

Effect of Essential Oil of *Mentha piperita* and *Ziziphora clinopodioides* on *Lactobacillus acidophilus* Activity as Bioyogurt Starter Culture

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Abstract: Due to flavoring and antioxidant characteristics of herbs and essential oils in addition to their vital role in preservation of foods, applying these antimicrobial compounds has met a growing interest in food industrial and related sciences. In addition, probiotic products highly affect the health of consumers by reducing risk of heart attacks and improving micro flora in intestinal tract. This study was designed to evaluate the effect of *Mentha piperita* and *Ziziphora clinopodioides* on growth of *Lactobacillus acidophilus* as yoghurt biostarter culture. Probiotic yoghurts produced in the standard procedure and essential oil of *Mentha piperita* and *Ziziphora clinopodioides* were added to inoculated milk in different concentration (0, 25, 40, 70, 100, 130 µg/L). All treatments were incubated at 42°C until medium acidity (amount of Lactic acid) reached to 0.6 %. Later than they were refrigerated at 4°C. Viability of *Lactobacillus acidophilus* during the storage of bio yoghurt at 4°C at different time intervals was investigated. The results indicated that the number of starter culture significantly decreased (P<0.05) after 7 days storage. Also there was no significant difference in viability of *Lactobacillus acidophilus* among samples contained various concentration of essential oil of *Mentha piperita*, *Ziziphora clinopodioides* and control (P< 0.05).

Key words: *Mentha piperita* % *Ziziphora clinopodioides* % Essential oil % Probiotic % Bio yoghurt % *Lactobacillus acidophilus*

INTRODUCTION

Of a long ago essential oils have been considered as "natural preservatives". Also it can be used as an alternative method to control pathogens activities. [1]. Applying natural antimicrobial compounds, such as herbs and spices extracts to preservative foods against microbial reorders due to their special characteristics such as flavoring effects and even sometimes antioxidant consequences has been considered increasingly [2]. Different concentrations of some essential oils affect the activity of starter culture bacteria in yoghurt variously, which has been studied by Boyoumi [3] and Hadad Khoodaparast *et al.* [4]. Among the Gram-positive bacteria, lactic acid bacteria are most resistant species to antimicrobials compounds [5].

Mentha piperita and *Ziziphora clinopodioides* belong to the *Lamiaceae* family. They are widespread all over Iran [6]. For about hundred

years *Lamiaceae* family have been used as flavoring agent or spice in different foods and also has been applied as a traditional medicine for treatment of digestive and viral diseases but unfortunately a few studies have been done on the possible interactions between these compounds and yogurt starter culture. The purpose of this work was to investigate the effects of essential oil of *Mentha piperita* and *Ziziphora clinopodioides* on yoghurt bio starter culture during storage.

MATERIALS AND METHODS

Yoghurt Production: For yoghurt production, milk was obtained from Pegah Dairy Plant (2.5% fat). *Lactobacillus acidophilus* as starter culture (LA-5) was supplied by Chr. Hansen, Denmark. The essential oil of *Mentha piperita* and *Ziziphora clinopodioides* were purchased from Tak Osareh Company (Mashhad, Iran).

Milk was pasteurized at 85°C for 5 min, then cooled to 42°C and inoculated with activated starter culture [7]. At this stage, essential oil of *Mentha piperita* and *Ziziphora clinopodioides* were added to inoculated milk in different concentration (0, 25, 40, 70, 100, 130 µg/L). All treatments were incubated at 42°C until medium acidity reached to 0.6% (amount of Lactic acid). Afterward they were refrigerated at 4°C.

Bacteriological Analysis: The LAB count was performed alternately until day 21 to prevent the growth of essential oils, *Mentha piperita* and *Ziziphora clinopodioides*. 1 grams of each yoghurt sample was suspended in 9 mL of physiological serum. The suspension was serially diluted. Then 0.1 mL of the appropriate diluted solution was plated on the MRS Agar media in triplicate. All plates were incubated at 42°C for 72 hr. subsequently; the colonies were enumerated [8].

Statistical Analysis: All determinations were carried out in triplicate samples and data were based on the analysis of variance. Statistical analysis was performed according to the MSTAT-C software. Significant differences between means were determined by Duncan's multiple range test. P value less than 0.01 were considered statistically significant.

RESULTS AND DISCUSSION

The viability of *L. acidophilus* in bio yoghurt containing various concentration of essential oil of *Mentha piperita* and *Ziziphora clinopodioides* (0, 25, 40, 70, 100, 130 µg/L) was significantly decreased (P<0.05) after 7 days storage (Table1 and 2). These results are comparable to those obtained for mint, thyme and garlic by Simsek *et al.* [9] and also those belongs to *Ziziphora* by Hadad Khodaparast *et al.* [4].

Yoghurt Bio starter cultures were examined for inhibition by different concentrations of *Mentha piperita* and *Ziziphora clinopodioides* essential oil during storages. As outlined in Table 3 and 4, however yoghurt bio starter cultures were affected by all concentration of essential oils but no significant differences were observed (control (P<0.05)). Similar observations were also reported by Simsek *et al.* [9]. They could found that the effect of mint, thyme and garlic on LAB in ayran were not differed significantly from the control sample (P<0.05). Also Agboola and Tesic [10] reported similar results.

Table 1: Effect of storage time periods in counts of *L. acidophilus* in bio yoghurt containing *Mentha piperita* essential oil

Time	The number of <i>L. acidophilus</i> (*10 ⁷ cfu/ml)
Immediately after production	34.77
7 days after production	17.72
14 days after production	8.84
21 days after production	12.78

Table 2: The effect of storage time periods in counts of *L. acidophilus* in bio yoghurt containing *Ziziphora clinopodioides* essential oil

Time	The number of <i>L. acidophilus</i> (*10 ⁷ cfu/ml)
Immediately after production	42.72
7 days after production	17.66
14 days after production	16.99
21 days after production	11.91

Table3: The effect of different concentration of *Mentha piperita* in counts of *L. acidophilus*

Concentration of <i>Mentha piperita</i> (µg/L)	The number of <i>L. acidophilus</i> (*10 ⁷ cfu/ml)
0	24.10
25	17.39
40	25.51
70	18.41
100	16.58
130	9.18

Table4: The effect of different concentration of *Z. clinopodioides* in counts of *L. acidophilus*

Concentration of <i>Z. clinopodioides</i> (µg/L)	The number of <i>L. acidophilus</i> (*10 ⁷ cfu/ml)
0	24.10
25	24.21
40	19.07
70	27.57
100	17.44
130	21.53

They also found that Lactic acid bacteria counts in all cheese samples with various spices (mint, lemon myrtle and bush tomato) were not significantly changed during maturation. But Bayoumi [3] reported that essential oils of clove, cinnamon, cardamom and peppermint reduce final population of Lactic acid cultures in flavored yoghurt till 1.5-3 cycles.

CONCLUSIONS

This report is based the first illustration of *Mentha piperita* and *Ziziphora clinopodioides* essential oils usage in bio yoghurt to investigate interactions between these compounds and *Lactobacillus acidophilus* activity.

The results indicated that there is no significant differences between concentrations of *Mentha piperita*, *Ziziphora clinopodioides* and control sample although the number of starter culture decreased seriously during storage.

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