Journal of Chemical Technology and Metallurgy, 59, 1, 2024, 53-60

APPLICATION OF DILL ESSENTIAL OIL AS ADDITIVE TO BULGARIAN YOGHURT

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Received 14 June 2023
Accepted 17 August 2023
DOI: 10.59957/jctm.v59.i1.2024.6

ABSTRACT

In this study, the influence of the essential oil extracted from dill fruits and fronds on lactic acid bacteria, aroma profile and sensory characteristics of the obtained yoghurt was investigated. The addition of essential oils in amounts of 30 μl kg^{-1} fermented milk affects the pH and aroma profile, which is due to the different compositions of the oils. The obtained yoghurts were sensory well evaluated by the testers during the tasting performed.

Keywords: *yoghurt, essential oil, dill, aroma profile.*

INTRODUCTION

Increasing consumer attention to the health and the role of food in improving the quality of life determine the need to develop new products with improved characteristics that exhibit beneficial effect on human health. Modern consumers are looking for natural products that have a positive impact on the functioning of the body. The focus is on introducing harmless natural additives that improve the quality and nutritional value of traditional products.

Essential oils are multicomponent mixtures containing various compounds, e.g. hydrocarbons and their derivatives, which determine their properties.

In recent years, the interest of the food producers towards essential oil and their application as bio preservatives is also increasing [1, 2].

Dairy products are perishable by nature, and they need to be preserved during their processing, storage and distribution. Some researchers have reviewed the use of essential oils and their preserving effect on dairy products. The addition of essential oils to dairy products (milk and cheese) plays the role of biopreservatives, antioxidants and certain antimicrobials. They can be used to improve their aroma. The fermentation of milk caused by lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* gives a new type and composition of yoghurt [3, 4]. It has been proven that the use of essential oils in lactic acid milk products improves their nutritional, organoleptic, and health-related properties [5, 6]. The essential oils consisting of terpenoids, aldehydes, ketones, esters, alcohols and benzene compounds exert significant effects on the sensory properties of the milk in concentrations of about 1.0 μl L^{-1}. Dill seed and its essential oil contain biologically active ingredients of high nutritional value, and they have antimicrobial and antifungal effects [7].

The extract obtained from dill fronds using 1,1,1,2-tetrafluoroethane as extractant possesses certain antimicrobial properties towards the gram-positive and gram-negative bacteria studied. Beside the antibacterial effect, plant extracts can prevent the spoilage of dairy products [8]. The plant extracts act as internal filters that catch the free radicals and prevent oxidation. These properties are inherent to the flavonoids, as well as quercetin [9].

The aim of this work is to study the effect of essential oil taken from dill fruits and fronds on the lactic acid bacteria, aroma profile and sensory characteristics of the milk products obtained.

EXPERIMENTAL

The dill fruits and fronds used for this study were purchased from a retail shop. The essential oils were obtained by aqueous distillation of the raw material in a laboratory glass distiller of the British pharmacopeia modified by Balinova-Tsvetkova and Dyakov [10]. The distillation duration was 3 h and it was terminated when 2 consecutive samples taken at 30 min period did not show increase of the amount of essential oil.

The gas chromatographic (GC) analysis of the essential oil, carried out at the Agrobio Institute - Sofia, used an Agilent 7890A apparatus with a flame-ionization detector; column HP-INNOWax Polyethylene Glycol (60 x 0.25 mm; film 0.25 μm); temperature conditions: 70°C - 10 min; 70 - 240°C-5°C min^{-1}, 240°C-5 min; 240 - 250°C-10°C min^{-1}, 250°C - 15 min; carrier gas helium, 1 cm^{-3} min^{-1} constant velocity; injector: 250°C, split ratio 50:1. For the mass spectral/gas chromatographic (MS/GC) analysis, an Agilent 5975 C instrument, helium carrier gas, column and temperature conditions as for GC analysis were used; detectors: FID, 280°C, MSD, 280°C transfer line.

