

## An Introductory Review on Increasing the Survival of Probiotic Bacteria in Dairy Products Using Essential Oil

This article was published in the following Scient Open Access Journal:  
Journal of Dental and Oral Health

Received March 05, 2017; Accepted March 20, 2017; Published March 29, 2017

Razzagh Mahmoudi<sup>1</sup>, Masoud Kazemina<sup>2</sup>,  
Peyman Ghajarbeygi<sup>3</sup> and Babak Pakbin<sup>4</sup>

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

<sup>2</sup>Department of Foods Hygiene and Safety, Qazvin University of Medical Sciences, Qazvin, Iran

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

<sup>4</sup>Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

### Abstract

Increasing the survival of probiotic bacteria and improve the hygienic quality of dairy products using natural preservative agents is widely accepted today. It has long been recognized that some essential oils (EOs) have antimicrobial properties, so that they can be used as food flavoring agents, preservatives, also for medicinal purposes. However, to establish the usefulness of natural preservatives, they must be evaluated alone and in combination with other preservative factors (such as probiotic bacteria) to determine whether there are synergistic effects and to devise effective combinations. This study is a review on investigation of feasibility in using of EOs (including: *Teucrium polium*, *Cumin*, *Allium ascalonicum*, *Mentha longifolia*, *Ferula sharifi* and *Pimpinella anisum*) in probiotic dairy products. In most of these research survival of *L. casei* decreased throughout the storage period. Nevertheless, probiotic dairy products and treatment containing medium concentration EOs had the highest viable count of probiotic bacteria. But the lowest concentration of these EOs was the most appropriate treatment in sensory assessment. Based on our review, dairy products such as yoghurt, cheese and dairy drinks can be a suitable food matrix to survive probiotic bacteria while certain herbal EOs is added.

**Keywords:** Essential oil, Herbal plants, Functional dairy products, Natural preservative

### Introduction

Foodborne diseases recognized as one of the major public health problems worldwide, especially in developing countries and on the other hand, increasing incidence of foodborne disease along with its social and economic consequences have led to conducting extensive research in order to produce safer food and develop new antimicrobial agents; among them, extensive use of probiotics and bacteriocins as biological additives is of considerable importance [1]. Concerns about the safety of some chemical preservatives and negative consumer reactions to chemical and artificial preservatives rose, and more options include 'natural' and 'green' alternatives for the maintenance or extended of product shelf life have been considered. Particular interest has focused on the potential of applications of plant EOs [2,3]. Leistner and Gorris (1995) [4] suggested that to secure microbial stability and consumer safety while maintaining sensory, nutritious and economical properties of foods, multiple preservatives material in small amounts were superior to preservation by a large amount of a single preservative. The synergistic effect between EOs and other antimicrobial substances such as GRAS metabolites produced by lactic acid bacteria has been proven. It has been noted that the activities of the EOs and their constituents are enhanced by the presence of nisin [5]. Herbal EOs are aromatic oil liquids, extracted from various parts of plants, are used as flavoring agents in foods thus the importance of use of medicinal plants in food products can be multiple times [6].

### Cuminum Cyminum

*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 monocytogenes* and *S. aureus* [7]. *C. cyminum* with the vernacular name of "Zireh e sabs" (in Farsi, 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 kind's food. The spice contains EO that imparts a characteristic aroma to it [9].

\*Corresponding Author: Razzagh Mahmoudi, Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran, Tel: +98-9127868571, Email: r.mahmodi@yahoo.com

Major constituents in *C. cyminum* EO are cuminal, cuminic alcohol, gammaterpinene and beta-pinene [9].

### **Mentha Longifolia**

*Mentha longifolia* L. belongs to the Lamiaceae plant family. This genus includes 25 species that grow wild in the humid zones of central and southern Europe, the south-west of Asia and northern Africa. The stems of the plant are erect, and the leaves are simply attached to the stem. The plant also bears white small blossoms with a light-purple tinge. Different parts of this plant are used in commercial spices as flavouring. Widely used in herbal medicine and believed to be particularly beneficial in building the immune system and fighting secondary infections. The plant is used for the treatment of coughs, colds and influenza. Externally, wild mint is used to treat wounds and swollen glands [10] and also this plant bears medicinal characteristics and has proven to be of benefit for digestive system disorders, vomiting and loss of appetite, ulcerative colitis and liver malfunctions. It also bears antimicrobial and antioxidant activity. Other reported inhibitory effects have been reported towards micro-organisms causing foodborne diseases, for example, *S. aureus*, *E. coli*, *Bacillus* subsp., *Salmonella* subsp. and *Aspergillus* subsp [11-13].

