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MAKING YOGHURT FLAVOURED WITH ESSENTIAL OILS

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ABSTRACT

Essential oils are gaining interest from the academic and industrial communities as they have been associated with a possible antimicrobial activity against a wide range of microorganisms. Not many studies are known about the effect of essential oils on useful bacteria in fermentative processes. The present study investigated the possibility of flavouring yoghurt with essential oils. Five oils (mint, clove, cinnamon, sage, orange) extracted by hydrodistillation in a Clevenger-type equipment were tested. Each oil was tested in three concentrations (0.01%, 0.05% and 0.1%). The coagulation capacity of the milk was monitored by measuring the pH at regular intervals (1 hour) and it was found that at no oil concentration was the fermentative capacity of the lactic acid bacteria used in this study inhibited. Sensory analysis led to the conclusion that yoghurt flavoured with orange oil scored highest.

INTRODUCTION

Essential oils are a diverse group of natural products that are important sources of chemicals and flavourings in food, industrial and pharmaceutical products. They are largely composed of terpenes and aromatic polypropanoid compounds derived from the acetate-mevalonic acid and shikimic acid pathways respectively. The composition of plant essential oils varies and is due to genetic and environmental factors influencing gene expression (Ze-Hua Li et al. 2019). Yoghurt, also called trapped milk or curd, is a milk product resulting from the lactic fermentation of milk with the help of lactic acid bacteria (*Lactobacillus spp., Streptococcus spp*.). It has a creamy texture and a slightly sour taste due to the conversion of lactose into lactic acid. It is preferred by different consumer groups because of its creamy feel and texture, which make it an attractive dairy product. Starting from the classic yoghurt, many yoghurt varieties have been developed over time in which optional ingredients have been included such as: different strains of probiotic microorganisms (Abdelazez et al. 2017, Akan et al. 2022, Atallah 2016, Dimitrellou et al. 2019, Douglas et al. 2022, Ekinci et al. 2008, Khademi et al. 2022, Khalili et al. 2020, Saleena et al. 2022, Sarwar et al. 2019, Warmi et al. 2003), vitamins (Sobczak et al. 2022) grains (Soltani et.al. 2017), seeds, fiber (Issar et al. 2017), fruits (Abou El-Ez et al. 2017, Al-Dhabi et al. 2022, Buchilina & Aryana 2022; Melia et al. 2022, Melia et al. 2022) algae (Shazly et al. 2022), walnut (Baba et al. 2018), honey (Rotar et al. 2015), essential oils (Mishra et al. 2020, Douglas et al. 2017).

In the current study, we tried to develop a special yoghurt that is naturally flavoured using different nanoemulsions of essential oils and functionally using lactic acid bacteria.

MATERIAL AND METHODS

Pasteurized milk with 1.8% fat, which was seeded with freeze-dried lactic acid bacteria, was used to obtain flavored yogurt. Immediately after the addition of lactic acid bacteria, essential oil was added in three concentrations: 0.01%; 0.05%, 0.1% respectively. This resulted in 15 experimental variants which were followed by comparison with the control variant (without oil): M = no oil; $V1_1$ = 0.01% clove oil; $V1_2$ = 0.05% clove oil; $V1_3$ = 0.1% clove oil; $V2_1$ = 0.01% peppermint oil; $V2_2$ = 0.05% peppermint oil; $V2_3$ = 0.1% peppermint oil; $V3_1$ = 0.01% cinnamon oil; $V3_2$ = 0.05% cinnamon oil; $V3_3$ = 0.1% cinnamon oil; $V4_1$ = 0.01% sage oil; $V4_2$ = 0,05 % sage oil; $V4_3$ = 0,1 % sage oil; $V5_1$ = 0,01 % orange oil;$V5_2$ = 0,05 % orange oil;$V5_3$ = 0,1 % orange oil. Thermostating was carried out at 40ºC for 4 hours. At 1-hour intervals, notes were made on the ability of the bacteria to coagulate the milk: pH evolution, assessment of the degree of coagulation and characteristics of the coagulum formed. After completion of the thermostatting, the obtained yoghurt was kept under refrigerated conditions and observations were made for 21 days.

Statistical Analysis

All tests and analyses were performed in triplicate, with the mean values for each sample used for statistical analysis. To summarize the variability in the datasets, standard deviation was used using Microsoft 365 Excel and the data are presented as means ± SD.

