

## COMPARATIVE STUDY ON THE POSSIBILITIES OF INCORPORATING OLIVE OIL AND NATURAL FENNEL EXTRACT IN FERMENTED MILKS

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### Abstract

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It is well-known that olive oil consumption is associated with a number of health benefits to humans due to its fatty acid composition. Because of these characteristics, olive oil can be suitably included in dairy products in order to improve their fatty acid composition. The present study investigated the possibilities of incorporating olive oil in the manufacture of fermented milk with a set coagulum, directly in the form of an emulsion or encapsulated (with and without the addition of an antioxidant). A significantly higher content ( $p < 0.05$ ) of unsaturated fatty acids and a lower peroxide value of extracted fat was established in the samples supplemented with  $2 \text{ g} \cdot 10^{-2} \text{ g}^{-1}$  encapsulated oil and an antioxidant in an amount of  $0.03 \text{ cm}^3 \cdot 10^{-1} \text{ dm}^{-3}$ . The sensory profile of the fermented milks containing encapsulated olive oil was better in comparison with the fermented milks with directly added olive oil. At the end of the shelf-life, the chemical indicators active and titratable acidity, and the microbiological characteristics – lactobacilli and streptococci counts – remained comparable to those of the control.

*Key words:* functional dairy products; yoghurt; unsaturated fatty acids; olive oil; encapsulation

### Introduction

Naturally-occurring fats are essential to human nutrition. They perform a number of functions: energy supplying, constructive and regulative, which have a specific physiological significance to the human body (Joshi, 2010). The high calorie factor of fats encourage consumers to choose products with low fat content and traditional products with improved fatty acid profile, containing omega fatty acids.

A number of clinical trials have been carried out that prove the benefits of replacing milk fat with vegetable oils rich in omega fatty acids (Dawczynski et al., 2009, 2010).

Vegetable oils are a source of essential polyunsaturated fatty acids (Hunter, 1989). Linoleic and alpha-linolenic acids cannot be synthesized in animal cells (Belitz et al., 2009), and are therefore identified as an essential ingredient for the human body. It has been shown that the consumption of oils

with high oleic acid content favourably reduces LDL cholesterol and blood pressure (Lopez-Huertaz, 2010).

Furthermore, as a source of essential fatty acids, vegetable oils provide the human body with oil-soluble vitamins, tocopherols, phytosterols, lecithin, pigments and other unsaponifiable components present in trace amounts (Gunstone, 2004).

Olive oil contains large amounts of oleic acid ( $C_{18:1}$ ) ( $50\text{--}83 \text{ g} \cdot 10^{-2} \text{ g}^{-1}$  extracted fat) and polyunsaturated fatty acids ( $7\text{--}16 \text{ g} \cdot 10^{-2} \text{ g}^{-1}$  extracted fat). The favourable unsaturated to saturated fatty acid ratio and the presence of all vitamins and about 100 trace elements needed by humans increase the nutritional and biological value of olive oil (Kiritsakis et al., 1998).

Lipid oxidation of fats found in foods is a technological problem that has a significant effect on the shelf-life and organoleptic properties of products (Min and Lee, 1999).

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Given this fact, possibilities are increasingly sought to limit lipid oxidation in products. Encapsulation of essential oils is one possibility of limiting the contact of oil with oxygen, while the addition of a natural antioxidant further limits the oxidation processes (Bermúdez-Aguirre and Barbosa-Cánovas, 2011).

The aim of this study was to carry out comparative characteristic between the possibilities of incorporating olive oil in the form of emulsion or in encapsulated form during the manufacture of fermented milks, and the influence of the added natural antioxidant – fennel extract.

## Materials and Methods

Fermented milk was prepared with raw whole and skimmed cow's milk in compliance with the requirements of Regulation 853/2004 of the European Commission, and cream made from raw cow's milk under Regulation 853/2004, in the amounts given in Table 1. Starter composition for Bulgarian yoghurt: *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, exceeding  $9.5 \times 10^9 \text{ g}^{-1}$  in an amount of  $20 \text{ cm}^3 \cdot \text{dm}^{-3}$ , manufactured by Lactina LTD.