### **Allium Ascalonicum**

*Allium ascalonicum* Linn. (Liliaceae) is an annual herbaceous plant, smaller than *Allium cepa*, belonging to the family Liliaceae. The shallot is commonly grown in West Africa, more than *Allium cepa*, being more suited to the climate. Various species of *Allium* are of considerable importance in African cooking and in salads. The pounded leaves of shallot are rolled in balls and sold in North-ern Nigerian markets and in Sudan [14].

In Northern Nigeria, some traditional herbalists pound the *Allium ascalonicum* dry skin, and dry leaf into a powder, and mix it with honey and carrot juice for the treatment of cancer. For the treatment of jaundice, *Allium ascalonicum*, *Ziziphus mucronata* (root), *Khayag randiflora* (roots) and *Moridia whitei* plant decoctions are taken orally. It has also been combined with *Carica papaya* (old leaves), *Eliaeis guineensis* (roots), *Citrullus vulgaris* (roots), *Aniophyllea* species (seeds), *Capsicum frutescens* (unripe fruits) and *Securi-daca longipedunculata* (root) taken with pap made from *Zea mays*, for the treatment of malaria [15].

### **Pimpinella anisum**

*Pimpinella anisum* L. Umbelliferae is an annual herb and a grassy plant with white flowers and small green to yellow seeds, which grows in Turkey, Iran, India, Egypt, and many other warm regions of the world [16,17]. *P. anisum* had several therapeutic effects on several conditions such as digestive, gynaecologic, neurologic, and respiratory disorders [18]. The aqueous extract of this plant has been reported to delay the onset of picrotoxin-induced seizures in mice [19].

### **Teucrium Polium**

*Teucrium polium* L. locally named Kalpooreh has been known as an important traditional medicinal plant in Khuzestan, South West of Iran. *T. polium* L. is the member of Lamiaceae family, a grass plant, durable, with 10 to 30 cm in height and callous with exterior that ordinarily have dispersal in rocky and sandy area of Europe zones, North of Africa and Southwest of Asia like Iran.

Medical of reputation of this plant was noticed in traditional medicine by Socrates and Jalinous [17]. Researchers showed that this plant have antidiabetic, anti-inflammatory, antispasmodic, analgesic and antioxidant effects [20,21].

### **Ferula sharifi**

The genus *Ferula*, belonging to the family Apiaceae, comprises about 170 species. These are produced from central Asia westward to northern Africa. The Iranian flora comprises of 30 species of *Ferula*, of which some are endemic. The popular Persian name of the most species is "Koma" [22]. The chemistry of this genus has been studied by many investigators. To date, more than 70 species of *Ferula* have been investigated chemically [23,24]. The plants of this genus are well documented as a good source of biologically active compounds such as Sesquiterpenoids and sulfur containing compounds [23]. Several species of this genus have been used in traditional medicine for the treatment of various organ disorders. Among different *Ferula* species that have been used as natural remedies, *F. assa-foetida* used as anticonvulsant, carminative, antispasmodic, diuretic, aphrodisiac, antihelmintic, tonic, and laxative, alterative, *F. badrakema* and *F. gummosa* both used as anti-convulsant, tonic, anti-hysterical, decongestant, treatment of neurological disorders, and stomachache, and *F. persica* used as laxative, carminative, antihysterical, treatment of lumbago, diabetes, rheumatism, and backache are most famous [17,22].

### **Lactobacillus**

*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 [25].

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 [26]. *L. casei* is a homofermentative micro-organism, it is acid tolerant and could thus survive during yoghurt production and storage [27].

### **Due to the use of EOs combined with probiotics**

An application performance of botanical extracts EOs is in food preservation. However, to create a beneficial natural antimicrobial preservative, they must be evaluated alone and in combination with other preservation factors such as probiotic bacteria to determine whether there are synergistic effects and to devise effective compounds [28-30].