RESULTS AND DISCUSSIONS

pH evolution

The pH value of the raw milk was recorded at 1, 2, 3, 4 hours of thermostatting at 400C and after 12 hours of refrigeration for all experimental variants. The results obtained are shown in Figures 1-5.

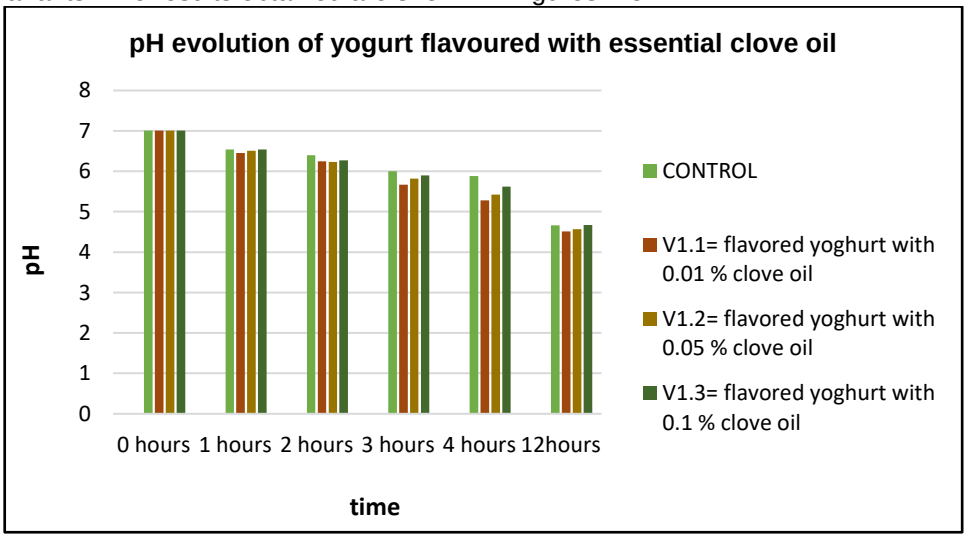

Figure1. pH evolution of experimental V1 (yoghurt flavoured with clove oil)

The Polish median test

Variant	0 hours	1 hours	2 hours	3 hours	4 hours	12hours	*Effect of refrigeration time*
CONTROL	-0.093	-0.071	0.022	0.0467	0.266	-0.027	0.093
V1.1= flavored yoghurt with 0.01 % clove oil	0.077	0.009	0.043	-0.112	-0.1626	-0.007	-0.077
V1.2= flavored yoghurt with 0.05 % clove oil	0.031	0.023	-0.022	-0.008	-0.0685	0.007	-0.031
V1.3= flavored yoghurt with 0.1 % clove oil	-0.031	-0.009	-0.045	0.0085	0.068	0.044	0.031
The effect of incorporating essential clove oil into yoghurt	0.955	0.462	0.229	-0.195	-0.535	-1.460	6.05

*The effects of refrigeration time are in the right column. The effects of incorporating essential clove oil into yoghurt are in the bottom row. The global effect =6.05 is shown at the extreme end of the last row.

From the graphical representation it can be seen that the experimental variant with the addition of 0.01% clove oil recorded the lowest pH value, close to the standard value for yoghurt, while the control and the experimental variant with the addition of 0.1% oil recorded the highest pH values at the end of thermostatting. The pH decrease in the experimental variant $V1_1$ (0.01% clove oil) was constant throughout the observation period. Observations made on the appearance of the coagulum showed as the best the same experimental variant, $V1_1$, in which the coagulum was compact, without whey separation, compared to the other two experimental variants and the control in which whey separation (control) and broken coagulum ($V1_3$) were observed. From the recorded data, it can be concluded that clove oil in concentrations higher than 0.01% can produce changes on the activity of *Lactobacillus* bacteria used in yoghurt production.

