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Improvement the hygienic quality and organoleptic properties of bioyoghurt using *Cuminum cyminum* L. essential oil

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Abstract

In this study organoleptic properties, microbial quality (behavior growth of *Salmonella typhimurium* and survival of *Lactobacillus casei*) and pH changes of bioyoghurt sample prepared with adding different concentration *Cuminum cyminum* L. essential oil (50, 100 and 200 ppm EO) were investigated during 10 day storage. S. *typhimurium* was shown to survive for 72h in control and some treated yoghurt samples (treatment 50 and 100 ppm EO without Probiotic). The significant ($P<0.05$) main and interactive inhibitory effects of probiotic and EO (even at its lowest concentration, 50 ppm) on this organism were conclusively demonstrated. According to the results, this inhibitory effect was obviously affected by increasing of EO concentration to 100 and 200 ppm combined with *L.casei* ($P<0.05$). Survival of *L.casei* decreased throughout the storage period. Nevertheless, yoghurt sample containing 200 ppm EO had the highest ($P<0.05$) viable count of probiotic bacteria (7.10 Cfu/g). Yoghurt sample contains 50 ppm EO and probiotic was the best treatment with acceptable flavor, good appearance without any signs of spoilage. The pH values showed no inhibitory effects of EO on *L.casei* growth during storage of yoghurt.

Keywords: Bioyoghurt, *Cuminum cyminum* essential oil, Shelf life.

1. Introduction

Food Food spoilage is an enormous economic problem worldwide. Through microbial activity alone, approximately one-fourth of the world's food supply is lost [1]. Undesirable microbes that can cause spoilage of dairy products include Gram-negative psychrotrophs, coliforms, lactic acid bacteria, yeasts, and molds. In addition, various bacteria of public health concern such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, pathogenic strains of *Escherichia coli* and enterotoxigenic strains of *Staphylococcus aureus* may also be found in milk and dairy products [1].

Salmonella is also the most common cause of diseases associated with the consumption of dairy products. The occurrence of *Salmonella* in yogurt seems to be possible. It may be a result of exceptionally heavy raw milk contamination, inadequate heat treatment and secondary contamination arising from lack of hygiene during packaging. Based on literature data it can be concluded that *Salmonella* cells have not favorable conditions for growth in yogurt. However, it may survive in final product for some time dependent on the type of product, its pH, storage temperature and other environmental conditions [2].

Concerns over the inevitable side effects of chemical food preservatives have prompted on increased interest in more ‘‘natural-green’’ alternatives as a

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suitable approach, Particular interest has focused on the potential applications of plant EOs [3]. Despite the reportedly strong antimicrobial activity of EOs against foodborne pathogens and spoilage micro-organisms, their practical application as preservatives is currently limited owing to the undesirable flavour changes they cause in food products [4]. Leistner and Gorris (1995) [5] suggested that to secure microbial stability and consumer safety while maintaining the sensory, nutritive and economic properties of foods, multiple preservatives in small amounts were superior to preservation by a large amount of a single preservative. The synergistic effect between essential oils and other antimicrobial substances such as GRAS metabolites produced by lactic acid bacteria has been conclusively demonstrated, and it has been noted that the activities of the essential oils and their constituents are enhanced by the presence of nisin [6].

Cuminum cyminum L. has been allocated the topic of some recent studies in addition to its well documented traditional usage for treatment of toothache, dyspepsia, diarrhoea, epilepsy and jaundice and indicated drastic inhibitory effects on *Escherichia coli, Listeria monocytogenws* and *S. aureus* by disc diffusion method [7]. *C. cyminum* with the vernacular name of "Zireh e sabs" (in Iran), is a plant belonging to the Apiaceae family applied in Iranian folk medicine since more than 200 years ago [8]. Besides its use in traditional medicine in the treatment of some ailments, C. cyminum is widely used as a spice (flavoring agent) in different kinds food. The spice contains essential oil that imparts a characteristic aroma to it [9]. Major constituents in *C. cyminum* essential oil (EO) are cuminal, cuminic alcohol, gamma-terpinene and beta-pinene [9]. *Lactobacillus* strains have been utilized as dairy starter and may act as both probiotic and bioprotective culture (one possible mode of action for probiotics is the production of antimicrobial compound) as well as fermenting agent in fermented products. Studies showed the inhibitory effect of these bacteria against the growth of various foodborne bacterial pathogens [10]. Probiotic species such as *Lactobacillus casei and Lactobacillus acidophilus* have been safely used for more than 70 years and are available in conventional foods and dietary supplements [11]. *L. casei* is a homofermentative micro-organism, It is acid tolerant and could thus survive during yoghurt production and storage [12]. The aim of this study was to evaluate the behaviour of *L.casei* and *S. typhimurium* during the storage of yoghurt made with *Cuminum cyminum* L. essential oil at different concentrations. In addition, pH value and Sensory properties of yoghurt were also investigated.