Probiotics are food supplements containing live microorganisms bringing beneficial effects to consumer host through balancing intestine micro flora [31]. The idea of probiotics was primarily introduced by Russian scientist Metchnikoff in 1907. According to his observations, consuming large amounts of fermentative dairy products leads to a long life. That was the first scientific interpretation related to useful effects of lactic acid bacteria available in fermented milks. Along with the discovery of useful effects of probiotics, many efforts were focused on the production and process of fermentative products containing probiotic microorganisms. More than 90% of probiotic products include various species of *Lactobacilli* and *Bifidobacteria* [32]. Different concentrations of herbal EOs influence the activity of

EO and dairy product	chemical composition	probiotic microorganisms	effect on growth probiotics	pathogenic microorganisms	effect on inhibition pathogenic	source
Cuminum cyminum L. (yoghurt)	cumin aldehyde, alpha-Terpinene, gamma Terpinene, gamma Terpinene, para-Seaman and beta-pinene	Lactobacillus casei, Streptococcus salivarius and Lactobacillus delbrueckii	+	Salmonella typhimurium	+	Mahmoudi 2013 [37]
Mentha longifolia L. (Iranian white-brined cheese)	pulegone, 1,8-cineole and menthofuran	Lactobacillus casei	+	Staphylococcus aureus and Listeria monocytogenes	+	Ehsani, et al. 2013 [38]
Mentha longifolia L. (feta cheese)	Pulegone, 1,8-Cineole, Menthofuran and cis-Isopulegone	Lactobacillus casei	+	**	—	Mahmoudi, et al. 2013 [39]
Mentha longifolia L. (Bio-Ayran*)	Pulegone, 1,8-Cineole, Menthofuran, β-Pinene and cis-Isopulegone	Lactobacillus casei	+	—	—	Mahmoudi 2014 [40]
Allium ascalonicum (Iranian white-brined cheese)	Diallyl disulphide, Trisulfide, methyl 2-propenyl, Trisulfide, di-2-propenyl and Disulfide, methyl 1-propenyl	—	—	Escherichia coli	+	Ehsani and Mahmoudi 2014 [41]
Pimpinella anisum (Iranian white-brined cheese)	Benzene, 1-methoxy-4-(1-propenyl), Longifolene-(V4) and Phenol, 2-methoxy-4-(1-propenyl)	—	—	Escherichia coli	+	Ehsani and Mahmoudi 2014 [41]
Teucrium polium (yoghurt)	Spathulenol, Beta-pinene, Beta-myrcene, Germacrene B, Bicyclogermacrene and Germacrene D	Lactobacillus casei	+	—	—	Mahmoudi, et al. 2014 [42]
Teucrium polium (yoghurt)	Spathulenol, Beta-pinene, Beta-myrcene, Germacrene B, Bicyclogermacrene and Germacrene D	Lactobacillus casei	+	Salmonella typhimurium	+	Mahmoudi, et al. 2015 [43]
Ferula sharifi (MIC*** and MBC**** method)	β-Pinene, α-Pipene, Naphthalene, Isolongifol, Sabinene, trans-Pinocarveol and Myrtenol	Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus plantarum and Lactobacillus ramnus	+	—	—	Mahmoudi, et al. 2013 [44]

\*Ayran is a fermented milk beverage traditionally manufactured by mixing yoghurt with water and salt

\*\*Not mentioned

\*\*\*Minimal Inhibition Concentration (MIC)

\*\*\*\*Minimal Bactericidal Concentration (MBC)

**Table 1:** Review on chemical constituent and effects of EOs on probiotic & pathogenic bacteria in dairy product.

starter bacteria in fermentative dairy products and this has been investigated by some researchers [32,33]. Among Gram-positive bacteria, lactic acid bacteria are often known as the most resistant species against antimicrobial agents of herbs [34]. However, one of the major challenges in production and processing of probiotic products is low viability of these bacteria due of their sensitivity to difficult situation within food products, powerful enzymes of stomach and small intestines. According to FAO, a standard probiotic product must contain a minimum of  $10^6$  to  $10^7$  CFU/g live and active probiotic microorganisms at the moment of consumption [35]. Thus, one of the main research fields is the introduction of products capable of providing a more suitable medium for survival and maintenance of probiotic microorganisms within standard range for a longer time. Physicochemical characteristics of food products such as lipid level, type and concentration of proteins, sugars, buffering capacity and pH are the major factors contributing to the survival and function of probiotics [1,36].

In the below Table 1 showed EOs effect on pathogenic microorganisms and probiotic bacteria in dairy product.

## Conclusion

The results obtained in our studies showed significant inhibitory effects of EOs, probiotic bacteria, time and the overall interactions including EO concentrations with the presence of probiotic on the growth rate of pathogenic microorganisms.

The combined effect of EOs and probiotic bacteria can be improve the scope of EO function in the food industry, especially in processing dairy and fermented dairy products. Thus the use of synthetic or chemical preservatives can be reduced or replaced by using the natural ones. Finally, there are numerous advantages of probiotics and herbal EOs. The use of these bacteria or bacteriocins purified from them in combination with herbal extracts and EOs as biological preservatives may revolutionize the food industry.