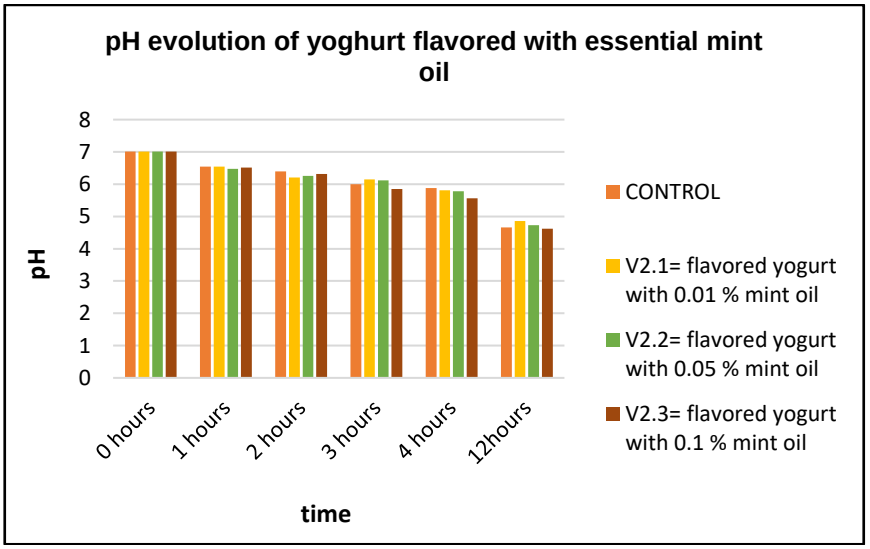

Figure 2. pH evolution of experimental variant V2 (yoghurt flavoured with mint oil)

The Polish median test

Variant	0 hours	1 hours	2 hours	3 hours	4 hours	12hours	*Effect of refrigeration time*
CONTROL	-0.005	0.004	0.075	-0.065	0.084	-0.049	0.0003
V2.1= flavored yogurt with 0.01 % mint oil	-0.014	-0.004	-0.123	0.075	0.005	0.141	0.009
V2.2= flavored yogurt with 0.05 % mint oil	0.005	-0.055	-0.054	0.065	-0.005	0.030	-0.009
V2.3= flavored yogurt with 0.1 % mint oil	0.054	0.033	0.054	-0.1559	-0.176	-0.030	-0.058
The effect of incorporating essential mint oil into yoghurt	0.827	0.3481	0.137	-0.122	-0.391	-1.477	6.186

*The effects of refrigeration time are in the right column. The effects of incorporating essential mint oil into yoghurt are in the bottom row. The global effect =6.186 is shown at the extreme end of the last row.

When using mint oil it was found that the best results were recorded in variant $V2_3$ (0.1% mint oil). The decrease in pH value for this variant was recorded after 3 hours of thermostating and at the end of the recordings. From the analysis of the recorded data regarding the antimicrobial activity of peppermint oil, it can be seen that it does not influence the Lactobacillus bacteria at all, so it can be used for flavouring yoghurt or other fermented products with the help of lactic acid bacteria. Analysis of the coagulum showed that in the case of variant $V2_3$ there was no whey separation, the coagulum was compact, as in the other two experimental variants in which mint oil was used. Therefore, we find that peppermint essential oil can be successfully used for yoghurt flavouring, the optimal concentration being determined only by sensory analysis.

Cinnamon oil in the concentrations used did not inhibit the activity of the lactic acid bacteria used to make the yoghurt, noting as in the case of peppermint oil that at the concentration of 0.1% the greatest decrease in pH occurs. The appearance of the coagulum formed shows that it has the characteristics described by the standard in force, it is compact and does not separate from the whey.

Sage, unlike mint and cinnamon, gave the best results when used in the lowest concentration (0.01%) - Fig.4. As the oil concentration increased, the activity of the bacteria was slowed down, and higher pH values were finally observed. However, there were no major differences between the experimental variants in terms of coagulation appearance and whey separation, with all variants to which sage oil was added showing good results (compact coagulation, no whey separation).

Comparative analysis of the five oils tested at 0.01% concentration showed that the best pH evolution was recorded in V4 (sage oil), while peppermint oil at the same concentration recorded the highest pH value. The control, V1 (clove oil), V3 (cinnamon oil) and V5 (orange oil) recorded very close pH values, which proves that this oil concentration cannot affect the activity of lactic acid bacteria responsible for milk fermentation. Increasing the oil concentration up to 0.05% did not influence the activity of the bacteria very much, finding that the lowest pH value is recorded in V1 (clove oil), very close to V4 (sage oil).