2. Materials and Methods

*2.1. Essential oil extraction.*The powdered dry *Cuminum cyminum* L. plant was subjected to steam distillation for 3 h using a Clevenger-type apparatus. The obtained EO was dried over anhydrous sodium sulfate until the last traces of water was removed, and then stored in dark glass bottles at 4 °C.

2.2.Gas chromatography - mass spectrometry. The EO was analyzed by gas chromatography – mass spectrometry (GC-MS). The chromatograph instrument (Agilent 6890 UK) was equipped with an HP-5MS capillary column (30 × 0.25 mm ID × 0.25 mm film thickness) and the data were taken under the following conditions: initial temperature 50°C, temperature ramp 5°C.min, 240°C.min to 300°C (holding for 3 min), and injector temperature at 290°C. The carrier gas was helium and the split ratio was 0.8 mL^{-1}/min. For confirmation of analysis results, EO was also analyzed by gas chromatography–mass spectrometry (Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass-selective detector; Agilent UK) and the same capillary column and analytical conditions as above. The MS was run in electron-ionization mode with ionization energy of 70 eV. Confirmation of the components was performed by referring to kovats index.

2.3.Bacterial strains. Lyophilized cultures of *S.typhimurium* ATCC 13311 was obtained from the culture collection of the Department of Microbiology, Faculty of Veterinary Medicine, Urmia, Urmia, Iran. Subcultivation and preparation of the inocula were conducted according to Parsaeimehr et al. (2010) [13].

2.4.Starter and probiotic bacteria. Freeze dried yoghurt inoculants (Christian Hansen Co., R 704, Denmark) containing *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp.

bulgaricus (1:1) was used as a starter. A commercial lyophilized culture of the probiotic *L. casei* ATCC 3939 was obtained from the Iranian Organization of Industrial Research. Subcultivation and preparation of the probiotic bacteria were conducted according to Phillip *et al.* (2006) [14].

*2.5.Preparation and inoculation of yoghurt.*Raw cow milk was subjected to a heat treatment at 90°C for 20 min, followed by cooling to 40 – 45°C. It was inoculated with the test organisms at 10^5 CFU⁄mL in separate groups, the EO was added to milk before processing with different concentrations (50, 100, 200 ppm) followed by mixing. As starter culture yoghurt (*L.bulgaricus* and *S. thermophilus*) was added (1.5%) to the milk, followed by mixing, finally *L.casei* (10^8-10^9 Cfu/ml) added. then packed in sterilized glass capped cups 250 mL capacity, followed by incubation at 40°C for 3 hours till gel forms (pH 4.5). Freshly yoghurt was cooled and stored at refrigeration at 4°C for 10 days.

All of the manufacturing procedures were also carried out for preparation of uninocultad (without *S. typhimurium*) yoghurt which used for *L. casei* enumeration and sensory evaluation.

2.6. Microbial analysis. Samples (10 g) from yoghurt samples were pooled in 90 mL of sterile 0.1% (w⁄ v) peptone water (Merck, KGaA) in sterile 500-mL stomacher bags. Samples were blended in a Stomacher 400 (Interscience, Saint-Nom-La- Breteche, France) for 3 min (Nunez et al., 1985). *S.typhimurium* counts were determined on salmonella shigella agar (Merck) after incubation at 37 °C for 48 h.

Survival of probiotic bacteria during storage period of yoghurt samples (on days 1, 2, 3, 5 and 10) was conducted according to standard method.

One gram of yoghurt sample were homogenised aseptically in a stomacher with 9 mL of sterile peptone water (Merck, Germany) (0.1%) and 10-fold (10^2 – 10^8) serial dilutions were prepared. The enumeration was carried out using spread plates with a 100 ppm inoculums on RCA with bromocresol green and vancomycin medium (RCABV).