The emulsion was prepared with glycerol monostearate as emulsifier in an amount of  $1 \text{ g} \cdot \text{dm}^{-3}$  according to Hasenhuettl and Hartel (2008) and commercially available olive oil in an amount given in Table 1.

**Table 1**  
**Experimental variants of different yoghurt samples**

*Sample	Fat content of product, $\text{g} \cdot 10^{-2} \text{ g}^{-1}$	Olive oil amount, $\text{g} \cdot 10^{-2} \text{ g}^{-1}$	Milk fat amount, $\text{g} \cdot 10^{-2} \text{ g}^{-1}$	Antioxidant amount, $\text{cm}^3 \cdot 10^{-1} \text{ dm}^{-3}$
Y	4	0	4	0
YA	4	0	4	0.03
E	4	2	2	0
EA	4	2	2	0.03
C	4	2	2	0
CA	4	2	2	0.03

\*Y – control sample (yoghurt); YA – control sample (yoghurt) with added antioxidant (fennel extract); E – yoghurt with directly added olive oil; EA – yoghurt with directly added olive oil and antioxidant (fennel extract); C – yoghurt with encapsulated olive oil; CA – yoghurt with encapsulated olive oil and antioxidant (fennel extract)

The alginate beads were prepared with: medium viscosity sodium alginate supplied by Sigma-Aldrich. For the purposes of the experiment, a solution with a concentration of  $1.37 \text{ g} \cdot 10^{-2} \text{ cm}^{-3}$  (Kostov et al., 2016) was prepared. The alginate was soaked in appropriate quantity of distilled water for 24 h until complete dissolution, and then was sterilized in a

microwave oven (800 W for 5 min) to ensure microbiological stability of the solution. Olive oil in a concentration of  $30 \text{ cm}^3 \cdot 10^{-2} \text{ cm}^{-3}$  was added to the finished alginate solution and the emulsion was homogenized at 17 150 rpm. The homogenized emulsion was added dropwise to a  $2 \text{ g} \cdot 10^{-2} \text{ cm}^{-3}$   $\text{CaCl}_2$  solution using a peristaltic pump. Afterwards, the gel beads remained for a further 30 min in the gelling solution to achieve the required mechanical stability. The resulting capsules were washed with distilled water and left for 12–24 hours to dry under refrigeration conditions and were then added to the product.

The antioxidant was fennel extract used in an amount of  $0.03 \text{ cm}^3 \cdot 10^{-1} \text{ dm}^{-3}$  with known characteristics (Nenov et al., 2013). The experimental variants are given in Table 1.

### Preparation of fermented milk

The experimental samples were obtained by the classical method for production of fermented milk with set coagulum (Tamime and Robinson, 2007). The antioxidant in the control sample was added during normalization of milk.

The direct addition of olive oil and the addition of olive oil with an antioxidant to the milk were performed prior to pasteurization in the form of emulsion. Emulsions were prepared by mixing the oil phase in the dispersion medium under constant stirring (speed – 15 000 rpm) for 5 min using a IKA® T18 digital Ultra-Turrax laboratory homogenizer at 55–60°C until the emulsifier was completely dissolved (Vlaseva et al., 2014).

The addition of olive oil capsules and olive oil and antioxidant capsules to the milk was performed during inoculation.

### Titrateable acidity

The acidity of the samples was determined by Thorner's method (BNS 1111-80), and presented as a percentage of lactic acid.

### Active acidity

pH of the samples was measured by a pH meter (model MS 2000, Mycrosist, Plovdiv, Bulgaria) with a glass electrode (Sensorex, Garden Grove, USA) standardized at 20°C over the range of 7.0–4.0. Active acidity (pH), potentiometrically measured with a 7110 WTW (Germany) pH-meter.