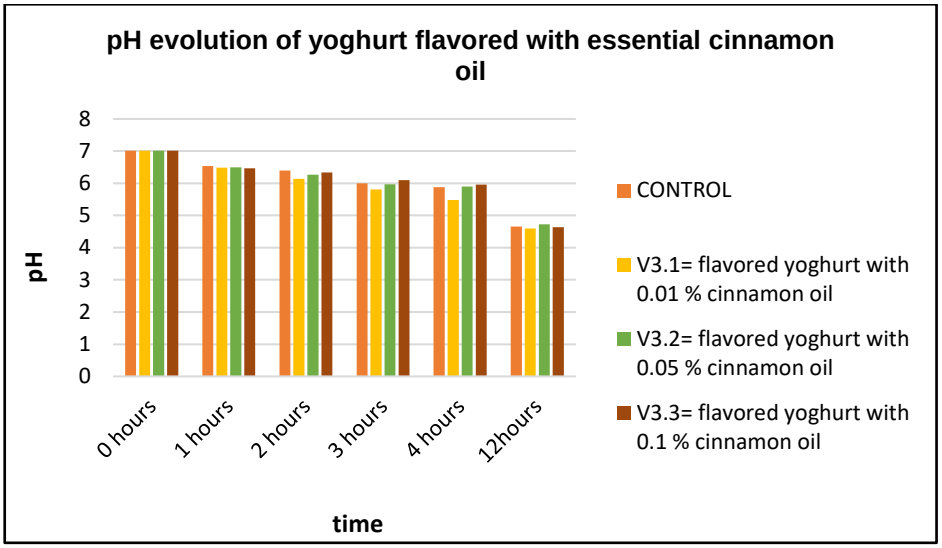

Figure 3. pH evolution of experimental variant V3 (yoghurt flavoured with cinnamon oil)

The Polish median test

Variant	0 hours	1 hours	2 hours	3 hours	4 hours	12hours	*Effect of refrigeration time*
CONTROL	-0.007	0.012	0.093	0.007	-0.017	-0.037	0.004
V3.1= flavored yoghurt with 0.01 % cinnamon oil	0.127	0.097	-0.031	-0.047	-0.282	0.037	-0.130
V3.2= flavored yoghurt with 0.05 % cinnamon oil	0.007	-0.012	-0.021	-0.007	0.017	0.047	-0.010
V3.3= flavored yoghurt with 0.1 % cinnamon oil	-0.019	-0.069	0.021	0.095	0.0509	-0.069	0.0164
The effect of incorporating essential cinamon oil into yoghurt	0.850	0.360	0.140	-0.174	-0.269	-1.469	6.161

*The effects of refrigeration time are in the right column. The effects of incorporating essential cinamon oil into yoghurt are in the bottom row. The global effect =6.161 is shown at the extreme end of the last row.

The orange peel oil showed the most uniform action on the activity of Lactobacillus lactic acid bacteria. The pH values recorded throughout the experiment were very similar for the three oil concentrations, finding that at the end both the control and the experimental variants recorded almost the same pH value - Fig. 5.

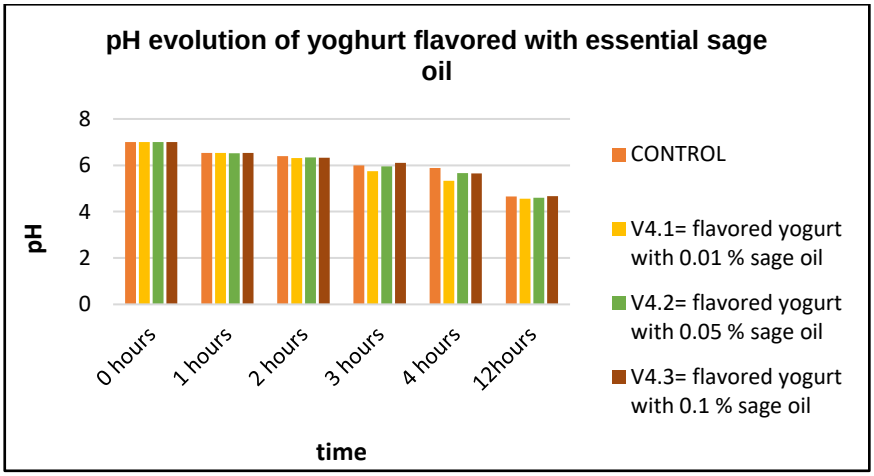

Figure 4. pH evolution of experimental variant V4 (yoghurt flavoured with sage oil)