The pH of the RCA agar base was adjusted to 5.5 prior to autoclaving and then bromocresol green stock 0.2% w/v (prepared as previously described) added at the rate of 20 ml/l. Vancomycin stock solution (2% w/v) was prepared with distilled water and filter-sterilized through a 0.45-µm membrane. This was added at the rate of 0.5 ml/l to the molten agar. The plates were incubated anaerobically in gas jars using the GasPak System, (Oxoid) for 48 h at 37 °C prior to observation. All plate counts were carried out in triplicates. Plates containing 25–250 colonies were enumerated and recorded as colony forming units (CFU/g) of the product [14].

2.7.Calculation of pH. The pH values of yoghurt samples were measured using a digital pH meter (Nick, 776, Germany) fitted with a standard, combined glass electrode. The pH meter was calibrated with buffer solutions of pH 4 and pH 9 prior to use.

*2.8.Evaluation of the sensory quality.*The sensory effects of adding of *C.cyminum* EO and probiotic bacteria to yoghurt were evaluated using an acceptance test. Yoghurt samples with various amounts of EO as described previously were equally divided into seven parts of 20 g each and placed on white plates coded with three-digit random numbers. The sensory evaluation was performed by a panel of seven judges consisting of the scientific staff of the Department of Food Hygiene, Faculty of Veterinary Medicine, University of Tabriz, experienced in the sensory analysis of food. Each panelist evaluated the samples by rating them using a nine-point scale, where 9= like extremely and 1= dislike extremely, for various characteristics such as (appearance) colour, odour and flavour [15].

The variability of acceptance or liking of the samples was analysed by ANOVA and Fisher's least significant difference procedure (LSD), using the SPSS 17 statistical software package (SPSS 17 for Windows; SPSS Inc.).

3.Statistical Analysis

All the tests were conducted in triplicate, and results were computed as mean standard deviation and were subjected to one-way analysis of variance to establish whether the differences in experimental results were significant or not. The Statistical significance was determined at $P<0.05$.

4. Result and Discussion

The chemical composition *C.cyminum* EO was analyzed by GC–MS (Figure 1). The cumin aldehyde (29.02%), alpha-Terpinene (20.70%), gamma Terpinene (12.94%), gamma Terpinene (8.90%), para-Seaman (8.55%) and beta-pinene (7.73%) most component were found in *C. cyminum* EO.

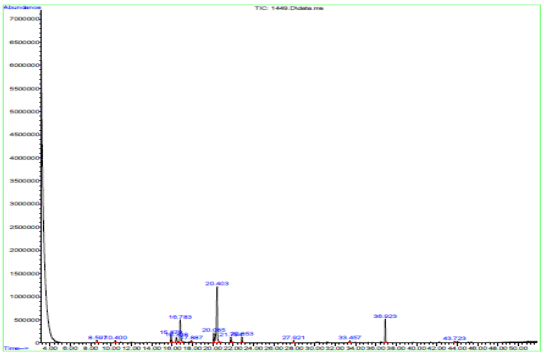


Figure 1. Chromatogram of *Cuminum cyminum* L. EO

The growth behavior of *S.typhimurium* during 10 daye storage of different yoghurt samples at 4ºC is shown in Table1. *S.typhimurium* was not isolated in pasteurized milk before inoculation.

Table 1 represents growth response of *S.typhimurium* affected by EO and probiotic during 10 days storage of yoghurt. The significant ($p<0.05$) main and interactive inhibitory effects of probiotic bacteria and EO (even at its lowest concentration, 50 ppm) on this organism were conclusively demonstrated. The growth of *S.typhimurium* was inhibited during the cold storage of the different yoghurt samples, *S.typhimurium* counts decreased sharply from 5.02 log cfu/g to 3.25 log cfu/g (Sample A, contaminated yoghurt without EO and probiotic bacteria) and from 5.02 log cfu/g to 2.47 log cfu/g (Sample C, bioyoghurt with 200 ppm EO) at the first days of storage.

Based on the results obtained from this study *S.typhimurium* was not found after 5 days of storage in samples A and G, also was not found after 3 days of storage in samples B, D, E and F,

and G. yoghurt sample containing EO (200 ppm) and probiotic bacteria (sample C) after 48 *h, S.typhimurium* was not found.