The oil from dill fruits has 12 components, 5 of them have concentrations higher than 3 %. The main components (above 3 %) are: carvone (46.89 %), limonene (28.93 %), p-cymene (5.03 %), dihydrocarvone (4.22 %) and dihydrocarveol (3,10 %). In the oil extracted from the fronds, 14 components were identified and 9 of them have concentrations higher than 3 %. The main component (above 3 %) are: carvone (27.81 %), limonene (16.94 %), α-phellandrene (5.87 %), p-cymene (14.18 %), dihydrocarvone (5.78 %) and dihydrocarveol (3.68 %).[11]. The essential oils were stored at temperature of 4°C until the analysis.

The yoghurt was prepared from raw cow milk from the Yambol region. The chemical composition of the raw material was determined by ultrasonic analyzer Lactoscan MCCW-V1 (Milkotronic Lactoscan, Varna, Bulgaria) (Table 1).

Table 1. Chemical composition of raw cow‘s milk..

Composition	Cow milk
Fast, %	3,45
Solids Not Fat (SNF), %	8,65
Density, g./cm^3	$1,029.10^{-3}$
Lactose, %	4,56
Proteins, %	2,84
Solids, %	0,65

The raw milk was subjected to thermal treatment at temperature of 95°C for 15 min, and after that cooled to temperature of 43 ± 1°C. A lyophilized sourdough from the company Laktina Ltd., Bankya, Bulgaria, with a composition of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, was used to ferment the milk. The milk was left to ferment at temperature of 43 ± 1°C until pH reached 4,6 ± 0,1 (260 minutes), then the fermentation was terminated and the product was cooled to 4 ± 1°C. Essential oils from the fruits (Sample 1) and fronds (Sample 2) of dill were added to the yoghurts in concentrations of 30 μl per 1 kg fermented milk. The coagulum was stirred and well homogenized. The yoghurts obtained were stored at 4 ± 1°C and the following properties were determined at certain time interval (1^{st}, 5^{th}, 10^{th} and 15^{th} day): titratable acidity expressed as percentage of lactic acid, aroma profile, microbiologic and sensory properties.

Chemical analyses

Determination of pH

During the fermentation period, the pH of the yoghurt was measured by pH meter (Model MS 2011, Microsyst, Plovdiv, Bulgaria) equipped with electrode (pH electrode Sensorex, Garden Grove, CA, USA).

Titratable acidity (TA)

The titratable acidity (TA) of the samples was determined by the Thörner method and expressed as lactic acid percentage [12].

$$\text{Gres. lact.} = G_{lac.} - G_{degr.lac.} \quad (1)$$

$$\text{Gdegr.lac.} = \frac{[(\Delta T_{beg.} - \Delta T_{cur}) * 0{,}009 * 342]}{360} \quad (2)$$

where: Gres. lac. - residual amount of lactose, %; $G_{lac.}$ - initial amount of lactose %; $G_{degr.lac.}$ - decomposed amount of lactose, %; ΔTbeg. - initial titratable acidity, %; ΔTcur. - current titratable acidity, %.

Aroma profile

The aroma profiles of yoghurt samples obtained were determined on a gas chromatograph with mass spectrometric detector TSQ Quantum XL (Thermo-

Scientific, USA). The chromatograph column was TG-WAX (Thermo-Scientific, USA), length 30 m, inner diameter $2.5.10^{-4}$ m, thickness of the stationary phase 0.20 µm. The temperature regime was from 40°C to 220°C with gradient of 10°C min^{-1}, mobile phase (helium) flow rate was 1.10^{-3} $m.min^{-1}$, amount of 1.10^{-3} L of the vapour phase by automated Head-space device for the thermostatting the vials at 80°C with 2.10^{-3} kg samples. The results were processed with Xcalibur software (Thermo-Scientific, USA).

Determination of the number of lactic acid bacteria

The amount of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* during storage of yoghurts was determined according to BDS EN ISO 7889:2005 [13].

The number of viable lactic acid bacteria after the fermentation process and after storage was carried out by resuspending 10 mg of milk samples in 100 mL of sodium chloride 0.85 % and diluting to the extent of 10^{-5} using 0.85 % sterile NaCl solution. 0.5 mL of diluted samples were used to inoculate by plating on MRS agar for counting *Lactobacillus bulgaricus* colonies at 42°C for 24 hours and M17 agar for counting *Streptococcus thermophilus* at 37°C for 48 hours for streptococci.