### Microbiological analysis

Total lactobacilli and streptococci count – sample preparation was conducted according to IDF Standard 122C:1996. The suitable dilutions were inoculated into selective agars M17 and MRS, as described in IDF Standard 117B:1997.

### Peroxide value

Fat extraction for determination of the peroxide value was performed by the method of Bligh and Deyer, 1959. The iodometric method based on the interaction of active peroxides or hydroperoxides with oxygen in the presence of iodide acetic acid (ISO, EN 3960:2007) was used.

Fatty acid composition – The preparation of methyl esters of fatty acids was in accordance with ISO 5509:2000. The analysis of the methyl esters of the fatty acids was carried out by gas chromatography (ISO 5508:2004). Fatty acid composition of the obtained yoghurts was determined after extraction of fat by the method of Schmidt-Bonzynsky-Ratzaloff (ISO 1735 | IDF 5:2004).

Organoleptic assessment was conducted according to BNS 15 612-83. The evaluation criteria included the following indicators: flavour and aroma – 35 points, density and fracture of coagulum – 30 points, texture – 25 points, appearance – 10 points (maximum overall score – 100 points). The results were equated to a 10-point evaluation scale with a view to being presented in a spider diagram.

### Statistical analysis

Computer processing of the results was performed using the program Microsoft Excel 2010 (ANOVA). Multiple comparisons were made by the LSD method. The results are presented as mean value  $\pm$  SD ( $n = 4$ ).

## Results and Discussion

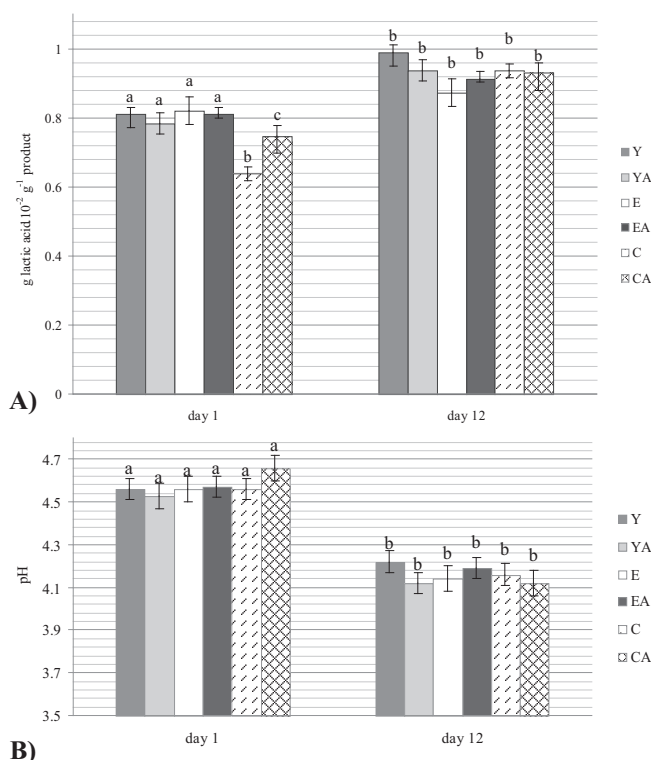
### Chemical indicators of fermented milks

The changes in the indicators – pH and lactic acid amount of the experimental samples, were monitored and determined over the twelve-day storage at 4°C (Figure 1 A, B).

The results show that on the first day of storage the amount of lactic acid in samples Y, YA, E and EA was in the range of approximately  $0.80 \text{ g} \cdot 10^{-2} \text{ g}^{-1}$ , while the active acidity values were in the range between 4.5–4.6. It is noteworthy that in the samples containing encapsulated oil (variant C) and encapsulated oil and antioxidant (variant CA) the amount of lactic acid that was formed was slightly lower, while the reported values for the pH indicator of the same variants were higher. This is probably due to the presence of other substrates (free carboxyl groups of the alginate) which slow down lactose absorption.