The Polish median test

Variant	0 hours	1 hours	2 hours	3 hours	4 hours	12 hours	*Effect of refrigeration time*
CONTROL	-0.039	-0.029	0.005	0.0007	0.185	0.003	0.024
V4.1= flavored yogurt with 0.01 % sage oil	0.052	0.052	0.008	-0.156	-0.272	-0.003	-0.068
V4.2= flavored yogurt with 0.05 % sage oil	0.008	-0.001	-0.005	-0.0007	0.013	-0.007	-0.0242
V4.3= flavored yogurt with 0.1 % sage oil	-0.008	0.001	-0.032	0.1320	-0.0135	0.045	-0.0070
The effect of incorporating essential sage oil into yoghurt	0.843	0.363	0.187	-0.207	-0.511	-1.550	6.182

*The effects of refrigeration time are in the right column. The effects of incorporating essential sage oil into yoghurt are in the bottom row. The global effect =6.182 is shown at the extreme end of the last row.

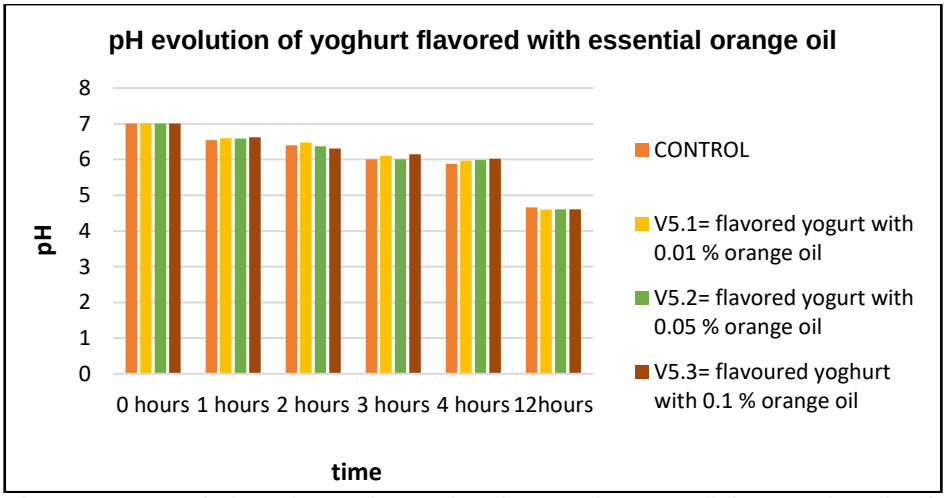

Figure 5. pH evolution of experimental variant V5 (orange oil flavoured yoghurt)

The Polish median test

Variant	0 hours	1 hours	2 hours	3 hours	4 hours	12 hours	*Effect of refrigeration time*
CONTROL	0,028	-0,026	0,028	-0,034	-0,071	0,083	-0,020
V5.1= flavored yogurt with 0.01 % orange oil	-0,002	0,002	0,076	0,034	-0,012	-0,007	0,010
V5.2= flavored yogurt with 0.05 % orange oil	0,002	-0,002	-0,028	-0,060	0,012	0,007	0,005
V5.3= flavoured yoghurt with 0.1 % orange oil	-0,012	0,012	-0,103	0,074	0,027	-0,007	0,020
The effect of incorporating essential orange oil into yoghurt	0,787	0,372	0,178	-0,159	-0,242	-1,617	6,214

*The effects of refrigeration time are in the right column. The effects of incorporating essential orange oil into yoghurt are in the bottom row. The global effect =6.214 is shown at the extreme end of the last row.

Compared to the pH values recorded at 0.01%, these were higher. When used at 0.1%, the tested oils did not influence the activity of Lactobacillus bulgaricus bacteria, with the final pH values being the closest between the variants. The lowest pH value was recorded for V5 (orange oil).

A comparative analysis of the five oils tested at 0.01% concentration showed that the best pH evolution was recorded in V4 (sage oil), while peppermint oil at the same concentration recorded the highest pH value. The control, V1 (clove oil), V3 (cinnamon oil) and V5 (orange oil) recorded very similar pH values, which proves that this oil concentration cannot affect the activity of lactic acid bacteria responsible for milk fermentation.

Increasing the oil concentration up to 0.05% did not influence the activity of the bacteria very much, finding that the lowest pH value is recorded in V1 (clove oil), very close to V4 (sage oil). Compared to the pH values recorded at 0.01%, these were higher. When used at 0.1%, the tested oils did not influence the activity of Lactobacillus bulgaricus bacteria, with the final pH values being the closest between the variants. The lowest pH value was recorded for V5 (orange oil).