The number of colonies grown on the media from three parallel inoculations was counted using a microorganism colony counter according to BDS EN ISO 7889:2005 [13].

The number of cells in a unit volume of starting suspension is calculated according to eq. 3:

$$N = \frac{a.10^n}{V}, CEU.g^{-1} \quad (3)$$

where: N is the amount (number) of cells in 1 ml of the initial suspension; a - arithmetic mean number of colonies when seeded at a certain dilution; 10 - dilution coefficient; V - the amount of seed material (inoculum) in mL; n - the order number of the dilution from which the sowing was carried out.

Sensory evaluation

The sensory evaluation of the yoghurts was performed by calculating the points of a hedonic scale, as well as by a modified method introduced by Nakov [14, 15]. The yoghurts were given estimations from 1 to 9 of their properties: aroma, taste, texture, aftertaste and appearance. The evaluators gave their estimates using a 9-point hedonic scale from 1 - “extremely unacceptable” to 9 - “extremely good”. The sensory analysis was carried out on the first and tenth day after obtaining the yoghurt. The study was carried out according to Resolution No P2-0209/2022 of the Ethic Commission at FTT - Yambol, Trakia University Stara Zagora, which complies with the directives for the ethics and research related to food testing approved by the EU and the participants have given their informed consent for participation [16].

Statistical analysis

The results obtained were processed using Microsoft Excel 2010 (ANOVA). The multiple comparisons were made by the LSD method. The results obtained are presented as average values ± SD (n = 3**).**

RESULTS AND DISCUSSION

pH Change

On the first day, the pH of the control was 4.50, while for sample 1 and sample 2 it was higher - 4.85 and 4.75, respectively, Fig.1. During milk storage, a decrease in active acidity was observed in both control and essential oil enriched milks. On the 15th day of storage, the pH values were: 4.25 for the reference sample, 4.35 and 4.41 for samples 1 and 2, respectively.

Similar decrease of pH values was observed with the addition of essential oils of thyme, black cumin, dill, spearmint and chamomile [17].

The decrease in the active acidity of the obtained milks is probably due to the increased activity of lactic acid bacteria, the formed lactic acid and the presence of organic acids introduced with the essential oils. The obtained results correlate with the results of other scientists [18].

Fig. 2 shows the change of acidity in the yoghurts obtained expressed as lactic acid percent. The results indicate that the amount of lactic acid accumulated during the first day of storage was 0.63 % for the reference sample and for samples 1 and 2. At the end of the experimental period (15 days), the acidity increased to 1 % for sample 1 and 1.1 % for sample 2 while that of the reference sample was 0.83 %. Therefore, the addition of essential oil does not affect the vital activity of lactic acid bacteria.

