

AN OVERVIEW OF USING SOME ESSENTIAL OILS IN FUNCTIONAL DAIRY PRODUCTS FROM IRAN

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ABSTRACT In the last few years more and more studies on the biological properties of essential oils (EOs) especially antimicrobial and antioxidant properties in vitro and food model have been published in all parts of the world. But so far no comprehensive reports of these studies have been reported in food model from Iran. The focus of this overview lies in the using of EOs from some indigenous medicinal plants of Iran (including: *Mentha longifolia*, *Cuminum cyminum*, *Teucrium polium*, *Pimpinella anisum* and *Allium ascalonicum*) in probiotic dairy products (especially cheese, yoghurt and Aryan) in recent years. Recently, consumers have developed an ever-increasing interest in natural products as alternatives for artificial additives or pharmacologically relevant agents. Among them, EOs has gained great popularity in the food, cosmetic, as well as the pharmaceutical industries. Despite the reportedly strong antimicrobial activity of EOs against food-borne pathogens and spoilage microorganisms, their practical application as preservatives is currently limited owing to the undesirable flavor changes they cause in food products. Nonetheless, more studies are necessary to the applicability of various EOs on other food models in Iran and other countries.

(**Keywords:** EOs, Functional Dairy Foods, Natural preservative, Sensory quality)

INTRODUCTION

EOs are composed of lipophilic and highly volatile secondary plant metabolites [1]. As defined by the International Organization for Standardization (ISO), the term “EOs” is reserved for a product obtained from vegetable raw material, either by distillation with water or steam, or from the epicarp of citrus fruits by a mechanical process, or by dry distillation [2], that is, by physical means only. EOs has been proposed as natural preservatives and is used as alternatives for the control of pathogenic microorganisms. Concerning their dietary intake, EOs are generally considered as safe (GRAS) for their intended use by the U.S. Food and Drug Administration. EOs have uses in human health such as functional food, food additives, medicine, nutritional supplements and cosmetic manufacturing [3, 4, 5]. The use of natural antimicrobial compounds such as herbal extracts and spices for the protection of food against microbial activity has led to the identification of some of their unique features such as taste effects and antioxidant activity [6]. Probiotic bacteria defined as living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition, have become a major topic of LAB research over the past 20 years [7]. More than 90% of probiotic products contain various species of *Lactobacilli* and *bifidobacteria* [8]. Viable LAB of

probiotic foods has several scientifically established and/or clinically proved health effects, such as reduction and prevention of diarrheas of different origin, improvement of the intestinal microbial balance by antimicrobial activity, alleviation of lactose intolerance symptoms, prevention of food allergy, enhancement of immune potency and antitumorigenic activities [5, 9, 10]. Moreover, some studies have shown that certain LAB possesses antioxidative activity [5, 11]. Despite the reportedly strong antimicrobial activity of EOs against foodborne pathogens and spoilage microorganisms [12], their practical application as preservatives is currently limited owing to the undesirable flavor changes they cause in food products [4, 13, 14]. Many researcher have 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 [13, 14, 15, 16]. Since a specific knowledge on the use of EOs in food products, especially dairy foods and its effects on hygienic and organoleptic properties of dairy products are fundamental for an adequate use, the present review compiles and discusses the hitherto

scattered data from Iran on dairy products Processed with probiotics and Eos.

EOS FROM INDIGENOUS MEDICINAL PLANTS OF IRAN

Mentha longifolia L. from *Laminaceae*, essentially grows in wet river banks of temperate areas of Central and South Europe, Australia, South-West Asia and Iran [4, 17]. The EOs of this plant varying in quantity according to variety and characteristic of growing site is composed of cationic compounds especially Pulegone, 15–40% total alcohols, 7–12% Limonene and Dipanten [4, 17]. Traditionally, plants from *Laminaceae* were used as flavour and spice in many foods, 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 [4, 17].

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 jaundice and indicated drastic inhibitory effects on *E.coli*, *L. monocytogenes* and *S. aureus* by disc diffusion method [18]. *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 [19]. Besides its use in traditional medicine in the treatment of some ailments, *C. cyminum* is widely used as a spice (flavoring agent) in different types food. The spice contains essential oil that imparts a characteristic aroma to it.

