** Effect of temperature of storage on sensory features

Tabela 4. Wpływ temperatury przechowywania na zmiany właściwości sensorycznych

| Days | Appearance and color, pts<br>Wygląd i barwa, pkt. |        |      | Consistency, pts<br>Konsystencja, pkt. |                   |       | Aroma and taste, pts<br>Smak i zapach, pkt. |       |                   | Desirability, pts<br>Pożądalność, pkt. |            |       |            |       |            |       |
|------|---|--------|------|--|-------------------|-------|---|-------|-------------------|--|------------|-------|------------|-------|------------|-------|
| Dni  | Temp  | o. 2°C | Temp | . 8°C                                  | Temp              | . 2°C | Temp  | . 8°C | Temp.             | . 2°C                                  | Temp       | . 8°C | Temp       | . 2°C | Temp       | . 8°C |
|      | - x   | s      | x    | s                                      | x                 | s     | x   | s     | x                 | s                                      | x          | s     | x          | s     | x          | s     |
| 0    | 5.00  | 0.00   | 5.00 | 0.00                                   | 5.00 <sup>b</sup> | 0.00  | 5.00  | 0.00  | 5.00 <sup>b</sup> | 0.00                                   | 5.00a      | 0.00  | 5.00a      | 0.00  | 5.00a      | 0.00  |
| 7    | 4.60  | 0.00   | 4.60 | 0.00                                   | $4.70^{b}$        | 0.00  | 4.40  | 0.00  | $4.96^{b}$        | 0.05                                   | $4.40^{b}$ | 0.07  | $4.84^{b}$ | 0.03  | $4.43^{a}$ | 0.04  |
| 14   | 5.00  | 0.00   | 5.00 | 0.00                                   | $5.00^{b}$        | 0.00  | 4.67  | 0.00  | $3.53^{\rm c}$    | 0.18                                   | 1.94°      | 0.13  | 4.12°      | 0.11  | $3.08^{b}$ | 0.08  |
| 21   | 4.26  | 1.65   | 5.00 | 0.00                                   | $3.66^{a}$        | 1.48  | 4.33  | 0.00  | 3.87°             | 0.30                                   | 2.47d      | 0.60  | 3.87°      | 0.80  | $3.31^{b}$ | 0.36  |

a, b, c – Means marked by different lower-case letters differ significantly ( $P \le 0.05$ ).

Table 5. Results of chemical composition analysis of natural yoghurts on the day of purchase and the 21st day of storage

Tabela 5. Wyniki analizy składu chemicznego w dniu zakupu i 21 dniu przechowywania jogurtów naturalnych

| Traits<br>Cechy  | Chemical composition as declared<br>by producer, g<br>Skład chemiczny deklarowany<br>przez producenta, g | Chemical composition assayed on day<br>of purchase, g<br>Skład chemiczny oznaczony w dniu<br>zakupu, g | Chemical composition assayed on the 21st day of storage, g Skład chemiczny oznaczony w 21 dniu przechowywania, g |
|------------------|--|--|--|
| Protein – Białko | 3.6  | 3.37   | 3.37   |
| Cukier – Sugar   | 6.1  | 5.03   | 3.90   |
| Fat-Tluszcz      | 2.5  | 2.00   | 1.95   |

In our study, the number of lactic acid bacteria decreased faster at the lower (2°C) than higher (8°C) temperature, inversely than in the Cais-Sokolińska and Pikula [2001] studies, which showed that the decrease in number of lactic acid microorganisms proceeds more slowly in yogurts stored at  $6\pm1^{\circ}C$  than when stored at higher temperature (10  $\pm1^{\circ}C$ ). The storage temperature of fermented beverages is very important, as it determines the size of population at an appropriate level, and should be kept at below 6°C [Zaręba and Ziarno 2013].

Many studies clearly state that the storage time of dairy products is very important and has a decisive impact on the number of characteristic microflora of yogurt. Sady et al. [2007] showed that the level of L. bulgaricus and S. thermophilus increased slightly until the 3<sup>rd</sup> day of storage, after which it decreased to the lowest level on the 14<sup>th</sup> day. In addition, they observed that the number of bacilli was decreasing more slowly than streptococci. In fresh products, the ratio of L. bulgaricus to S. thermophilus was 1:1.46, while after 14 days it changed into 1.23:1. This change is associated with the process of acidification during cool storage, as S. thermophilus is less tolerant to high acidity. Similar results were obtained by Laye et al. [1993] and Beal et al. [1999], while Kneifel et al. [1993] and Barrantes et al. [1994] did not observe any significant changes in the number of bacteria during yogurt storage. Numerous studies confirm a decrease in the number of bacteria during cool storage [Beal et al.

1999, Cais Sokolińska and Pikul 2001, Sady et al. 2007, Zareba et al. 2008, Zareba and Ziarno 2013].