The observed trend changed during storage, when the values of lactic acid increased in all experimental samples and reached values of approximately  $1.0 \text{ g} \cdot 10^{-2} \text{ g}^{-1}$  in the control, and approximately  $0.9 \text{ g} \cdot 10^{-2} \text{ g}^{-1}$  in variants YA, E, EA, C and CA. The active acidity of these samples was between 4.1–4.22, respectively.

The nutritional and biological value was determined by the amount and ratio of saturated to unsaturated fatty acids



**Fig. 1. Changes in acidity (A) and pH (B) of different yoghurt samples during 12 days of storage**  
**Y – control sample (yoghurt); YA – control sample (yoghurt) with added antioxidant (fennel extract); E – yoghurt with directly added olive oil; EA – yoghurt with directly added olive oil and antioxidant (fennel extract); C – yoghurt with encapsulated olive oil; CA – yoghurt with encapsulated olive oil and antioxidant (fennel extract).**  
 Different letters in the same row show statistically significant differences ( $p < 0.05$ ) during storage

contents, and the ratio between essential and non-essential fatty acids. The fatty acid composition of the experimental samples is given in Table 2.

The results reveal that the content of short-chain saturated fatty acids – caproic, caprylic and capric – is the highest in samples YA and Y, followed by variants E and C, and the lowest levels are in samples EA and CA. At the end of storage, the contents of palmitic, lauric and stearic fatty acids account for the largest share of the total saturated fatty acids content in all experimental samples. The content of palmitic acid is higher in samples Y, YA and E in comparison with variants EA, C and CA, while lauric acid is present in a greater amount in YA, Y and E compared to samples EA, C and CA.

In the group of unsaturated fatty acids, the oleic acid content is the highest and is approximately twice higher in sam-

