

QUALITY OF YOGURT BLENDED WITH *THYMUS KOTSCHYANUS* ESSENTIAL OIL

Razzagh Mahmoudi^{a*}, Payman Gajarbeygi^b, Foad Mahmoodzadeh^b, Mohsen Hassanzadeh^a, Raziye Kiyani^a and Mohammad Reza Asadi Nadari^a

^a Department of Food Hygiene & Aquatics, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

^b School of Public Health, Qazvin University of Medical sciences, Qazvin, Iran

* Corresponding Author: r.mahmoudi@yahoo.com

ABSTRACT In agreement with the current trend of giving value to natural and renewable resources, the use of natural antimicrobial compounds, particularly in food and biomedical applications, becomes very frequent. The behaviour of *Listeria monocytogenes* during the cold storage (10 days) of yoghurt which was produced with *Thymus Kotschyanus* essential oil (0, 50, 150 and 300 ppm EO) with sensory attributes of yoghurt were investigated, in addition, The viability of starter culture bacteria were studied. Viable counts of *L. monocytogenes* fall was sharp from 3.00 to 1.07 (log cfu/g) at the first day and was not present at the other storage day in yoghurt with 300 ppm EO. In spite of *L. monocytogenes* could be isolated and counted in days 7 from control sample (1.19 log cfu/g). Yoghurt made with 150 ppm of the EO had the highest starter culture bacteria count at the end of storage. Considering the organoleptic effect of the EO, the best treatment with acceptable flavor, good appearance was obtained at 50 ppm EO. In conclusion, the combination of *T. Kotschyanus* EO treatment and other natural preservative was significantly effective in the inactivation of foodborne pathogens while maintaining acceptable yoghurt quality.

(Keywords: Yoghurt, *Thymus kotschyanus* EO, *Listeria monocytogenes*)

INTRODUCTION

Many food products are perishable by nature and require protection from spoilage during their preparation, storage, and distribution to give them desired shelf life [1-3]. *L. monocytogenes* is a most important food-borne bacterial pathogen and can lead to food borne diseases though consumption of contaminated milk and fermented milk products including yoghurt [4].

To prevent growth of spoilage and pathogenic microorganisms in foods, several preservation techniques, such as heat treatment, salting, acidification, and drying have been used in the food industry [5,6]. In recent years, because of the great consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural additives have become very popular [7,8]. Main natural compounds are EOs derived from plants (e.g., basil, thyme, oregano, cinnamon, clove, and rosemary), enzymes obtained from animal sources (e.g., lysozyme, lacto-ferrin), bacteriocins from microbial sources (nisin, natamycin), organic acids (e.g., sorbic, propionic, citric acid) and naturally occurring polymers (chitosan). In this context, plant EOs are gaining a wide interest in food industry for their potential as

decontaminating agents, as they are Generally Recognized as Safe (GRAS) [9].

The genus *Thymus* (Persian name: Avishan) belongs to Lamiaceae family and is a well-known aromatic perennial herb originated from Mediterranean region. Among 215 species of this genus grown in the world, 14 species are distributed in Iranian flora [10]. *Thymus* species are commonly used as herbal teas, flavoring agents (condiment and spice) and medicinal plants because of their biological and pharmacological properties [11]. *T. Kotschyanus* is one of the well-known species in this genus. The antioxidant and antimicrobial activity of *Thymus* species has been extensively investigated in vitro [12,13]. *Thymus* EOs and extracts are widely used in pharmaceutical, cosmetic, and perfume industry, also for flavoring and preservation of several food products [14,15]. But limited information is exists on biological activities of *T. Kotschyanus* EO in food models.

This research was aimed to evaluate the antimicrobial effect of *T. Kotschyanus* EO on *L. monocytogenes* growth in yoghurt stored under refrigeration (4°C) for 10 days. In addition, this study also aimed evaluated the

sensorial acceptability of the yoghurt treated with this EO at different concentrations.

MATERIAL AND METHODS

Plant material and EO extraction

The aerial parts of *T. Kotschyanus* was ground and subjected to hydro distillation for 3 h using Clevenger type apparatus [3]. The isolated EO was dried over anhydrous sodium sulfate and stored in dark at 4°C.

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 [16].

Bacterial strains

Lyophilized cultures of *L. monocytogenes* ATCC 19118 were obtained from the culture collection of the Department of Microbiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. Subcultivation and preparation of the inocula were conducted according to Parsaeimehr et al. (2010) [17].