Table 1. Survival of *S. typhimurium* in yoghurt samples over 10 days at 4°C

Yoghurt samples	*Number of counts (log CFU/g)*				
	24h	*48h*	*72h*	*5 days*	*10 days*
A	3.25±0.03[a]	2.99±0.05[a]	2.39±0.08[b]	ND	ND
B	3.07±0.09[a]	2.54±0.04[a]	ND	ND	ND
C	2.47±0.05[a]	ND	ND	ND	ND
D	2.74±0.13[a]	1.92±0.09[a]	ND	ND	ND
E	2.85±0.10[a]	2.32±0.04[a]	ND	ND	ND
F	2.69±0.11[a]	1.62±0.18[a]	ND	ND	ND
G	2.79±0.07[a]	2.54±0.10[a]	1.37±0.06[b]	ND	ND
H	3.20±0.08[a]	2.91±0.05[a]	1.90±0.08[b]	ND	ND

ND, not detected. The mean values followed by the difference letter in the column are significantly different ($P<0.05$). Sample A = Yogurt with no additive; Sample B = Probiotic yoghurt; Sample C =Probiotic yoghurt with 200 ppm EO; Sample D = Probiotic yoghurt with 100 ppm EO; Sample E = Probiotic yoghurt with 50 ppm EO; Sample F = Yoghurt with 200 ppm EO; Sample G = Yoghurt with 100 ppm EO; Sample H = Yoghurt with 50 ppm EO.

Table 2. Changes in pH value of yoghurt samples during the 10 days storage

Yoghurt samples	*Storage period*				
	24h	*48h*	*72h*	*5 days*	*10 days*
A	4.56±0.03[a]	4.45±0.07[a]	4.25±0.09[c]	4.12±0.03[d]	3.90±0.02[f]
B	4.54±0.01[a]	4.40±0.09[a]	4.29±0.03[c]	4.05±0.06[e]	3.81±0.04[f]
C	4.47±0.01[a]	4.38±0.12[a]	4.15±0.05[c]	4.15±0.09[d]	3.92±0.07[f]
D	4.51±0.02[a]	4.35±0.07[b]	4.22±0.07[c]	4.08±0.13[d]	3.90±0.05[f]
E	4.49±0.05[a]	4.31±0.15[b]	4.20±0.10[c]	4.00±0.18[e]	3.78±0.10[f]
F	4.55±0.09[a]	4.32±0.02[b]	4.26±0.14[c]	4.15±0.05[d]	3.89±0.18[f]
G	4.58±0.07[a]	4.42±0.11[a]	4.26±0.06[c]	4.10±0.03[d]	3.85±0.04[f]
H	4.55±0.04[a]	4.36±0.08[b]	4.29±0.02[c]	4.02±0.07[e]	3.80±0.07[f]

The mean values followed by the difference letter in the column are significantly different ($P<0.05$). Sample A = Yogurt with no additive; Sample B = Probiotic yoghurt; Sample C =Probiotic yoghurt with 200 ppm EO; Sample D = Probiotic yoghurt with 100 ppm EO; Sample E = Probiotic yoghurt with 50 ppm EO; Sample F = Yoghurt with 200 ppm EO; Sample G = Yoghurt with 100 ppm EO; Sample H = Yoghurt with 50 ppm EO.

The decrease count of *S.typhimurium* at 4ºC storage were different in yoghurt produced by adding EO and probiotic bacteria compared to contaminetd yoghurt without any adding ($p<0.05$).

According to the results, this inhibitory effect was obviously affected by increasing of EO concentration to 100 and 200 ppm.

Singh et al. (2011) demonstrated that incorporation of *Anis* EO (1 g/l) and oleoresin is quite effective in controlling the growth of spoilage microorganisms in yoghurt, also addition of thise EO has no adverse effect on the physicochemical properties of yoghurt [16].

Hudson et al. (1997) reported that the population of *E.coli* inoculated in plain live yoghurt constantly decreased from an initial inoculums level of 5×10^7 cfu/g to 30 cfu/g after storage for 8 days at 4 °C [17].

The behaviour of *E. coli* O157:H7 during the storage of plain yoghurt at 4, 8, 17 and 22 °C was investigated by Bachrouri et al. (2002) [18]. Counts of *Lactobacillus* and *Streptococcus* were about 9 log cfu/g during this study. The TA and pH development in the contaminated samples were not statistically different from their own control groups, *E. coli* O157:H7 does not survive during the fermentation process of yoghurt, and the presence of this organism in ready-to eat yoghurt indicates the post processing contamination [19].