## References

- Mahmoudi R, Tajik H, Ehsani A, Zare P. Physicochemical and hygienic effects of Lactobacillus acidophilus in Iranian white cheese. *Vet Res Forum*. 2012;3(3):193-197.
- Tassou C, Koutsoumanis K, Nychas GJ. Inhibition of Salmonella enteritidis and Staphylococcus aureus in nutrient broth by mint essential oil. *Food Res Int*. 2000;33(3-4):273-280.
- Smith-Palmer A, Stewart J, Fyfe L. The potential application of plant essential oils as natural food preservatives in soft cheese. *Food microbial*. 2001;18(4):463-470.
- Gachkar L, Yadegari D, Rezaei MB, et al. Chemical and biological characteristics of Cuminum cyminum and Rosmarinus officinalis essential oils. *Food chem*. 2007;102(3):898-904.
- Misaghi A, Basti AA. Effects of Zataria multiflora Boiss. Essential oil and nisin on Bacillus cereus ATCC 11778. *Food control*. 2007;18(9):1043-1049.

6. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. *Int J Food Microbiol.* 2004;94(3):223-253.
7. Singh G, Kapoor I, Pandey S, Singh UK, Singh RK. Studies on essential oils: part 10; antibacterial activity of volatile oils of some spices. *Phytother Res.* 2002;16(7):680-682.
8. Khatibi A, Haghparast A, Shams J, Dianati E, Komaki A, Kamalinejad M. Effects of the fruit essential oil of *Cuminum cyminum* L. on the acquisition and expression of morphine-induced conditioned place preference in mice. *Neurosci Lett.* 2008;448(1):94-98.
9. Charlier C, Cretenet M, Even S, Le Loir Y. Interactions between *Staphylococcus aureus* and lactic acid bacteria: an old story with new perspectives. *Int J Food Microbiol.* 2009;131(1):30-39.
10. Van Wyk BE, Oudtshoorn BV, Gericke N. Medicinal Plants of South Africa. Briza, 1997.
11. Mitchell S. Wild Iris Herbal Handbook. Quivertree Publications, 2005.
12. Gulluce M, Sahin F, Sokmen M, et al. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. *Food Chem.* 2007;103(4):1449-1456.
13. Van Wyk BE, Gericke N. People's plants: A guide to useful plants of Southern Africa. Briza Publications. 2000.
14. Irvine FR. The edible cultivated and semi-cultivated leaves of West Africa. *Materiae Vegetabiles.* 2:35-42.
15. Akinniyi JA, Manawadu D, Sultanbawa M. Ethnobotany and ethnopharmacology of Nigerian medicinal plants. *The State of Med Plant Res in Nigeria.* 1986;154-165.
16. Pourgholami M, Majzoob S, Javadi M, Kamalinejad M, Fanaee GH, Sayyah M. The fruit essential oil of *Pimpinella anisum* exerts anticonvulsant effects in mice. *J Ethnopharmacol.* 1999;66(2):211-215.
17. Zargari A. Medicinal plants. Vol 2. Tehran University Press, Tehran, 1992.
18. Aboabrahim Z. Zakhirah Kharazmshahi, vol 2. Nacional Works. 1970.
19. Abdul-Ghani AS, El-Lati S, Sacaan A, Suleiman MS, Amin RM. Anticonvulsant effects of some Arab medicinal plants. *Int J of Crude Drug Res.* 1987;25(1):39-43.
20. Shahraki MR, Arab MR, Mirimokaddam E, Palan MJ. The effect of *Teucrium polium* (Calpoureh) on liver function, serum lipids and glucose in diabetic male rats. *Iranian Biomedical J.* 2007;11(1):65-68.
21. Hasani P, Yasa N, Vosough-Ghanbari S, Mohammadirad A, Dehghan, G, Abdollahi M. In vivo antioxidant potential of *Teucrium polium*, as compared to  $\alpha$ -tocopherol. *Acta pharm.* 57(1): 123-129.
22. Sahebkar A. Biological activities of essential oils from the genus *Ferula* (Apiaceae). 2011;4(6):835-847.
23. Iranshahi M, Hassanzadeh-Khayyat M, Sahebkar A, Famili A. Chemical composition of the fruit oil of *Ferula flabelliloba*. *J Essential Oil Bearing Plants.* 2008;11(2):143-147.
24. Iranshahi M, Kalategi F, Sahebkar A, Sardashti A, Schneider B. New sesquiterpene coumarins from the roots of *Ferula flabelliloba*. *Pharm Biol.* 2010;48(2):217-220.