Major constituents in *C. cyminum* essential oil (EO) are cuminal, cuminic alcohol, gammaterpinene and β -pinene [14, 20]. *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 10–30 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 [21]. Researchers showed that

this plant has anti-bacterial, anti-diabetic, anti-inflammatory, anti-spasmodic, analgesic and antioxidant effects [4, 21, 22, 23].

Pimpinella anisum L. is a plant with white leaves and small green yellowish seeds and is from *Umbelliferae* family. This plant grows in countries such as Iraq, Turkey, Iran, India, Egypt and many tropical areas of the world [18, 24]. Cultivation of this plant is important because of producing aromatic seeds by this plant having many uses in medicine for treatment and relieving of digestive disorders, headache and cough. In addition, EOs of some species of this plant is used in treating diseases such as pilepsy [25].

Plant *Allium ascalonicum* is originally from West Asian areas, some consider Palestine Mountains as its origin, this plant also grows in Iran. In genus *Alliums* more than 500 species have been identified, only few of them are used as food stuff. The most important species existing on this genus includes garlic's, onions and leek, that have long being used as spice and drugs [18, 26]. Numerous studies conducted in the field of antimicrobial and preservative characteristics of herbal essential oils from *A. ascalonicum* and *P. anisum* L. [18, 25, 26].

PROBIOTIC DAIRY FOODS

Probiotics are food supplements containing live microorganisms bringing useful effects to host through balancing intestine microflora [27]. 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 contain various species of *Lactobacilli* and *Bifido* bacteria [5, 28].

However, one of the major challenges in producing and processing of probiotic products is low viability of these bacteria because of their sensitivity to difficult conditions within food products powerful enzymes of stomach and small intestines. 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 [29]. Thus, one of the major 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 fat level, proteins, sugars, buffering capacity and pH are among the major factors influencing probiotics' survival and function [30]. Some properties of cheese like high pH (nearly neutral), high fat, dense and compact texture Has led to a suitable foods carrying probiotics with respect to durability and keeping biological activity in all stages of passing through digestive system[4, 15, 31]. Reported other fermentative dairy products such as yoghurt and Ayran can also be a suitable carrier for probiotic bacteria [4, 15].

PROBIOTIC DAIRY FOODS AND EOS

The effects of essential oil on starter culture activity of fermented foods reported by many researchers [33, 34, 35, 36]. Among Gram-positive bacteria, lactic acid bacteria are often known as the most resistant species against antimicrobial agents of herbs [36, 37].

Kivanc et al. (1991) also showed that EOs of *M. longifolia* and *C. cyminum* in low concentrations lead to stimulation of growth and acid production;

in high concentrations, they prevented *L. plantarum* growth [37]. In a study conducted by Simsek et al. (2007), essential oils of spearmint, thyme and garlic had no inhibitory effects on growth and durability of lactic acid bacteria present in Ayran [35]. Agboola and Tesic (2002) showed that lactic acid bacterial count in all cheese samples prepared with various spices (spearmint, green lime and tomato skin) was not significantly different during ripening period [38]. Zaika and Kissinger (1981) showed that *M. longifolia* can motivate, delay or prevent lactic acid bacteria according to application case [39]. EOs from plants and bacteriocins from probiotic bacteria (especially various species of *Lactobacillus*) have well-known antimicrobial effects which can substitute chemical preservatives to control and prevent the activity of foodborne pathogens. Besides, they exert positive effects on consumer's health [3]. Research by Zaika et al. (1978) and Zaika and Kissinger (1979) on the effect of some EOs and spices on lactic starter cultures is among the studies supporting the results of the present research. They showed that some spices have stimulating effects on growth and acid production in some lactic starter cultures [40, 41]. Some research had shown the effect of plant-derived volatile oils on growth and viability of some pathogenic and lactic acid bacteria in dairy foods from Iran (Table 1).

