



## ORIGINAL ARTICLE

## *Ziziphora clinopodioides* Essential Oil Effects on the Physicochemical and Microbial Characteristics of Cow Milk Butter during the Storage at 4°C

Razzagh Mahmoudi<sup>\*1</sup>, Sasan Khezri<sup>2</sup>, Peyman Ghajarbeygi<sup>3</sup>, Ata Kaboudari<sup>4</sup>, Mohammad Panahzadeh<sup>2</sup>, Shaghayegh Mousavi<sup>5</sup>, Behnaz Familsatarian<sup>6</sup>

<sup>1</sup>Professor, Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

<sup>2</sup>Department of Food Hygiene and Safety, School of Public Health, Qazvin University of Medical Sciences, Qazvin, Iran

<sup>3</sup>Assosiated Professor, Health Products Safety Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

<sup>4</sup>Ph.D. Candidate of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia Iran

<sup>5</sup>Ph.D. Student in Molecular Medicine, Faculty of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran

<sup>6</sup>Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

(Received: 24 June 2019

Accepted: 21 September 2019)

## KEYWORDS

Butter;  
Essential oil;  
Improvement of health  
quality;  
GC/MS;  
*Ziziphora  
clinopodioides*

**ABSTRACT:** This study was conducted with the aim of investigating the effect of *Ziziphora clinopodioides* on the physicochemical and microbial properties of Iranian traditional cow milk butter. The *Z. clinopodioides* essential oil (EO) was extracted by Cleveenger. The EO constituents were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Three butter samples with concentrations of 300, 600, and 900 ppm were prepared from the EO. The variation of physicochemical and microbial properties of butter was evaluated on days 1, 3, 5, 7, and 10 at 4°C. Based on GC-MS analysis results, the major EO compounds were Carvacrol (40.1%), Linalool (8.30%), and Isoborneol (4.90%). The results of the microbial evaluation showed that the amount of microbial load is significantly reduced by increasing concentrations of EO and the maintenance time of the butter. The logarithmic reduction of the total count of bacteria, *Escherichia coli*, and the total number of mold and yeast were 3.68, 1.76 and 3.83 (log<sub>10</sub> CFU/mL) at 900 ppm, respectively. The peroxide and acid values of butter samples were also decreased significantly. Additionally, the sensory evaluation showed that the EO had acceptable desirable sensory acceptance at 300, 600, and 900 ppm, and the lowest sensory acceptance was observed at 900 ppm. Considering the antimicrobial and antioxidant effects of *Z. clinopodioides* EO, as well as improving the organoleptic properties of various foods such as butter and its abundance as a native plant in the country, it can be used as a natural preservative and flavoring agent instead of chemical ones.

## INTRODUCTION

Today, the demand for natural products with the lowest processing rate has increasingly grown. One of the challenges ahead of this demand estimation in food industry is the reduction of chemical synthetic

additives in the formulation of food products. In this regard, the use of herbal and natural products has attracted a lot of attentions. Among the natural antimicrobial compounds, essential oils (EO) have a

\*Corresponding author: r.mahmodi@yahoo.com (R. Mahmoudi)  
DOI: 10.22034/jchr.2019.669294

wide range of functional effects. Therefore, the existing volatile and aromatic compounds in different organs of the plant are effective against microorganisms that are responsible for food spoilage and disease [1]. One of these plants is *Ziziphora clinopodioides*, which has 9 native subspecies in Iran. This plant, which belongs to the genus *Ziziphora*, has been used in traditional medicine since ancient times and used as an analgesic, anti-inflammatory substance in treatment of gastrointestinal infections. Today, this plant is used in restaurants and homes in foods, and to treat colds and heart disorders, depression, diarrhea, coughing, migraine, and fever [2].