Farrag (1992) reported that population of the pathogenic microorganisms in yoghurt and kefir samples decreased at various levels during the cold storage [20].

This study has shown that effects of the yoghurt with EO and *L.casei* bacteria on *S.typhimurium* were different from control samples. However, in general, yoghurt samples had inhibitory effects on *S.typhimurium* and this result might be significant for the post-contamination of the infectious dose of *S.typhimurium* which might be very low [6,16]. The obtained results suggest that the *S.typhimurium* populations were not inhibited by low concentrations of the EO after 72 h. However, increases in the EO concentrations lead to decreases in bacterial counts. Burt (2004) reported that essential oils contain phenolic compounds that are primarily responsible for their antimicrobial properties [21].

Generally, the results obtained showed similar regularities to those found by others author. Antibacterial effect of lactic acid bacteria (LAB) on pathogenic microorganisms were observed in many works and this fact is well known [2, 22].

The viability of *L.casei* in bio yoghurt containing various concentration of *C.cyminum* EO was significantly decreased (P<0.05) after 10 days of storage (Table 3). The viability of *L.casei* in the contaminated samples was not statistically different from yoghurt samples without *S.typhimurium.*

Different concentrations of herbal EO can influence the activity of starter bacteria and LAB in fermentative dairy products and this has been investigated by some researchers [23, 24].

Table 3. Enumeration of *L.casei* in yoghurt samples over 10 days storage

Yoghurt samples	*Number of counts* (log CFU/g)				
	24h	*48h*	*72h*	5 days	10 days
A	8.57 ± 0.13^{a}	8.33 ± 0.11^{b}	8.00 ± 0.08^{c}	7.32 ± 0.03^{e}	6.77 ± 0.12^{f}
B	8.48 ± 0.09^{a}	8.30 ± 0.14^{b}	7.93 ± 0.10^{c}	7.13 ± 0.08^{e}	6.69 ± 0.10^{f}
C	8.61 ± 0.08^{a}	8.49 ± 0.10^{a}	8.12 ± 0.10^{c}	7.65 ± 0.10^{d}	7.10 ± 0.09^{e}
D	8.50 ± 0.13^{a}	8.39 ± 0.06^{b}	8.05 ± 0.10^{c}	7.40 ± 0.11^{e}	6.82 ± 0.11^{f}
E	8.31 ± 0.10^{b}	7.91 ± 0.09^{c}	7.71 ± 0.08^{d}	7.00 ± 0.07^{e}	6.35 ± 0.08^{g}
F	8.54 ± 0.10^{a}	8.30 ± 0.13^{b}	8.09 ± 0.12^{c}	7.63 ± 0.09^{d}	7.02 ± 0.07^{e}
G	8.48 ± 0.10^{a}	8.35 ± 0.10^{b}	8.00 ± 0.08^{c}	7.49 ± 0.05^{e}	6.79 ± 0.14^{f}
H	8.25 ± 0.08^{b}	7.83 ± 0.08^{c}	7.75 ± 0.12^{d}	7.12 ± 0.09^{e}	6.40 ± 0.09^{g}

The mean values followed by the difference letter in the column are significantly different ($P<0.05$).Sample A = Probiotic yogurt without *S.typhimurium*; Sample B = Probiotic yoghurt contaminated *S.typhimurium* ; Sample C =Probiotic yoghurt with 200 ppm EO; Sample D = Probiotic yoghurt with 100 ppm EO; Sample E = Probiotic yoghurt with 50 ppm EO; Sample F = Yoghurt with 200 ppm EO and *S.typhimurium*; Sample G = Yoghurt with 100 ppm EO and *S.typhimurium*; Sample H = Yoghurt with 50 ppm EO and *S.typhimurium*.

It has previously been reported that addition of some essential oils to yoghurt and labneh cheese during its manufacture had a stimulatory effect on LAB by enhancing their growth and acid production [25].

Among Gram-positive bacteria, LAB is often known as the most resistant species against antimicrobial agents of herbs [26]. In present study with regarding result of enumeration of the *L.casei*, yoghurt containing *L.casei* and 50 ppm EO had the highest total viable count of probiotic bacteria (6.81 Cfu/g), Based on the results, *L. casei* populations were not inhibited by low concentrations of the *C.cyminum* EO. However, increases in the EO concentrations lead to decreases in bacterial counts (P<0.05).