**Table 2**  
**Fatty acids profile of different yoghurt samples after 12 days of storage**

Fatty acid	*Y	YA	E	EA	C	CA
Saturated fatty acids, g.10 <sup>-2</sup> g <sup>-1</sup> extracted fat						
Caproic C6:0	2.12±0.17 <sup>a</sup>	2.56±0.27 <sup>a</sup>	1.33±0.15 <sup>b</sup>	1.26±0.10 <sup>b</sup>	1.29±0.12 <sup>b</sup>	1.16±0.14 <sup>b</sup>
Caprylic C8:0	3.41±0.35 <sup>a</sup>	3.52±0.25 <sup>a</sup>	2.35±0.22 <sup>b</sup>	1.46±0.23 <sup>c</sup>	1.85±0.22 <sup>c</sup>	1.58±0.24 <sup>c</sup>
Capric C10:0	0.35±0.19 <sup>a</sup>	0.11±0.05 <sup>a</sup>	0.09±0.02 <sup>a</sup>	0.12±0.02 <sup>b</sup>	0.13±0.01 <sup>b</sup>	0.12±0.01 <sup>b</sup>
Lauric C12:0	11.92±0.25 <sup>a</sup>	12.8±0.25 <sup>a</sup>	8.58±0.22 <sup>c</sup>	6.10±0.36 <sup>d</sup>	5.72±0.25 <sup>d</sup>	4.83±0.24 <sup>c</sup>
Tridecylic C13:0	1.92±0.24 <sup>a</sup>	1.53±0.36 <sup>a</sup>	1.39±0.25 <sup>a</sup>	0.99±0.20 <sup>b</sup>	0.99±0.26 <sup>b</sup>	0.85±0.29 <sup>b</sup>
Myristic C14:0	1.45±0.20 <sup>a</sup>	1.24±0.19 <sup>a</sup>	1.02±0.15 <sup>a</sup>	0.65±0.14 <sup>b</sup>	0.69±0.13 <sup>b</sup>	0.60±0.12 <sup>b</sup>
Palmitic C16:0	35.41±3.45 <sup>a</sup>	36.05±2.21 <sup>a</sup>	28.63±2.23 <sup>b</sup>	24.19±2.25 <sup>b</sup>	21.92±2.00 <sup>b</sup>	20.05±1.95 <sup>c</sup>
Margaric C17:0	0.77±0.04 <sup>a</sup>	0.61±0.05 <sup>a</sup>	0.62±0.05 <sup>a</sup>	0.28±0.05 <sup>b</sup>	0.26±0.06 <sup>b</sup>	0.41±0.05 <sup>b</sup>
Stearic C18:0	7.35±0.89 <sup>a</sup>	6.71±0.75 <sup>a</sup>	5.58±0.45 <sup>b</sup>	4.08±0.45 <sup>c</sup>	4.76±0.35 <sup>c</sup>	3.60±0.36 <sup>d</sup>
Unsaturated fatty acids, g.10 <sup>-2</sup> g <sup>-1</sup> extracted fat						
Myristoleic C14:1	0.32±0.10 <sup>a</sup>	0.22±0.09 <sup>a</sup>	0.23±0.09 <sup>a</sup>	0.13±0.08 <sup>a</sup>	0.13±0.09 <sup>a</sup>	0.12±0.09 <sup>a</sup>
Palmitoleic C16:1	3.55±0.25 <sup>a</sup>	4.30±0.23 <sup>b</sup>	3.43±0.24 <sup>a</sup>	2.69±0.25 <sup>b</sup>	2.61±0.22 <sup>b</sup>	2.85±0.32 <sup>b</sup>
Heptadecenoic C17:1	0.34±0.04 <sup>a</sup>	0.22±0.08 <sup>a</sup>	0.27±0.03 <sup>a</sup>	0.28±0.03 <sup>a</sup>	0.16±0.04 <sup>b</sup>	0.11±0.02 <sup>b</sup>
Oleic C18:1	25.57±1.15 <sup>a</sup>	23.98±1.38 <sup>a</sup>	37.67±1.56 <sup>b</sup>	44.88±1.34 <sup>c</sup>	49.27±1.45 <sup>d</sup>	48.21±1.24 <sup>d</sup>
Essential fatty acids, g.10 <sup>-2</sup> g <sup>-1</sup> extracted fat						
Linoleic C18:2	5.52±1.00 <sup>a</sup>	6.75±1.21 <sup>a</sup>	8.80±1.00 <sup>b</sup>	13.03±1.10 <sup>c</sup>	10.62±1.43 <sup>c</sup>	15.30±1.10 <sup>d</sup>

Y – control sample (yoghurt); YA – control sample (yoghurt) with added antioxidant (fennel extract); E – yoghurt with directly added olive oil; EA – yoghurt with directly added olive oil and antioxidant (fennel extract); C – yoghurt with encapsulated olive oil; CA – yoghurt with encapsulated olive oil and antioxidant (fennel extract); Different letters in the same row show statistically significant differences ( $p < 0.05$ ) between samples

ples C and CA (about 50 g.10<sup>-2</sup>g<sup>-1</sup> extracted fat) compared to variants Y and YA (approximately 25 g.10<sup>-2</sup>g<sup>-1</sup> extracted fat). The total unsaturated fatty acids content is 29.78, 28.72, 41.6, 47.98, 52.17 and 51.29 g.10<sup>-2</sup>g<sup>-1</sup> extracted fat for variants Y, YA, E, EA, C and CA, respectively. The results obtained show that the fermented milk products enriched with olive oil are characterized by a higher content of unsaturated fatty acids compared to the control sample. The highest proportion of polyunsaturated fatty acids was established in the samples containing microencapsulated oil in comparison to the control sample and the samples with emulsified vegetable oil.