Milk and its products are one of the most important groups of foods people consume. In particular, butter has a special and exceptional position and is considered as one of the main foods in human diet and plays an important role in human nutrition. Accordingly, butter is a dairy product derived from cream. The quality of the produced butter is largely dependent on the quality of the milk used. The maintenance of butter also depends to a large extent on its bacteriological quality, which is itself influenced by the health conditions of the production process, as well as its maintenance [3]. Butter spoilage is often caused by the activity of microorganisms which are either capable of growing at low temperatures or functioning after melting. These microorganisms produce enzymes and cause lipolysis and result in the bad taste and color variation of the butter [4]. Oxidation and hydrostatic flavors happen due to fatty acids decomposition or oxidation of double bonds of unsaturated fatty acids in the butter structure. Hydrolytic degradation results from the activity of the lipase (microbial) enzyme. Fats hydrolysis releases fatty acids with a short carbon chain, which are associated with the presence of undesirable flavor. The proteins decomposition in the butter, though in small quantities caused by microorganisms, produces disintegration flavors. The microorganisms that make this flavor are often

*Coliform* and *Pseudomonas* species that infect the water used for butter production [5].

Considering the importance of butter in terms of nutrition and its sensitivity to microorganisms, especially *Coliform* and psychrotrophic bacteria, and factors such as light and heavy metals that exacerbate oxidation and spoilage of the butter, this study aims to investigate the effects of plant EO, *Ziziphora clinopodioides*, based on the physicochemical and microbial characteristics of Iranian traditional cow milk butter which is widely consumed in Iran.

## MATERIALS AND METHODS

### *Extraction of EOs*

The *Z. clinopodioides* was collected from Alamut district of Qazvin province, Iran, in spring 2018, and was certified in Qazvin University of Medical Science. The plant was dried, and then, grinded. Finally, using a Clevenger device for 3 hours, the EO was extracted by water distillation method. After drying using sodium sulfate, it was stored under refrigeration conditions [6].

### *Determination of the chemical composition of EO*

The EO compounds were determined by Gas Chromatography connected to a Mass Spectrometer (GC/MS). Agilent 6890 Gas Chromatograph specifications include a 30-meter-long column, an internal layer thickness of 0.25, and an internal diameter of 250 micrometers. The temperature of the injection chamber was 265 °C, and the helium gas velocity was 1.2 ml/min. Mass Spectrometry with ionization energy of 70 eV and ionization source temperature of 250 °C was of the Agilent type 5973[7].

### *Preparation of butter*

The cow milk butter was obtained from the central market of Qazvin city and was transferred under suitable conditions (at temperature of 4°C) to the

Health and Food Safety Laboratory in Faculty of Health, Qazvin University of Medical Sciences.

#### **Determination of the butter microbial population**

After the butter was transferred to laboratory under appropriate conditions, the total count of microorganisms, *E. coli*, *Pseudomonas* and the total count of mold and yeast were determined according to Iranian National Standard No. 2406 [8].

#### **Antimicrobial activity of *Z. cliniopodioides* EO in butter**

At first, three butter treatments with concentrations of 300, 600, and 900 ppm of EO were prepared and stored at 4 °C. The total count of microorganisms, *E. coli*, *Pseudomonas* and total mold and yeast count were assessed on days 1, 3, 5, 7, and 10, for each prepared concentration, using specific culture media, according to Iranian National Standard No. 2406 [8].

#### **Measuring the peroxide value and the acidity**

At the same time with evaluating the antimicrobial activity, the peroxide value and the acidity of the butter treatments containing different concentrations of EO were measured during the 10-day storage period in accordance with Iranian National Standards No. 4179 and 4178 [9, 10].

#### **Sensory evaluation**

To evaluate sensory features such as color, taste, smell, and general acceptance, the sensory evaluation was used. In this study, 8 trained evaluators were provided with treatments with concentrations of 300, 600, and 900 ppm of EO and control samples group, and the samples were evaluated in the form of a 9-item questionnaire (1: very undesirable to 9: quite desirable) [11].

#### **Statistical analysis**

Data were analyzed using one-way ANOVA in SPSS ver.24 software. All experiments were performed in three replications and the mean of data was compared by Duncan's multiple range test at the level of  $P < 0.05$ .

## **RESULTS AND DISCUSSION**

#### **GC/MS identification**

The results of GC-MS showed that the compounds listed in Table 1 are the most important components of *Z. cliniopodioides* EO. The antimicrobial property of the EOs depends on their components. The EOs of different herbs have different combinations. The major compounds of genus *Ziziphora* Eos are thymol and carvacrol. The major antimicrobial effects of carvacrol have been shown in various studies [12]. In the present study, carvacrol with 40.16% was the major component of the EO of *Z. cliniopodioides*.