25. Carey CM, Kostrzynska M, Ojha S, Thompson S. The effect of probiotics and organic acids on Shiga-toxin 2 gene expression in enterohemorrhagic *Escherichia coli* O157: H7. *J Microbiol Methods.* 2008;73(2):125-132.
26. Kasimoglu A, Goncuoglu M, Akgun S. Probiotic white cheese with *Lactobacillus acidophilus*. *Int Dairy J.* 2004;14(12):1067-1073.
27. Kourkoutas Y, Xolias V, Kallis M, Bezirtzoglou E, Kanellaki M. *Lactobacillus casei* cell immobilization on fruit pieces for probiotic additive, fermented milk and lactic acid production. *Process Biochemistry.* 2005;40(1):411-416.
28. López-Malo A, Alzamora S, Argaz A. Vanillin and pH synergistic effects on mold growth. *Food Sci.* 1998;63(1):143-146.
29. Pol I, Smid E. Combined action of nisin and carvacrol on *Bacillus cereus* and *Listeria monocytogenes*. *Let Appl Microbiol.* 1999;29(3):166-170.
30. Pol IE, Krommer J, Smid EJ. Bioenergetic consequences of nisin combined with carvacrol towards *Bacillus cereus*. *Innovative Food Sci & Emerging Technol.* 2002;3:55-61.
31. Fuller R. Probiotics in man and animals. *J Appl Bacteriol.* 1989;66(5):365-378.
32. Hadad Khodaparast, Mohammad Hosein, Mehraban Sangatash, et al. Effect of essential oil and extract of *Ziziphora clinopodioides* on yoghurt starter culture activity. *World Applied Sciences Journal.* 2007;2(3):194-197.
33. Simsek B, Sagdic O, Ozcelik S. Survival of *Escherichia coli* O157: H7 during the storage of Ayran produced with different spices. *J Food Engineering.* 2007;78(2):676-680.
34. Lemay MJ, Choquette J, Delaquis PJ, Garipey C, Rodrigue N, Saucier L. Antimicrobial effect of natural preservatives in a cooked and acidified chicken meat model. *Int J of Food Microbiol.* 2002;78(3):217-226.
35. Joint F. WHO working group report on drafting guidelines for the evaluation of probiotics in food. London, Ontario, Canada. 2002;30.
36. Ranadheera R, Ho P, Luo J, Adams, MC. Does the food substrate influence the functionality of probiotics? *Asia Pacific J of Clinical Nutr.* 2007;16:81.
37. Mahmoudi R. Improvement the hygienic quality and organoleptic properties of bioyoghurt using *Cuminum cyminum* L. essential oil. *J Agroalimentary Process Technol.* 2013;19(4):405-412.
38. Ehsani A, Mahmoudi R. Effects of *Mentha longifolia* L. essential oil and *Lactobacillus casei* on the organoleptic properties and on the growth of *Staphylococcus aureus* and *Listeria monocytogenes* during manufacturing, ripening and storage of Iranian white-brined cheese. *Int J Dairy Technol.* 2013;66(1):70-76.
39. Mahmoudi R, Tajik H, Ehsani A, et al. Effects of *Mentha longifolia* L. essential oil on viability and cellular ultrastructure of *Lactobacillus casei* during ripening of probiotic Feta cheese. *Int J Dairy Technol.* 2012;66(1):77-82.
40. Mahmoudi R. Effect of *Mentha longifolia* L. Essential Oil on Physicochemical Properties of the Bio-Ayran. *J Essential Oil Bearing Plants.* 2014;17(1):56-66.
41. Ehsani A, Mahmoudi R. Phytochemical Properties and Hygienic Effects of *Allium ascalonicum* and *Pimpinella anisum* Essential Oils in Iranian White Brined Cheese. *J Essential Oil Bearing Plants.* 2012;15(6):1013-1020.
42. Mahmoudi R, Zare P, Hassanzadeh P, Nosratpour S. Effect of *Teucrium polium* essential oil on the physicochemical and sensory properties of probiotic yoghurt. *J Food Processing and Preservation.* 2014;38(3):880-888.
43. Mahmoudi R, Zare P, Nosratpour S. Application of *Teucrium polium* Essential Oil and *Lactobacillus casei* in Yoghurt. *J Essential Oil Bearing Plant.* 2015;18(2):477-481.
44. Mahmoudi R, Kosari M, Barati S. Phytochemical and Biological Properties of *Ferula sharifi* Essential Oil. *J Biological Active Products from Nature.* 2013;3(5-6):331.