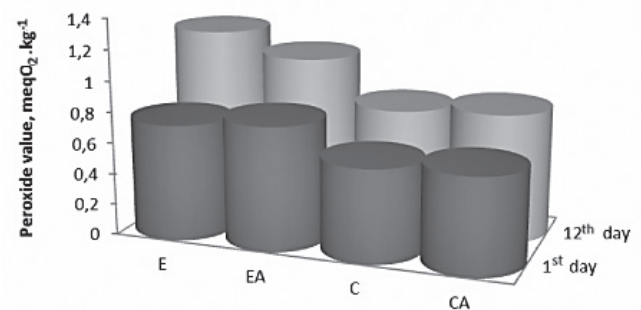
Polyunsaturated long-chain fatty acids – omega-3 and omega-6 fatty acids – are important to human nutrition. The experimental samples of fermented milks are characterized by a significantly high content of essential fatty acids ( $p < 0.05$ ). The data in Table 2 indicate that the amount of linolenic fatty acid is between 13 and 15 g.10<sup>-2</sup>g<sup>-1</sup> extracted fat in samples EA and CA, 8 and 11 g.10<sup>-2</sup>g<sup>-1</sup> extracted fat in samples E and C, and approximately 7 g.10<sup>-2</sup>g<sup>-1</sup> extracted fat in the control sample with and without added antioxidant.

Determination of the peroxide value indicator allows the determination of the degree of fat oxidation in the experimental samples.

Figure 2 shows the results obtained for the peroxide value indicator for samples E, EA, C, and CA determined in

over the period of storage at 4±1°C for 12 days.

The results presented in Figure 2 show that there is a statistically significant difference between the values of the peroxide value indicator obtained on the first and last day of storage. The higher oxidation state established in samples E and EA compared with variants C and CA is due to the fact that encapsulation restricts the contact of

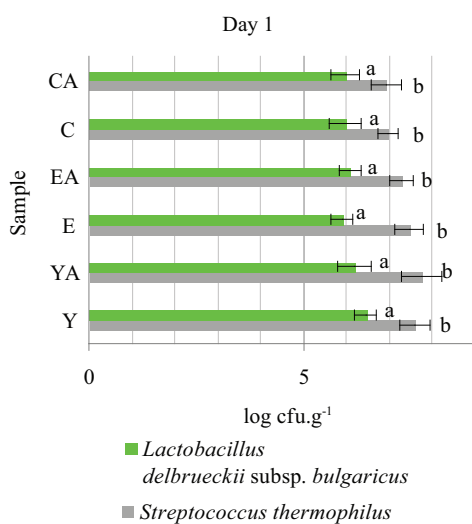
**Fig. 2. Peroxide value changes in different yoghurt samples during 12 days of storage**

E – yoghurt with directly added olive oil; EA – yoghurt with directly added olive oil and antioxidant (fennel extract); C – yoghurt with encapsulated olive oil; CA – yoghurt with encapsulated olive oil and antioxidant (fennel extract)

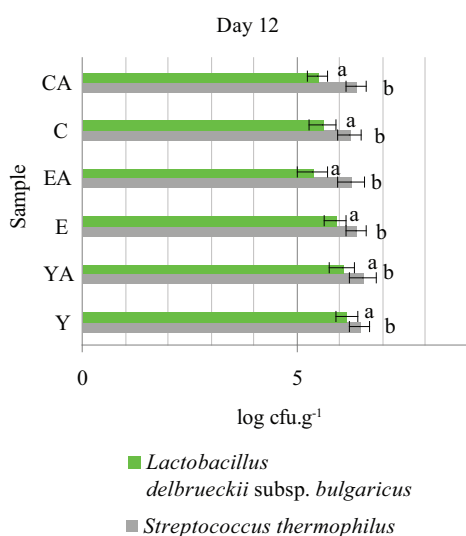
the oil with oxygen and the oxidation process. The data obtained are consistent with the results obtained by Tamjidi et al., 2012.

The results on the change in lactic acid bacteria count – lactobacilli and streptococci in the tested samples are presented in Figure 3.