Starter bacteria

Freeze dried yoghurt inoculants (Christian Hansen Co., R 704, Denmark) containing *S. salivarius* ssp. *thermophilus* (ST) and *L.delbrueckii* ssp. *bulgaricus* (LB) (1:1) was used as a starter.

Preparation and inoculation of yoghurt

Raw cow milk (composition shown in Table 1) 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 103 CFU/mL in separate groups, the EO was added to milk before processing with different concentrations (0, 50, 150 and 300 ppm) followed by mixing. As starter culture yoghurt (*L.bulgaricus* and *S. thermophilus*) was added (1.5%) to the milk, followed by mixing, 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 [18].

Table 1. Changes in pH values of the yoghurt samples during cold storage

Yoghurt Samples	Days				
	1	3	5	7	10
A	4.33 ^a	4.22 ^a	4.18 ^a	4.08 ^b	4.04 ^b
B	4.35 ^a	4.20 ^a	4.15 ^a	4.09 ^b	4.08 ^b
C	4.20 ^a	4.10 ^b	4.05 ^b	4.00 ^b	3.94 ^b
D	4.30 ^a	4.18 ^a	4.10 ^b	4.05 ^b	4.00 ^b

The mean values followed by the same letter in the column are nonsignificantly different (P<0.05). Sample A, yoghurt with 50 ppm EO; sample B, yoghurt with 150 ppm EO; sample C, yoghurt with 300 ppm EO; Sample D, yoghurt with no additive (Control).

Microbial analysis

L. monocytogenes enumeration

For microbial counts, 10 g of Ayran samples were homogenised with 90 ml of a 0.85% (w/v) sterilised sodium chloride (NaCl) solution. Decimal dilutions were prepared in sterile 9 ml of 0.85% (w/v) NaCl and plated on media. *L. monocytogenes* counts were determined on PALCAM Listeria agar (Merck) with PALCAM Listeria-selective supplement (Merck) after incubation at 37 °C for 48 h [16].

Lactic acid bacteria from yogurt samples were also studied at time zero and at the end of the storage period. The M17 agar (Oxoid) was used for the isolation of *S.thermophilus* incubating at 37 °C for 24-48 h, and MRS agar (Oxoid) for *L.bulgaricus* at 30 °C with 10% CO₂ for 72 h.

Chemical analysis

Changes in pH values of yoghurt samples were recorded using a digital pH meter (Nick, 776, Jena, Germany) in different steps of storage. The pH meter was calibrated with buffer solutions of pH 4 and pH 9 prior to use [18].

Sensory evaluation

Hedonic test was carried out by 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. Scores were awarded on a scale of 1–7, in which 1 indicated dislikes extremely and 7 like extremely [19].

The sensory attributes evaluated were color, aroma and overall acceptances.

Statistical Analysis

All statistical analyses were performed using software 17 SPSS. Data related to the mean values of microbial counts and physicochemical evaluations were subjected to ANOVA. Significant results were considered at $P < 0.05$. All experiments were performed in triplicate.

RESULT AND DISCUSSION

EO components

The Phenol (47.14%), Benzen (13.78%), alpha pinene (7.42%), 1,8 cineoloe (3.35%), gamma Terpinene (1.87%), carvacrol (1.41%) most component were found in EO.

Microbial analysis

The growth behavior of *L. monocytogenes* during 10 days storage of different yoghurt samples at 4°C are shown in Figure 1. *L. monocytogenes* was not isolated in pasteurized milk before inoculation. The growth of *L. monocytogenes* was inhibited during the fermentation of the yoghurt samples; these inhibitions are given in Figure 1. *L. monocytogenes* counts decreased sharply from 3.00 to 1.07 (log cfu/g) at the first days of storage and was not present at the other storage days in yoghurt with 300 ppm EO. The death curves of *L. monocytogenes* at the 150 and 300 ppm EO were significantly different from ($P < 0.05$) 50 ppm EO and control samples.