In our research, Lactobacillus (10<sup>5</sup>) was the dominant species, compared to Streptococcus (10<sup>3</sup>). Lactobacilli tolerate much lower pH levels and are able to survive in an environment with pH below 4.0, in contrast to the other yogurt bacteria [Jankowska and Reps 2013. According to Zareba and Ziarno [2013] during the yoghurt production process, the growth of Streptococcus thermophilus is inhibited at pH 4.2-4.4, whereas L. bulgaricus continue to grow by acidifying the environment and produce more lactic acid (lactobacilli tolerate pH 3.5–3.8). However, in this study the acidity of yoghurts ranged from 4.16 to 4.10 throughout the entire cool storage period, which may be the reason for a lower concentration of Streptococcus bacteria in the tested yogurt samples (Table 1). According to the research made by Ziarno and Zareby [2013], the pH of commercial yogurt can be from 3.5–3.7 to 4.3–4.4.

Analyzing the compliance of our results with the requirements of FAO-WHO [2000] and IDF/FIL which define the minimum amount of yogurt bacteria in total at  $10^7$  CFU  $\cdot$  g<sup>-1</sup>, the analyzed yogurt met the recommended criteria on the day of purchase, where total number of CFU was  $10^8$  (Table 3). However, with proceeding storage time, the concentration of bacteria was decreasing. After 7 days of storage, the minimum content of lactic acid bacteria ( $10^7$  CFU  $\cdot$  g<sup>-1</sup>) was met only by yoghurts

a, b, c – Średnie oznaczone różnymi małymi literami różnią się od siebie istotnie ( $P \le 0.05$ ).

stored at 8°C. In the next periods of cool storage and a week after the shelf life, there was no minimal normative number of live cells of lactic acid bacteria (Table 3). A decrease in lactic acid bacteria levels in commercial yoghurts was also noted by Zaręba and Ziarno [2013], but not to such the extent as in our analyzes. The authors analyzed the survivability of lactic acid bacteria and bifidobacteria in 70 commercial yogurt samples during their shelf life and 2 weeks after the shelf life. They found that the population of streptococci was decreasing regardless of their initial number and the percentage of samples containing less than the normative  $10^7$  CFU  $\cdot$  g<sup>-1</sup> of bacilli increased. In the discussed studies, on the last day of shelf life, only in 4.3% of yogurt samples the number of Streptococcus thermophilus cells was below 10<sup>6</sup> CFU  $\cdot$  g<sup>-1</sup>, and the number of *Lactobacillus* cells was below  $10^7$  CFU  $\cdot$  g<sup>-1</sup> in 10% of the examined samples. According to Zareba and Ziarno [2014], the highest reduction of bacterial populations in fermented milk drinks occurs immediately after their expiration date.

A diversified survivability of yogurt bacteria in this study may result from the different sensitivity of starter cultures used by the manufacturer, the technological treatments, and the conditions of storage of product in the warehouse before the purchase. Also some other factors like sugar concentration, dry matter content and access to nutrients are important for the amount of yoghurt bacteria in the final product. Level of pH of yogurt will play a very important role as well. In this study, the acidity of yogurt was from 4.16 to 4.10 throughout the entire cool storage period, hence perhaps the concentration of Lactobacillus bacteria in the tested samples was higher in comparison to Streptococcus (Table 1). Bolin et al. [1998] indicate that the yogurt fermentation process should end at pH 4.9-5.0, which ensures a correct bacterial survivability and normal metabolic processes, and guarantee the appropriate aroma and taste desirability.

The hygienic quality of the examined yogurts was very good. In the tested samples, no infections with mold and yeast or bacteria from the *Enterobacteriaceae* family were noted.

### Effect of storage time on sensory features

Natural yogurt should be characterized by a semi-smooth smooth consistency with a dense homogeneous curd, without protein lumps and air bubbles. The yogurt taste should be fresh, mild and pleasant and the aroma should have a characteristic yogurt flavor [Kudełka 2005]. According to the sensory analysis we carried out, the tested beverages were characterized by an appropriate color, appearance and consistency, and the analyzed features obtained very high scores, from 4.4 to 5.0 points during 14 days of storage at 2°C and 8°C (Table 4). Moreover, on the 21st day of storage after the expiry date,

the tested yogurt received slightly lower scores for these sensory parameters (notes from 3.66 to 5.0 points).

The results of organoleptic assessment did not show a statistically significant effect of storage time on the appearance and color of yoghurts at 2°C and 8°C (Table 4). The only significant differences (P  $\leq$  0.05) were found in the assessment of consistency of yoghurts stored at 2°C. Yoghurts rated on the 21st day of storage (3.66 points) obtained lower scores at this temperature compared to the yoghurts tested on the 7th and 14th day of storage (from 5.0 to 4.7 points).

Immediately after purchase and on the 7<sup>th</sup> day of storage, the yoghurts received the highest notes for taste and aroma (from 5.0 to 4.40 points), but together with the extension of the storage time a significant (P  $\leq 0.05$ ) deterioration of their taste and aroma at 2°C and 8°C was observed. The lowest notes for these parameters were recorded in yogurts stored at 8°C. On the 14th day of storage the score was 1.94 points, and on the 21st day of storage, 2.47 points. The statistical analysis of the general desirability of yogurt, which consisted of the results taken from all the assessed factors, showed that the yogurt is the most desirable immediately after purchase and in the first week of storage (Table 4). Products immediately after purchase and after the first week of storage, regardless of storage temperature, obtained significantly ( $P \le 0.05$ ) the highest notes (from 5.0 to 4.43 points) compared to yoghurts tested on 14th and 21th day of storage after the shelf life (4.12 to 3.08 points) (Table 4).