The data of Figure 3 indicate a higher concentration of lactic acid bacteria in samples Y and YA on the first day of storage as compared with the other samples. The more intensive microbiological and biochemical processes that occur during lactose fermentation in samples Y, YA, E and EA correlate with



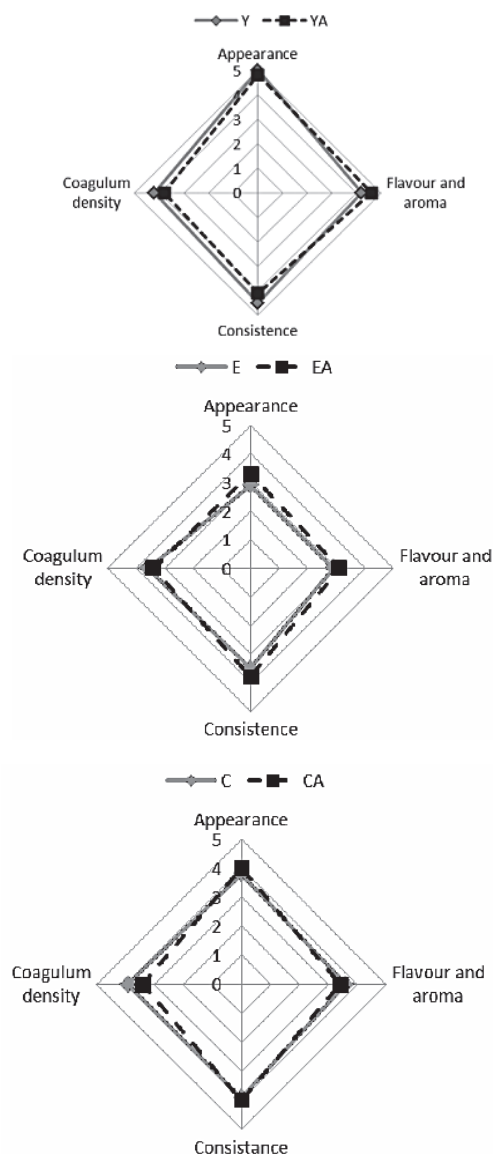
A)



B)

**Fig. 3. Changes in *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* number of different yoghurt samples at 1<sup>st</sup> (A) and 12<sup>th</sup> (B) days of storage**

the established higher levels of the indicators lactic acid and pH (Figure 1 A, B). The longer exponential phase established in the development of streptococci in samples Y and YA could be explained by the medium rate of milk acidification (Figure 1 A, B). A storage temperature below 5°C is unfavourable for the



**Fig. 4. Sensory profile of different yoghurt samples after 12 days of storage**

Y – control sample (yoghurt); YA – control sample (yoghurt) with added antioxidant (fennel extract); E – yoghurt with directly added olive oil; EA – yoghurt with directly added olive oil and antioxidant (fennel extract); C – yoghurt with encapsulated olive oil; CA – yoghurt with encapsulated olive oil and antioxidant (fennel extract)

development of lactic acid bacteria. Under low storage temperature conditions, lactobacilli and streptococci counts of fermented milks decrease. The established reduction is less pronounced in lactobacilli compared with streptococci, which indicates their lower sensitivity to the models developed here.

The results of the organoleptic evaluation of the studied samples, stored for 12 days at 4°C are presented in Figure 4.

The results obtained show that samples Y and YA of fermented milks are characterized by higher organoleptic evaluation in comparison with variants E, EA, C and CA. The variants with encapsulated olive oil C and CA were awarded a higher score by the tasting panel than the variants with emulsified oil E and EA.

## Conclusion

The enrichment of traditional dairy products with unsaturated fatty acids is an innovative approach to providing health ingredients beneficial to humans. The use of micro-encapsulated vegetable oils and natural antioxidants ensures increased stability of the unsaturated fatty acids, prolongation of the product shelf-life and preservation of the organoleptic properties of the product.

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