Table1. Some recent studies on application of vegetable oils in dairy products in Iran

Essential oil	Food model	Probiotic Organism	Pathogenic organism	Author, Year
<i>Z. multiflora</i>	Cheese	-	<i>S. aureus</i> , <i>L. monocytogenes</i>	Abbasifar et al., (2009)
<i>Cuminum cyminum</i> L.	Cheese	<i>L. acidophilus</i>	<i>S. aureus</i> ,	Sadeghi et al. (2012) ^[45]
<i>M. longifolia</i>	Cheese	<i>L. casei</i>	<i>S. aureus</i> , <i>L. monocytogenes</i>	Ehsani and Mahmoudi (2012)
<i>M. longifolia</i>	Cheese	<i>L. casei</i>	-	Mahmoudi et al, 2012 (2012)
<i>M. longifolia</i>	Bioayran	<i>L. casei</i>	-	Mahmoudi, 2013
<i>C. cyminum</i> L.	Bioyoghurt	<i>L. casei</i>	-	Mahmoudi, 2013
<i>A. ascalonicum</i> , <i>P. anisum</i>	Cheese	-	<i>E. coli</i>	Ehsani and Mahmoudi, 2012
<i>A. ascalonicum</i> , <i>P. anisum</i>	Cheese	-	<i>L. monocytogenes</i>	Ehsani, Mahmoudi, Zare and Hasani, 2012 ^[46]
<i>T. polium</i>	Yoghurt	<i>L. casei</i>	-	Mahmoudi et al., 2013

In a study, yoghurt samples treated with *T. polium* EO (40, 60 and 80 ppm) and *L. casei* (108–109 cfu/mL) were stored up to 28 days at 4°C. The physicochemical, viability of *L. casei* and

organoleptic properties of yoghurt samples were analyzed. Based on results, yoghurt can be a very suitable food product to carry relevant probiotic bacteria while adding certain herbal EOs. In this study, *T. polium* EO had adverse effect neither on physicochemical properties nor on the viability of *L.*

casei of yoghurt. In addition, *T. polium* EO is non-toxic and safe for public health, so it can be an acceptable alternative to chemical preservatives [42]. In another study the effects of *M. longifolia* L. EO during ripening and storage probiotic Feta cheese were studied, in relation to viability and cellular ultrastructure of *L. casei*. The addition of the EO in the concentrations from 0.0 to 0.03% was trialled: the 0.03% treatment resulted in the highest viability of *L. casei* at the low pH value compared with other treatments ($P < 0.05$). Electron microscopy showed that essential oil caused no harm to *L. casei*. This study demonstrated the favourable effects of *M. longifolia* EO on optimal maintenance of *L. casei* at the end of cheese storage period [36]. Organoleptic properties, microbial quality (behavior growth of *S. typhimurium* and survival of *L. casei*) and pH changes of bioyoghurt sample prepared with adding different concentration of *C. cyminum* L. 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 [43]. The result of the ayran samples treated with *M. longifolia* L. essential oil (EO) (50, 100, 200 and 300 ppm) and *L. casei* (stored to 15 days at 4°C) showed the gradual increase in acidity was observed, whereas a decline in pH and total solid (TS) contents was noted in all the samples ($P < 0.05$). Furthermore, ayran samples containing 100 ppm of EO combined with probiotic bacteria was the most appropriate treatment ($P < 0.05$) in sensory assessment, sample containing 200 ppm EO had the highest viability of *L. casei* at the end storage of period ($P < 0.05$). The addition of *M. longifolia* L. EO has no adverse effect on the physicochemical and organoleptic properties of ayran [44].

CONCLUSIONS

The natural antimicrobial is wide and there are still a great number of possibilities to explore. The tested plant derived oils must be thoroughly described and identified in the future studies as food preservation. The results of the present study showed that, the addition of essential oils can be used to increase the shelf life of dairy product. There are numerous benefits of probiotics, and therefore, 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. Many of dairy food such as yoghurt, chesses and ayran can be a very suitable food product to carry relevant probiotic bacteria while adding certain herbal EOs. In this over view, *M. longifolia*, *T. polium*, *C. cyminum*, *A. ascalonicum* and *P. anisum* EOs had adverse effect neither on physicochemical properties nor on the viability of probiotic bacteria of yoghurt, cheese and ayran. In addition, these EOs are nontoxic and safe for public health, Thus it can be concluded that EOs of these plants can be considered as proper natural preservatives, antibacterial and flavoring agents along with other natural preservation methods against some gram negative and positive bacteria in many food products e.g. cheese. So that the use of synthetic or chemical preservatives can be reduced or replaced by using the natural ones.

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