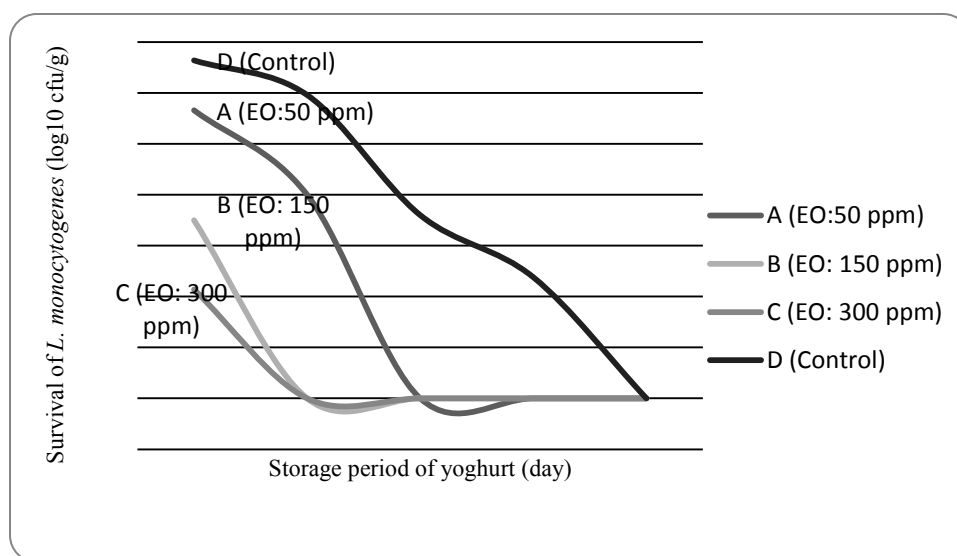


Figure 1. Survival of *L. monocytogenes* in yoghurt samples over 10 days at 4°C

T. Kotschyanus EO had the best *L. monocytogenes* growth inhibition at 150 and 300 ppm. However, *E. coli* O157:H7 could be isolated and counted in days 1, 3 and 7 from control group and in days 1 and 3 in 50 ppm EO. According to the results, this inhibitory effect was obviously affected by increasing of EO concentration to 150 and 300 ppm.

In many studies, growth and/or survival of *E. coli* O157:H7, *L. monocytogenes* and *Y. enterocolitica* in acidified dairy foods including yogurt (pH 4.0 to 4.5) even the 30 to 40 days has been stated [20-22]. In this study, *E. coli* O157:H7 grew during fermentation and survived during 7 days of cold storage in control sample. Contrary to our findings, *E. coli* O157:H7 has been suggested not to survive during fermentation process of yogurt and presence of this organism in ready to eat yogurt would indicate the postprocessing contamination [23].

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 this EO has no adverse effect on the physicochemical properties of yoghurt [18]. Reported that the population of *E. coli* inoculated in plain live yoghurt constantly decreased from an initial inoculum level of 7.69 to 1.47 (Log cfu/g cfu/g) after storage for 8 days at 4°C [24].

Investigation of the behaviour of *E. coli* O157:H7 during the storage of plain yoghurt at 4, 8, 17 and 22°C showed that *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 [25].

Farrag (1992) reported that population of the pathogenic microorganisms in yoghurt and kefir samples decreased at various levels during the cold storage [26].

The present study has shown that effects of the yoghurt with EO on *L. monocytogenes* were different from control samples. The obtained results suggest that the *L. monocytogenes* populations were not completely inhibited by low concentrations of the EO after 3 day. Hudson et al. (1997) reported that the population of this microorganism inoculated in plain live yoghurt constantly decreased from an initial inoculum level of 7.69 to 1.47 (Log cfu/g cfu/g) after storage for 8 days at 4°C [24].

However, our results showed the increases of the EO concentrations lead to decreases in *L. monocytogenes* counts, also the population of this microorganism in control sample constantly decreased and was not detected in 10th of storage days.

In order to know the behavior of the lactic acid bacteria present in the yogurt, counts of *Lactobacillus* and *Streptococcus* were carried out both at the beginning and the ends of the storage period.

LB and ST count of all the yoghurt samples decreased ($P < 0.05$) during the storage time. Additionally, effects of the EO on lactic acid bacteria were significantly different from the control sample. ST and LB counts of all yoghurt samples fell gradually from 7.82 to 5.36 log cfu/g and 7.96 to 5.97 log cfu/g during the storage period, respectively. Whereas yoghurt made with 150 ppm of the EO had the highest lactic acid bacteria count, those manufactured with 300 ppm of the EO had the lowest count. Hudson et al. (1997) reported that the population of this microorganism inoculated in plain live yoghurt constantly decreased from an initial inoculum level of 7.69 to 1.47 (Log cfu/g cfu/g) after storage for 8 days at 4°C [24]. The obtained results suggest that the LAB bacterial populations were not inhibited by low concentrations of the EO. However, increases in the EO concentrations lead to decreases in bacterial counts. It has previously been reported that addition of some EOs to yoghurt and labneh cheese during its manufacture had a stimulatory effect on LAB by enhancing their growth and acid production [27]. Notably, Mahmoudi et al. (2013) reported that the presence of *T. polium* EO, in the manufacture of bioyoghurt increased the counts of probiotic bacteria compared to untreated controls during cold storage [28].