From the beginning to the end of the study, yoghurts stored at 2°C received higher notes for the general desirability, compared to yoghurts stored at 8°C (Table 4).

Cais-Sokolińska and Pikul [2001] noticed the deterioration of sensory features of yogurt along with the extension of storage time. These authors have also shown a significant effect of temperature on the quality of yogurt. Just as in the above analysis, they proved that yoghurts stored at lower temperatures kept better desirability for longer than yoghurts stored at higher temperatures. Also Wichrowska and Wojdyła [2014] found that color, taste, aroma and texture of yoghurts deteriorated with the increasing storage time. However, according to research conducted by Chougrani et al. [2009] storage time had a positive effect on the taste of yogurt made from skim milk, where at the end of the study the taste of yogurt was rated better than at the beginning.

According to our studies, both storage time and storage temperature were the factors that differentiated the sensory quality of yogurt. The tested yoghurts stored at lower temperature were more stable in terms of the tested parameters than those kept at higher temperature. Keeping the appropriate sensory characteristics during the storage of yogurt is very important for the consumer.

Taste, aroma, texture or color are the reflection of their quality and play a decisive role in the final choice of products by the buyer.

Food manufacturers must pay attention to the sensory quality of produced food, to offer a competitive product with repeatable quality characteristics, that meets the growing requirements of consumers [Rój and Przybyłowski 2012].

### Effect of storage time on chemical composition

The results of protein and fat content tests in the examined samples on the day of purchase were close to those declared by the manufacturer (Table 5). However, the sugar content did not coincide with the data presented on the product label. Fluctuations in sugar content in samples tested on the day of purchase and during the cool storage can be explained by the fermentation activity of microorganisms added to dairy drinks during the production process, which at 4°C are still able to digest lactose [Cais-Sokolińska et al. 2009, Jankowska and Reps 2013].

### **CONCLUSIONS**

- 1. A significant effect of storage time on the titratable and active acidity of natural yoghurts was found.
- 2. There was no statistically significant effect of storage time on the color of yoghurts; only in yoghurts stored at 8°C a significantly ( $P \le 0.05$ ) lower proportion of green color (a) with the longer storage time was noted.
- 3. As the storage time increases, the number of yogurt microorganisms decreases. The normative amount of lactic acid bacteria was recorded on the day of purchase and after one week of storage at 8°C.
- 4. The hygienic quality of the determined yogurt samples was very good. In the tested samples no infections with molds, yeasts or bacteria from the *Enterobacteriaceae* family were found.
- As the storage time and the storage temperature increases, the sensory characteristics of yogurt deteriorates.
- Yoghurts had the best sensory quality immediately after purchase and after the first week of storage. These yoghurts were characterized by the best taste, aroma and general desirability.

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### WPŁYW CZASU PRZECHOWYWANIA I TEMPERATURY NA JAKOŚĆ JOGURTU NATURALNEGO

### **STRESZCZENIE**

Temperatura chłodni i czas przechowywania mają istotny wpływ na kształtowanie się jakości naturalnych jogurtów oraz zachowanie ich trwałości w handlu detalicznym. Celem pracy była ocena fizykochemiczna, mikrobiologiczna oraz organoleptyczna jogurtu handlowego, zaraz po zakupie oraz podczas 3-tygodniowego przechowywania w temperaturze 2°C oraz 8°C. W zakupionych jogurtach oznaczono następujące parametry fizykochemiczne: pH, kwasowość miareczkową, barwę (CIEL a\*, b\*) oraz podstawowy skład chemiczny. Wykonano ocenę organoleptyczną wyglądu i barwy, konsystencji, zapachu i smaku oraz pożądalności jogurtów naturalnych. Badania mikrobiologiczne obejmowały oznaczenie ilości bakterii z rodzaju *Lactobacillus, Streptococcus, Enterobacteriaceae* oraz obecności pleśni i grzybów. Stwierdzono istotny wpływ czasu przechowywania na kwasowość miareczkową i czynną jogurtów naturalnych. Nie stwierdzono istotnego wpływu czasu przechowywania na barwę jogurtów. Wraz z wydłużaniem czasu przechowywania zmniejszała się liczba drobnoustrojów jogurtowych. Minimalną normatywną łączną ilość bakterii kwasu mlekowego odnotowano w dniu zakupu i po tygodniu przechowywania w temperaturze 8°C. Wraz z wydłużaniem czasu przechowywania oraz podwyższaniem temperatury przechowywania następuje istotne pogorszenie smaku i zapachu jogurtu.

**Słowa kluczowe:** jogurt naturalny, temperatura, czas przechowywania, jakość: fizykochemiczna, sensoryczna, mikrobiologiczna

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