It is proven that some essential oils have been shown

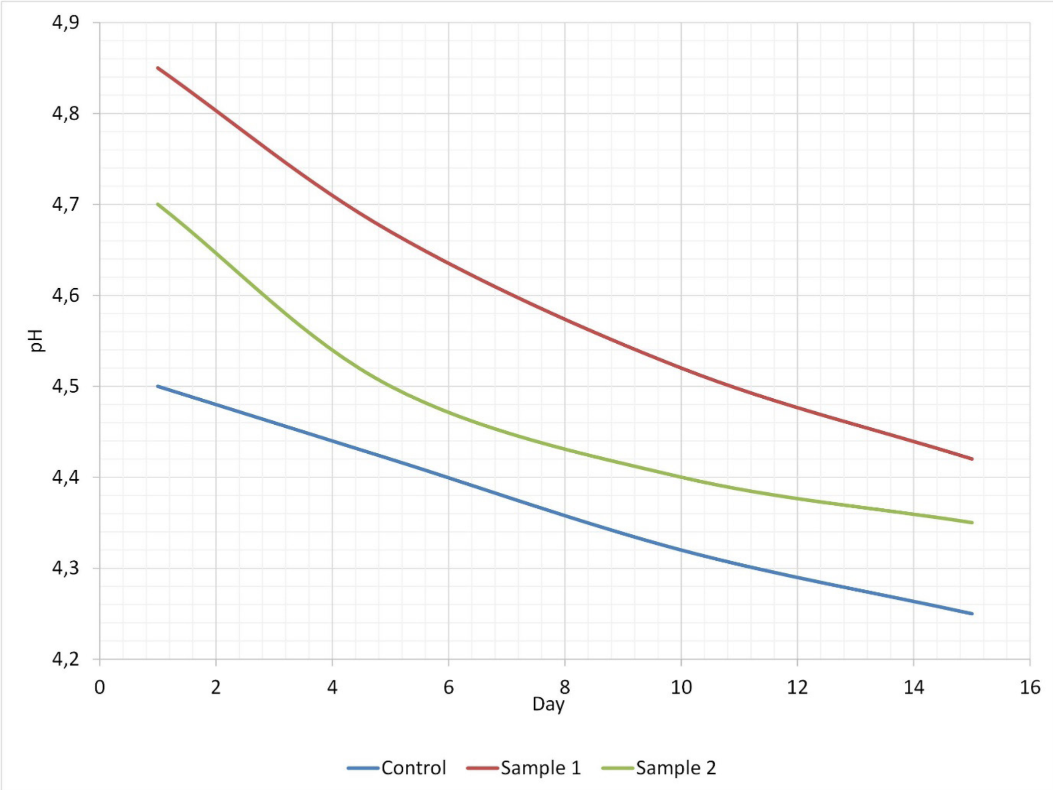

Fig.1. pH values during milk storage.

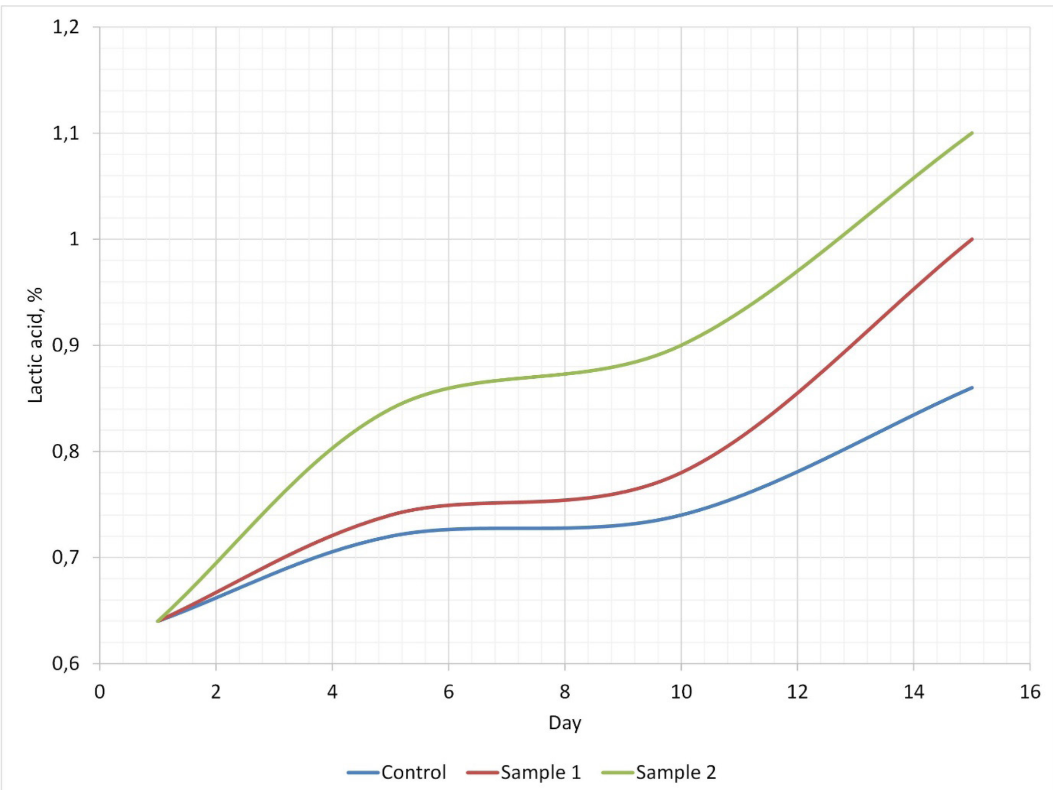

Fig.2. Changes in lactic acid values during storage.

to possess antibacterial and antimicrobial compounds that determine the ability of the oils to fight pathogens without affecting the growth of beneficial lactic acid bacteria [19].