According to FAO, a standard probiotic product must contain a minimum of 10^{6}–10^{7} Cfu/g live and active probiotic microorganisms at the moment of consumption [27]. Durability of *L. casei* at the end storage period of yoghurt in control and yoghurt containing *C.cyminum* EO (especially in 50 ppm) was sufficient to exert beneficial health effects.

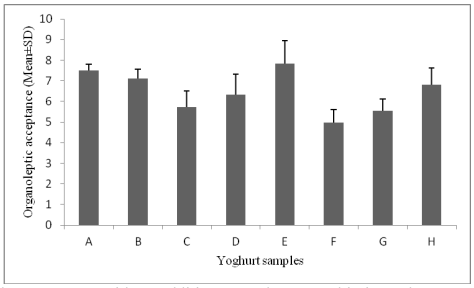
The results showing the effect of storage and treatments on the pH values of various yoghurt samples are presented in Table 2. A gradual and consistent decrease in pH along the storage was noted. The initial pH values for the different yoghurt types ranged from 4.45 to 4.65 in first day of storage.

In addition, According to the results, different concentrations of *C.cyminum* EO did not have any significant influence on pH and no significant difference was observed for pH fluctuation between the combinations. It can therefore be concluded that, in contrast to many gram positive bacteria, these

lactic acid bacteria are fortunately resistant to EO and treatment by EO had no negative effect on standard manufacture process. It has been also stated that compared to other gram positives, lactic acid bacteria are very resistant to EOs [28].

This result are in line with the findings of Mahmoudi et al. (2012), Singh *et al.* (2010) they reported a decrease in pH of yoghurt during storage [16, 23].

The pH evolution is correlated with the lactose fermentation intensity, Taken together, it appears that the composition of starter culture, fermentation temperature, storage duration, contamination, etc., could influence the overall level of acidity and pH of stored yoghurt samples [16].



Sample A = Yogurt with no additive; Sample B = Probiotic yoghurt; Sample C =Probiotic yoghurt with 200 ppm EO; Sample D = Probiotic yoghurt with 100 ppm EO; Sample E = Probiotic yoghurt with 50 ppm EO; Sample F = Yoghurt with 200 ppm EO; Sample G = Yoghurt with 100 ppm EO; Sample H = Yoghurt with 50 ppm EO.

Figure 2. Organoleptic properties of yoghurt formulated with *C. cyminum* EO and *L.casei*

Figure 2 shows the mean acceptability scores of the yoghurt samples containing of *C.cyminum* EO and *L.casei*. Indeed the samples which produced from milk containing 50, 100 and 200 ppm of the EO were acceptable to panelists. However, the most preferred sample was the yoghurt which prepared from the milk containing 50 ppm of EO combined with probiotic.

Although, according to the results of organoleptic analysis, adverse sensory effect of EO was obtained when the concentration of EO was increased from 100 ppm to 200 ppm. Therefore, in spite of the drastic suppressive effects of EOs (including this EO used in this experience) against foodborne pathogens and spoilage micro-organisms, practical application of these preservatives is currently confined due to undesirable flavor changes which they cause in food products [4]. In order to secure microbial stability and safety, and also maintain the sensory, nutritive and economic properties of foods, Leistner and Gorris (1995) have recommended applying multiple preservatives in small amounts is superior to preservation by a large amount of a single preservative [29].

The synergistic effect between EOs (and their constituents) and other antimicrobial substances such as GRAS metabolites produced by LAB (e.g. nisin) has been conclusively demonstrated and it has been noted that the activities of the EO and their constituents are enhanced by the presence of nisin [6]. In our previous work we found significant inhibitory effect of *Mentha longifolia* EO, *L.casei* and their synergistic effect on *S. aureus* and *L.monocytogenes* in Iranian with brined cheese during manufacturing and ripening period [23].

4. Conclusion

In this study, we found the significant ($p < 0.05$) synergistic inhibitory effect of *C. cyminum* EO (which is used as flavoring agent during process of different kinds food in Iran) and probiotic (*L.casei* which is available in conventional foods and dietary supplements) on growth of *S. typhimurium* during 10 days storage yoghurt. Consequently, food safety programs should be designed to ensure that this microorganism is absent from post pasteurization processes. Further investigations must be developed in order to find out more about the behavior of this microorganism in acidic dairy products.

Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

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