Sensory analysis of the yoghurts obtained

Sensory evaluation of the yoghurts obtained was carried out with volunteers Each volunteer individually completed an organoleptic tasting sheet. The evaluation was carried out using a 10-point hedonic scale, with 1 meaning "I don't like it at all" and 10 meaning "I totally like it". Attributes considered were: external appearance, consistency, colour, odors and taste of the flavoured yoghurt and general acceptance. Volunteers who carried out the tasting did not receive any information about the samples evaluated as they were coded with numbers. Volunteers were also asked to rinse their mouths with distilled water before each sample tasted. The results are shown in Tab.1 and Fig.6.

Table 1

Sensory analysis of yogurts flavored with essential oils

Variant	External appearance	Consistency	Color	Odors	Taste	General acceptance
$V1_1$	7	4,5	7,5	5,5	7,5	7,8
$V1_2$	6,3	6	6	5,5	5,5	5,5
$V1_3$	5,2	5	7	5,5	4	4,5
$V2_1$	9,9	8	8,5	9,5	7	8,8
$V2_2$	9,5	8,5	10	10	8,5	9,4
$V2_3$	8,2	6,5	5,5	7,5	6	5,8
$V3_1$	8,2	9,5	8	6	7,5	8,5
$V3_2$	7,2	7,5	6,5	5	7,5	9
$V3_3$	8	9	8,5	5,5	9,5	9
$V4_1$	6,4	8,5	8,5	8,5	6	6
$V4_2$	5,9	7,5	6,5	7,5	5,5	5,7
$V4_3$	4,9	7	7	6,5	4,5	5,3
$V5_1$	9,7	9	9	10	9	9
$V5_2$	9,4	9	10	10	10	10
$V5_3$	9,1	8,5	9,5	10	10	9,5

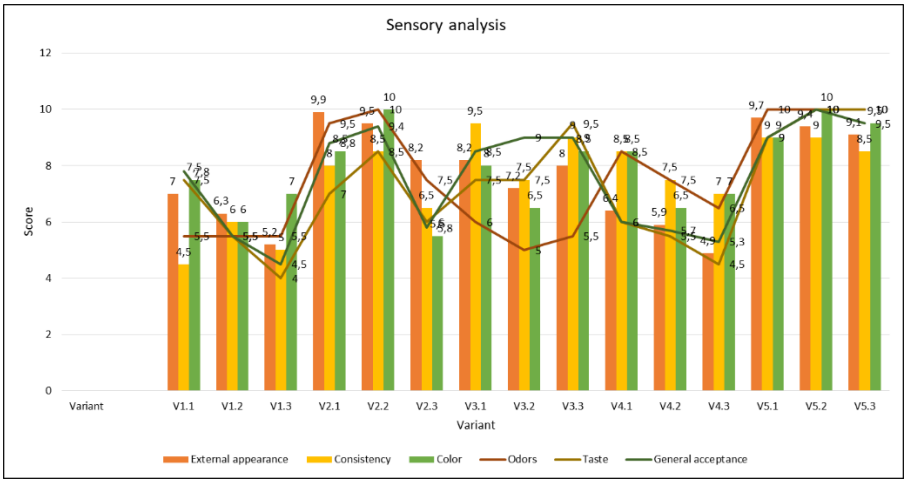

Figure 6. Senzory analysis of yogurts flavored with essential oils

CONCLUSIONS

Fermented dairy products are characterised by various nutritional and biological benefits that lead to the maintenance and promotion of human health.

Clove, peppermint, cinnamon, sage and orange essential oils do not show significant antimicrobial activity on Lactobacillus bulgaricus lactic acid bacteria unless in very high amounts.

The study compared the five oils and found that they do not influence the fermentative activity of Lactobacillus bacteria and can therefore be used to flavour yoghurt or other products fermented with lactic acid bacteria.

Fermentative activity followed by coagulation time and pH evolution showed that the best results were obtained at lower oil concentrations, except for mint, where the lowest pH value was recorded at the highest oil concentration.

The physico-chemical characteristics of yoghurt flavoured with essential oils were not modified compared to the control.

Sensory evaluation showed that orange oil-flavoured yoghurt obtained the highest score, followed by mint oil-flavoured yoghurt.

The current study would open a promising horizon for obtaining naturally flavoured dairy products based on essential oil nanoemulsions.

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