Aroma profile

The aroma profiles of the yoghurts obtained were determined and the results are shown in Figs. 3, 4 and 5.

For the reference sample, Fig.3, the main volatile components according to the retention times were as follows (%): acetaldehyde (2.20), acetone (2.63), 2-butanone (3.19), ethanol (3.46), diacetylene (3.92), acetone (8.11), acetic acid (10.28), propionic acid (11.17), lactic acid (12.45).

For the Bulgarian yoghurt with essential oil added from dill fruit sample 1, Fig.4, the main volatile

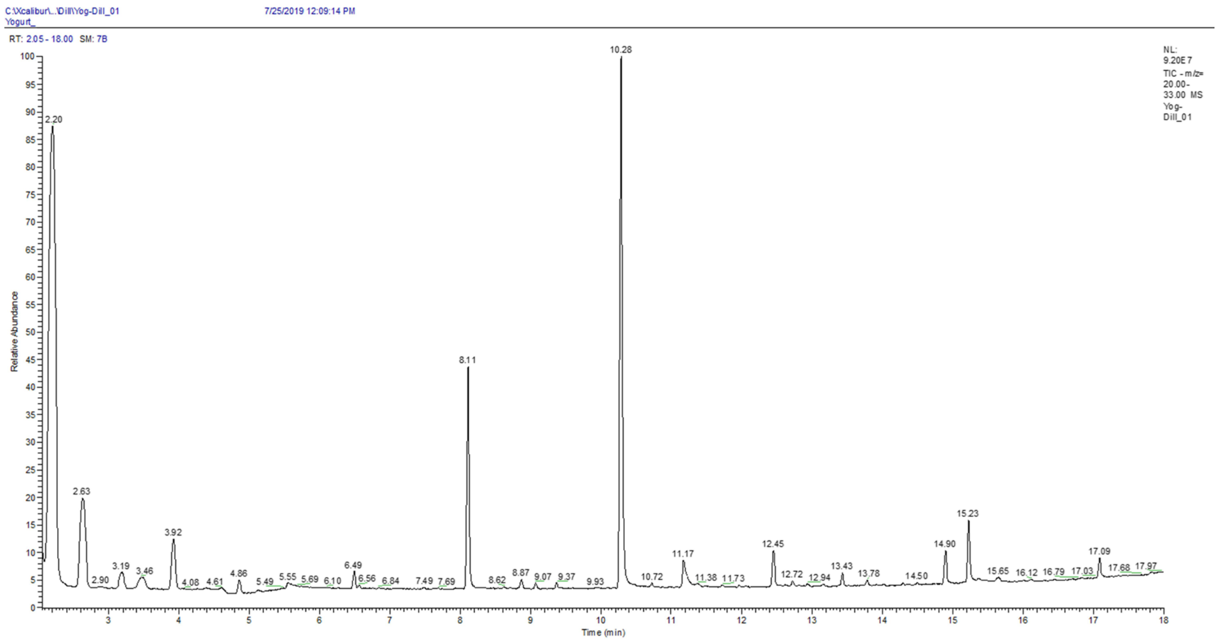

Fig.3. Bulgarian yoghurt chromatogram.