**Table 1.** Most important components of the EO of *Ziziphora cliniopodioides* by GC-MS

Identified component	Percent (%)
Carvacrol	40.1
Linalool	8.30
Isoborneol	4.90
Nerolidol	4.66
1,8-Cineole	3.51
Spathulenol	2.86

### Antimicrobial effects

The effects of various concentrations of EO in butter on the total count of microorganisms, *E. coli*, *Pseudomonas*, mold, and yeast are shown in Table 2. The results showed that there was a significant difference in different concentrations of EO between day 1 and day 10 ( $p < 0.05$ ). Also, there was a significant difference between the concentrations of 300 and 900 ppm on different days ( $p < 0.05$ ). Although the effect of herbs, plant extracts, and EOs has been known since thousands of years ago, in recent years, much attention has been paid to the effects of herbs and plant extracts on pathogens and microorganisms as the cause of food spoilage [13]. The results of the microbial evaluation of this study showed that the *Z. clinopodioides* EO significantly

reduces the microbial content of butter. In the meantime, logarithmic reduction of the total mold and yeast count was significant compared to bacteria, especially the gram-negative bacteria, *Pseudomonas*. However, various studies have suggested that gram-positive bacteria membrane structure is more susceptible to plant EOs than gram-negative bacteria [14]. However, in the present study, *E. coli* was also most susceptible after mold and yeast and logarithmic reduction was significantly affected by different concentrations of EO between days 1 and 10. The present study also showed that the microbial population decreased significantly by increasing the concentration of EO and storage time of butter containing EO.

**Table 2.** Results of microbial characterization (log<sub>10</sub> CFU/ml) under the influence of EO (on days 1, 3, 5, 7 and 10)

Concentration (ppm)	300				600				900				
	Period	3	5	7	10	3	5	7	10	3	5	7	10
Total count		0.07	0.13	0.14	1.22	0.04	0.11	0.18	1.37	0.17	0.39	0.67	1.76
<i>E. coli</i>		0.09	1.12	0.42	1.98	0.29	0.35	1.83	2.93	0.23	1.53	1.68	3.68
<i>Pseudomonas</i>		0.27	0.32	0.46	0.55	0.25	0.35	0.38	0.97	0.06	0.1	0.23	0.36
Total mold and yeast count		0.07	0.18	0.36	0.49	0.08	0.21	0.31	0.62	0.2	0.68	2.4	3.83

The effect of EO and extract of *Z. clinopodioides* on the activity of bacterial starter in yogurt showed that the number of bacterial starter in all samples decreased during storage period, which was similar with the results of the present study [15]. Additionally, the results of another study showed that the use of various concentrations of *Zataria multiflora* EO during the production and maintenance of white salted cheeses significantly reduced the population of *E. coli* O157: H7 [16]. The effects of *Pistacia atlantica* subsp *kurdica* on the growth of *Penicillium citrinum* and the organoleptic properties of UF cheese were investigated. It was concluded that EO of *Pistacia atlantica* subsp *kurdica* has a significant effect on the prevention of *Penicillium citrinum* growth and improvement of organoleptic properties of UF cheese [17].

### Peroxide value and acidity

The results for the peroxide value and acidity are given in Table 3. These results showed that at different concentrations of EO on days 1 and 10, the peroxide value was significantly decreased ( $p < 0.05$ ), but no significant difference was observed in acidity between different days ( $P > 0.05$ ). In addition to having antimicrobial effects, Herbal EOs have also antioxidant properties. That is why aromatic plants and spices are widely used in many foods containing fats such as meat, dairy and bakery products to protect oxidative damage [18, 19]. In the present study, the results of changes in peroxide value and acidity showed that the amount of peroxide and acidity in butter treatments decreased significantly by increasing EO and a storage time compared to the control sample ( $p < 0.05$ ). Meanwhile, the amount of peroxide value

reduction in comparison to acidity was significant, indicating that the EO of this plant has high antioxidant activity.

The study carried out by Amiri (2015) on the antioxidant effect of EO and extract of *Ziziphora clinopodioides* showed that the EO and methanolic extract of this plant have a good antioxidant activity

[18]. Another study was conducted to investigate the physicochemical and sensory properties of flavonoid-enriched yogurt extracted with orange skin, indicating that the addition of these compounds to yogurt reduced the acidity and deposition and increased pH, fat, and dry matter of yogurt [20].