Sensory evaluation

The results from sensory evaluation of yogurt samples are presented in Figure 2. The organoleptic evaluation indicated that there was significant difference ($P < 0.05$) in most of the attributes of control and EO 50 ppm of yogurt samples such as aroma, color and overall acceptance with other concentration of EOs. Aroma of control and 50 ppm EO yoghurt samples were more preferred (6.52) by consumers compared with other yoghurt samples. Overall acceptance of 50 ppm EO yoghurt sample followed by control yoghurt sample was respectively the most favorite by consumer.

Although adverse organoleptic effect of EO was obtained when the concentration of EO was increased from 150 ppm to 300 ppm. Therefore, in spite of the drastic suppressive effects of EOs against foodborne

pathogens and spoilage micro-organisms [29]. practical application of these preservatives is currently confined due to undesirable flavor changes which they cause in food products [30]. In order to secure microbial stability and safety, and also maintain the sensory,

nutritive and economic properties of foods have recommended applying multiple preservatives in small amounts is superior to preservation by a large amount of a single preservative [31].

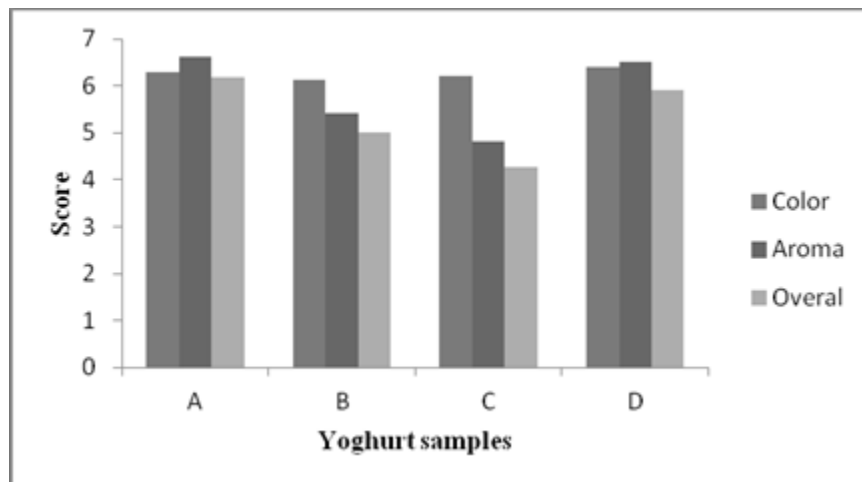


Figure 2. Mean of hedonic scores of various yoghurt samples A: EO 50ppm, B: EO 150ppm; C: EO300ppm and D: Control.

Physicochemical Properties

The change in total acidity (TA) and pH are very important factors, since their affects the shelf life and the acceptability of dairy product especially yoghurt. Based on the results presented in Table1, it is evident that pH values of the treated yoghurt decreased significantly with an increase in the storage period. However, the pH reduction trend between yoghurt samples produced with various concentrations of EO and control treatment was significantly different ($P < 0.05$). The highest values were obtained with yoghurt containing 150 ppm of the EO when fresh and it decreased up to the end of storage (day 10), suggesting that the EO had a stimulatory effect on the commercial culture and total viable count [32]. These results were in agreement with that obtained by mahmoudi et al. (2013), who reported that the TA increased and pH decreased gradually during cold storage period of yoghurt treated with different concentration of the *T. polium* EO [28]. Finally, reported 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 [18].

CONCLUSION

This study has shown that effects of the yoghurts with *T. Kotschyanus* EO on *L. monocytogenes* were different from control sample. However, in general, yoghurt sample had inhibitory effects on *L. monocytogenes* and this result might be significant for the post-contamination of the infectious dose of *L. monocytogenes* which might be very low. Consequently, food safety programs should be designed to ensure that this microorganism is absent from postpasteurization processes. Further investigations must be developed in order to find out more about the behavior of this microorganism in acidic dairy products.

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