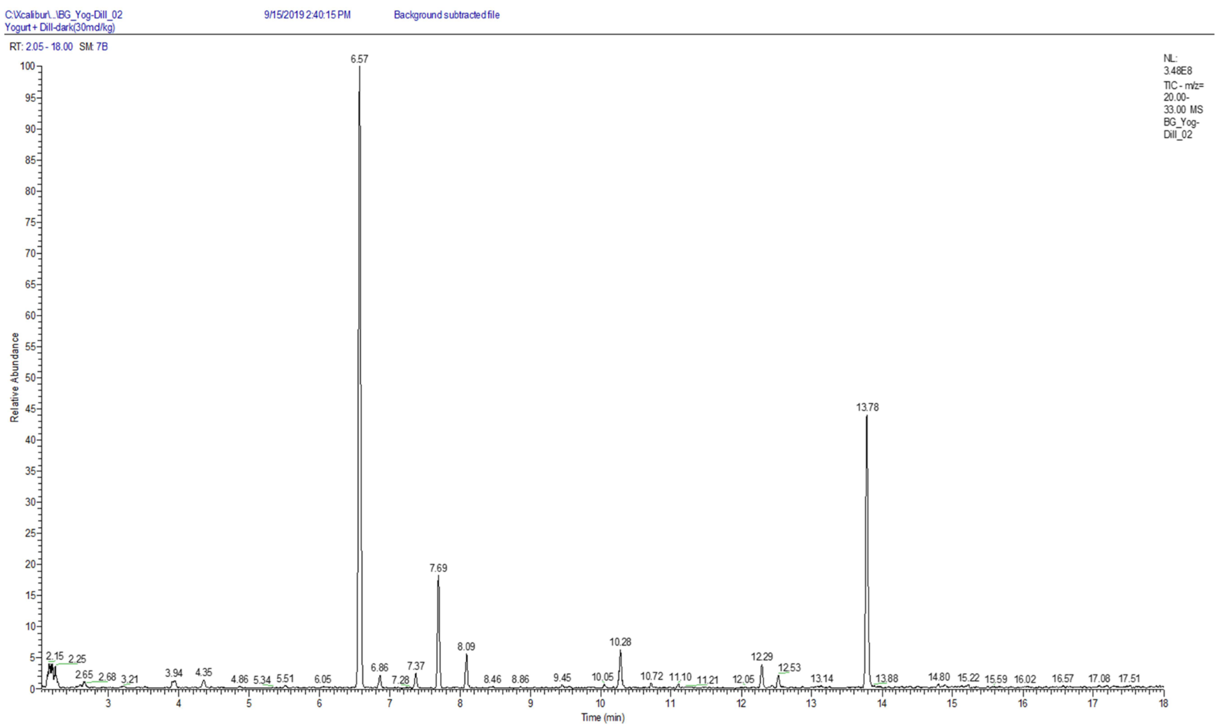

Fig. 4. Bulgarian yoghurt chromatogram with addition of dill fruit essential oil.