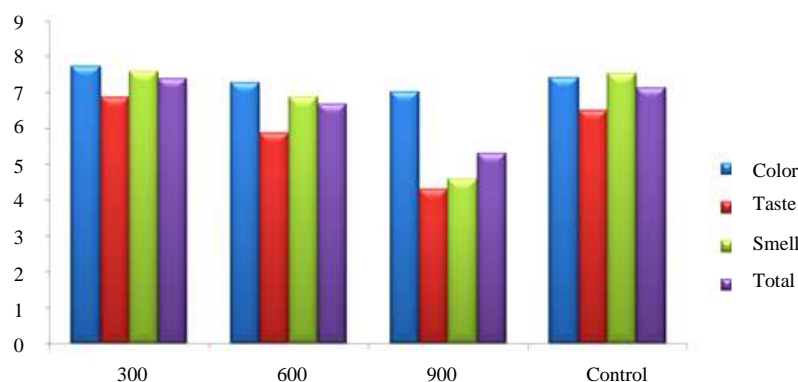
**Table 3.** Peroxide value and acidity of treatments under the influence of EO on days 1, 3, 5, 7 and 10)

Concentration (ppm)	300				600				900				
	Period	3	5	7	10	3	5	7	10	3	5	7	10
Peroxide value		0.6	0.43	1.03	1.33	0.92	0.97	1.33	2.28	0.27	0.63	0.79	1.03
Acidity		0.01	0.01	0.01	0	0.01	0.02	0.03	0.04	0.01	0.02	0.02	0.02

### Sensory evaluation

The results of sensory evaluation tests are shown in Figure 1. There were no significant changes in the color at different concentrations, although flavor tests were significant at concentrations of 300 and 900 ppm. The sensory evaluation results of the present

study showed that the concentrations of *Z. clinopodioides* EO in butter had desirable sensory acceptance, while the highest and the lowest sensory acceptance were observed at concentration of 300 ppm and 900 ppm, respectively.



**Figure 1.** The results of sensory evaluation of treatments.

In another study, the effect of *Teucrium polium* EO in probiotic yogurt was investigated in order to control *Salmonella Typhimurium LT2*. The results of this study showed that the best concentration of EO for inhibition of *S. Typhimurium* growth, as well as yoghurt production with optimum flavor properties was 40 ppm in combination with probiotic bacteria [21]. Results of another study showed that the addition of *Bunium persicum* Boiss EO improved the sensory properties of Gouda cheese [22].

In this study, the different concentrations of *Z. clinopodioides* EO in Iranian traditional cow milk butter reduced significantly the total microbial count, *E. coli* and total mold and yeast count. The EO also significantly reduced the peroxide value and acidity and improved the organoleptic properties of the butter treatments. Therefore, considering the antimicrobial and antioxidant effects of this EO in various studies, as well as the present study, and its abundance as a native plant in the country, it can be used as a natural preservative and flavoring compound instead of

chemical preservatives and flavors used in various foods. By comparing microbial, chemical, and organoleptic results, it can be concluded that by increasing the amount of EO, the antimicrobial and antioxidant properties increased, but the general acceptance and organoleptic properties decreased. However, the EO of this plant can be used up to a concentration of 900 ppm to improve organoleptic properties.

#### ACKNOWLEDGEMENTS

Thanks to the financial and spiritual support of Qazvin University of Medical Sciences.