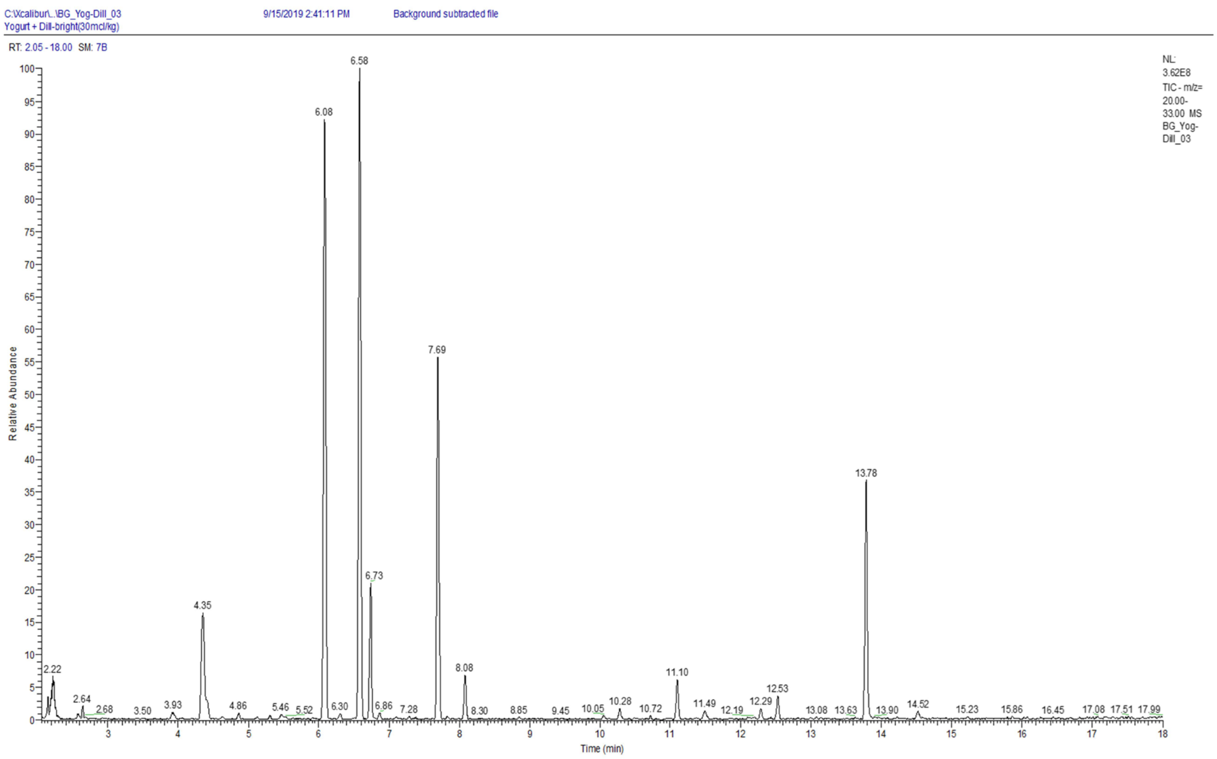

Fig.5. Bulgarian yoghurt chromatogram with addition of dill frond essential oil.

Table 2. Total number of *L. bulgaricus* and *Str.thermophilus* in yogurt during storage.

Sample	*L .bulgaricus*, CFU x10^{7}				*Str. thermophilus*, CFU x10^{8}			
	1 day	5 day	10 day	15day	1 day	5 day	10 day	15 day
Control	2.1±0.05	2.3±0.05	2.6±0.05	2.8±0.05	1.4±0.05	1.5±0.05	1.6±0.05	1.7±0.08
Sample 1	2.1±0.08	2.2±0.05	2.5±0.05	2.7±0.08	1.4±0.05	1.4±0.05	1.5±0.05	1.6±0.05
Sample 2	2.1±0.05	2.3±0.05	2.6±0.05	2.7±0.05	1.5±0.05	1.5±0.05	1.6±0.05	1.7±0.05

according to the retention times were as follows (%): D-limonene (6.57), m-cymene (7.69), acetone (8.09), acetic acid (10.28), trans-p-ment-8-en-2-on (12.29), ment-8-en-2-on (12.53), carvone (13.78).

In the Bulgarian yoghurt with added essential oil from dill fronds sample 2, Fig.5, the main volatile components according to the retention times were (%): α-pinene (4.35), α-phellandrene (6.08), D-limonene (6.58), terpinene (6.73), m-cymene (7.67), acetone (8.08), dimethyl-hexahydro-benzofuran (11.10), trans-p-ment-8-en-2-on (12.29), p-ment-8-en-2-on (12.53), carvone (13.78). The difference in the composition of the volatile fractions can be explained with the influence of the yoghurt main mass and its low pH.

Determination of the number of lactic acid bacteria

The influence of the essential oils added to the yoghurt on the lactic acid bacteria was studied by determining the bacterial number on the 1st, 5th, 10th and 15th day of the products' storage at 4^{o}C. The results obtained are presented in Table 2.

The results show that the number of *L. bulgaricus*, on the first day of storage, was the same for all samples and gradually increased, until the 15th day and reached 2.8 CFU x10^{7} for the reference sample and 2.7 CFU x10^{7} for the other samples.

These values suggest good growth of the starting lactic acid bacteria during the storage period. The addition of essential oil from dill fruits and fronds did not have statistically significant effect on the decrease of the number of *L. bulgaricu*s and *Streptococcus thermophiles*. In the yoghurts obtained, the number of viable strains of lactic acid bacteria is high enough to consider them probiotic [19]. Similar results for the increase number of lactic acid bacteria and the acidity were obtained by other researchers, too, when adding essential oils to yoghurt and cheese during their production [20].