#### REFERENCES

1. Cleveland J., Montville T.J., Nes I.F., Chikindas M.L., 2001. Bacteriocins: safe, natural antimicrobials for food preservation. *Int J Food Microbiol.* 71(1), 1-20.
2. Shafei M., Sharifan A., Aghazadeh M.M., 2012. Composition of essential oil of *Ziziphora clinopodioides* and its antimicrobial activity on *Kluyveromyces marxianus*. *Int J Food Sci Nut.* 9(1), 101-107.
3. Herreros M., Fresno J., Prieto M.G., Tornadijo M., 2003. Technological characterization of lactic acid bacteria isolated from Armada cheese (a Spanish goats' milk cheese). *Int Dairy J.* 13(6), 469-479.
4. Walstra P., Wouters J., Geurts T., 2005. *Dairy Science and Technology*. 2nd ed. CRC Press. pp: 197, 485.
5. Karim G., 2009. *Hygiene and Technology of milk*. 2nd ed. University of Tehran Press. pp: 30-35.
6. Dehghan G., Elmi F., 2015. Essential oil combination of three species of *Achillea* growing wild in East Azarbayjan-Iran. *Advanced Herbal Med.* 1(1), 22-8.
7. Ahmadi Z., Sattari M., Tabaraee B., Bigdeli M., 2011. Identification of the constituents of *Achillea santolina* essential oil and evaluation of the antimicrobial effects of its extract and essential oil. *Arak Med J.* 14(56), 1-10.
8. Iran NSO. Microbiology of milk and milk products Specifications and test methods. 2016. <http://www.isiri.gov.ir/>
9. Iran IASAIRO. Animal and vegetable fats and oils Determination of acid value and acidity Test method. 2011. <http://www.isiri.gov.ir/>
10. Iran IOSAIRO. Animal and vegetable fats and oils Determination of peroxide value Iodometric (visual) endpoint determination. 2008. <http://www.isiri.gov.ir/>
11. Hashemi M., Ehsani A., Jazani N.H., Aliakbarlu J., Mahmoudi R., 2013. Chemical composition and in vitro antibacterial activity of essential oil and methanol extract of *Echinophora platyloba* DC against some of food-borne pathogenic bacteria. *Vet Res Forum.* 4(2), 123-127.
12. Aghajani Z., Assadian F., Masoudi S., Chalabian F., Esmaeili A., Tabatabaei-Anaraki M., Rustaiyan A., 2008. Chemical composition and in vitro antibacterial activities of the oil of *Ziziphora clinopodioides* and *Z. capitata* subsp. *capitata* from Iran. *Chem Nat Compd.* 44(3), 387-389.
13. Valero M., Giner M., 2006. Effects of antimicrobial components of essential oils on growth of *Bacillus cereus* INRA L2104 in and the sensory qualities of carrot broth. *Int J Food Microbiol.* 106(1), 90-94.
14. Burt S., 2004. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbiol.* 94(3), 223-253.
15. Mehraban S.M., Karazhyan R., Hadad K.M., Habibi N.M., Beiraghi T.S., 2007. Effect of essential oil and extract of *Ziziphora clinopodioides* on yoghurt starter culture activity. *World Appl Scie J.* 2(3), 194-197.
16. Mohammadi K., Karim G., Hanifan S., Tarinejad A., Gasemnezhad R., 2011. Antimicrobial effect of *Zataria multiflora* Boiss essential oil on *Escherichia coli* O157: H7 during manufacture and ripening of white brined cheese. *J Food Hyg.* 1(2), 69-78.
17. Ostowar S., Bahramian S., Salehi R., 2014. Effect of essential oil of *Pistacia atlantica* Subsp. *Kurdica* gum on growth of *Penicillium citrinum* and

organoleptic properties of UF-cheese. J Food Hyg. 4(2), 39-46.

18. Amiri H., 2009. Composition and antioxidant activity of the essential oil and methanolic extract of *Ziziphora clinopodioides* Lam in preflowering stage. J Kerman Med Sci. 15(1), 79-86.

19. Keykavousi M., Ghiassi Tarzi B., Mahmoudi R., Bakhoda H., Kaboudari A., Rahimi Pir Mahalleh S.F., 2016. Study of antibacterial effects of *Teucrium polium* essential oil on *Bacillus cereus* in cultural laboratory and commercial soup. Carpathian J Food Sci Technol. 28(2), 176-183.

20. Zahedi H., Fadaei N.V., Khalafi L., 2015. Investigating physicochemical and sensory properties of yoghurt enriched with oil and flavonoid extracted from orange peel. J Research and Innovation Food Sci Technol. 4(1), 19-34.

21. Mahmoudi R., Zare P., Nosratpour S., Mardani K., Safari A., 2014. Hygienic effects of *Teucrium polium* essential oil against *Salmonella typhimorium* LT2 in probiotic yoghurt. J Urmia Med Sci. 25(8), 769-777.

22. Taherkhani P., Noori N., Akhondzadeh Basti A., Gandomi H., 2015. Antimicrobial Effects of *Kermanian Black Cumin (Bunium persicum* Boiss.) Essential Oil in Gouda Cheese Matrix. J Med Plants (JMP). 2(54), 76-85.