Sensory evaluation

Fig. 6 and Fig. 7 present the results obtained from the sensory evaluation of the yoghurt samples on the 1st and 15th days of storage at a temperature 1 ± 4°C. The results registered on the first day of storage showed a higher organoleptic value for the samples. The reference sample (yogurt without essential oil) was given a higher estimation. The probable reason for this is that yogurts enriched with biologically active substances are not very popular among consumers [21]. A change in taste and aroma was observed for sample 1, where the testers did not accept the strong smell and the specific taste of this kind of yogurt. The addition of essential oil from dill fruits and fronds had the strongest effect on taste and aftertaste, where the values given for sample 1 were lower.

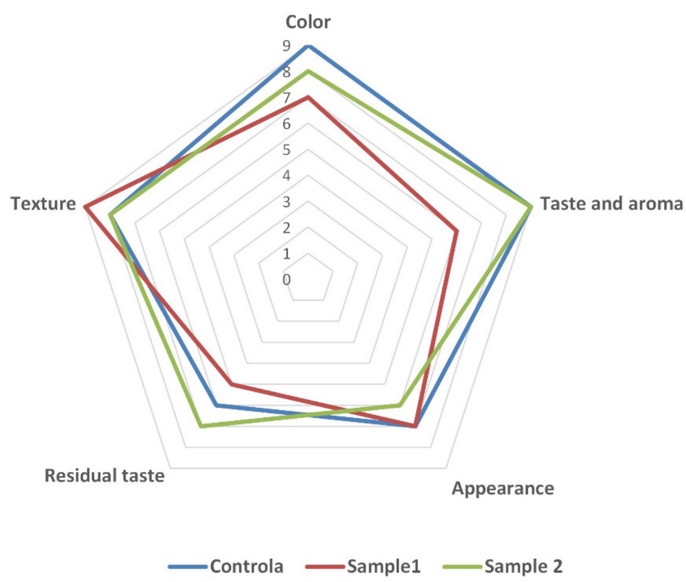

Fig. 6. Sensory evaluation of samples on day 1 of storage.

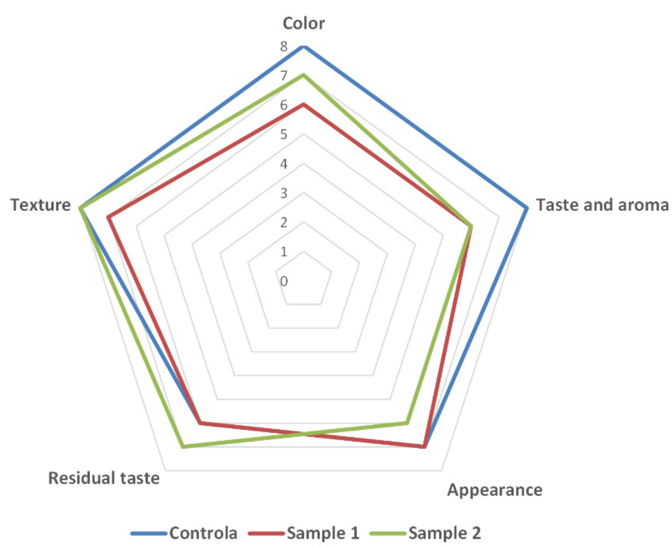

Fig.7. Sensory evaluation of samples on day 15 of storage.

CONCLUSIONS

Adding essential oils extracted from dill fruits and fronds into Bulgarian yoghurt in the concentrations mentioned above did not affect the number of live bacterial cells of *L. bulgaricus* and *Str. thermophilus,* and the smell of dill is sufficient, pleasant, and unobtrusive. The testers gave a comparatively good evaluation of the yoghurts with 30 μL кg^{-1} essential oil from dill fruits and fronds.

Acknowledgements

The work is supported by scientific project 3.FTT/2021 "Ultrasonic extraction of dill (Anethum graveolens L.) and white oregano (Origanum vulgare L.)"

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