REVIEW | UDK: 637.3.07 | DOI: 10.15567/mljekarstvo.2020.0102
RECEIVED: 24.07.2019. | ACCEPTED: 26.11.2019.

Antimicrobial efficiency of medicinal plants and their influence on cheeses quality

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Abstract

Milk and dairy products are the oldest and most widely consumed nutritious foods worldwide. They are highlighted as a source of high-quality proteins and the most important sources of bioactive peptides. Milk proteins have high nutritive value and remarkable medicinal properties. They are known as potential health-promoting ingredients of functional foods, and the dairy industry has already commercialized many milk proteins and peptide-based products which can be consumed as part of a regular daily diet. Besides, cheese is highly susceptible to contamination by pathogenic and spoilage microorganisms, which results in a decrease in its shelf life and cause serious risks to the consumers' health. Nowadays, the food industry is continuously becoming more specialized towards meeting consumer needs and demands. Consumers demand safe products, which are also preferably free of synthetic additives. Therefrom to the need to search for natural additives has emerged. Botanicals and their extracts came to an interest as a natural alternative for cheese preservation and quality enhancer. Some substances have demonstrated good effects against most pathogens of cheese such as *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* spp. Nevertheless, it is not quite clear do the botanical addition affect cheese quality characteristics. This review aims to present the effect of added botanicals such as medicinal plants, herbs, spices and essential oils to cheeses in the function of protection against pathogens and spoilage microorganisms, as well as for their influence on the cheese quality.

Key words: **milk, cheese, botanicals, essential oil, quality**

Introduction

Milk is an important component of a balanced diet and contains numerous valuable constituents (Malbaša et al., 2009). Considerable acclaimed health benefits of milk and its products are related to their proteins, not only for nutritive value but also for biological properties (Nagpal et al., 2011; Malbaša et al., 2015). Milk is a nutrient-dense food with important nutritional value due to its calcium, cholecalciferol, protein, cobalamin, retinol, riboflavin, potassium, and phosphorus (Arimond et al., 2015). Sufficient content of the amino acid tryptophan, a niacin precursor, highlights milk as an important source of niacin equivalents (Friedman and Levin, 2012). Additionally, it contains different bioactive compounds with medicinal effects (Davoodi et al., 2016). Cheese as a main product obtained from milk presents human food consumed all over the world (Ranadheera et al., 2019). Cheese production from different types of milk and different technologies makes possible to produce a large number of products variety (Fox et al., 2016; Tarakçı and Deveci, 2019). Consumption of cheese has increased during past years because of its taste, high biological protein value, and affordable price on the market (Agarwal et al., 2015; Jeske et al., 2018).

Beside all excellent properties, cheeses are very susceptible to contamination by pathogenic and spoilage microorganisms, which can decrease the shelf life, entailing recalls, leading to a risk to the consumer's health (Oliveira et al., 2016). The most important pathogens associated with food outbreaks from the consumption of cheese are *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* spp. (Asselt et al., 2017).

Consumers concerned about health and wellbeing demand safer and healthier food free of synthetic and chemical preservatives which are considered harmful and carcinogenic (Carocho et al., 2014).

The food industry is becoming more specialized, continuously being developed to meet consumer needs. Consumers demand products that are safe and preferably free of synthetic additives. These additives are associated with health effects, in most cases without reasonable justification (Carocho et al., 2015). Consequently, consumers are looking for clearly labelled products that guarantee the absence of synthetic additives (Schleenbecker and Hamm, 2013). This has led to the need to search for natural additives, which the food industry claims are natural preservatives (Puvača et al., 2018).

Many plants are used as natural preservatives and medicinal plants, herbs, species and essential oils are the most widely used (Kostadinović et al., 2016; Puvača et al., 2016; Popović et al., 2018). Moreover, the natural preservatives are often used in animal's diet. In the past thirty years because of their potential antimicrobial effects botanical with their bioactive substances have attracted the increased interest of food scientists and technologists, as natural preservatives and promising alternative to synthetic ones in milk industry (Bouarab Chibane et al., 2019).

The aim of this review is to present the effect of added botanicals such as medicinal plants, herbs, spices and essential oils to cheeses in the function of protection against pathogens and spoilage microorganisms, as well as for their influence on the cheese quality.

Characteristics of botanicals and essential oils

Nowadays botanicals are commonly used in a broad industry spectre such as the pharmaceuticals industry, agriculture and in food production (Popović et al., 2017). In recent years, the interest in biologically active plant substances has been increased in the European countries, Japan and the USA (Puvača et al., 2018). The specific characteristics of the botanicals are creditable for their use in distilling the essential oils. The botanicals have been used as a natural alternative to synthetic substances for almost a century. Botanicals are commonly used as a topical antiseptic and antibacterial because of its distinguishing antimicrobial features. They reduce inflammation and may be effective in fungal infection treatments (Kostadinović et al., 2016).

Essential or volatile oils are aromatic oily liquids, extracted by distillation from plant parts, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits and roots. Because of their valuable proper-

ties and active substances, they have found their path in many different industries as well as in the dairy industry (Naughton et al., 2019). The aromatic oils used in food production include oils from oregano (*Origanum vulgare*), mugwort (*Artemisia vulgaris*), tea tree (*Melaleuca alternifolia*), turmeric (*Curcuma longa*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), cinnamon (*Cinnamomum zeylanicum*), thyme (*Thymus vulgaris*), ginger (*Zingiber officinale*), eucalyptus (*Eucalyptus globulus*), and garlic (*Allium sativum*) (Rao et al., 2019). The most common plants used for essential oils production are cedar (*Juniperus virginiana*), lavender (*Lavandula angustifolia*), chamomile (*Matricaria chamomilla*), lemon myrtle (*Backhousia citriodora*) and orange (*Citrus sinensis*). They are used as nutritive additives to feed and drinking water, but also in maintaining facility sanitation (Puvača et al., 2013).

The main active substances in plants and their essential oils found in higher concentrations and related to the antimicrobial activity are phenolic compounds such as, thymol, eugenol, carvacrol, and also constituents such as linalool, sabinene, menthol, myrcene, and camphene which explains their different antimicrobial and antifungal properties (Puvača, 2018).

Factors that may affect the concentration of active compounds in essential oils are genetic factors, climate conditions, soil, plant management as well as phonological stage. As a consequence, there are usually a lack of a standardized concentrations of these compounds (Tongnuanchan and Benjakul, 2014) so it is difficult to find a proper quantity of essential oil to be added without precise analytical methods used for determination of quantity of active compounds (Cheesman et al., 2017).

A very important aspect in the use of botanicals and their constituents in food is the toxicity (Puvača, 2018). Although medicinal plants, aromatic plants, herbs and spices that can be used in food preparation have been applied for a long time. There is no reported evidence of typical toxicological information such as acceptable daily intake or observed the adverse effect of botanicals usage (Kumar et al., 2017). Conversely, the botanicals and essential oils are usually classified as substances generally recognized as safe (Malcolm and Tallian, 2018).

Botanicals and essential oils in cheese

Botanicals and essential oils such as thyme, oregano, rosemary, cumin, pepper, and sage during the history showed excellent *in vitro* antimicrobial activity against pathogens and spoilage microorganisms which are associated with cheese contamination and could be usefully used in cheese preservatives (Macwan et al., 2016). Results from *in vitro* investigation cannot be with certainty confirmed as valid when the food is in question, having in mind the complexity of the food matrix, in which many different factors interfere with the activity of present bioactive components. One of the factors is a glycoprotein produced by the parietal cells of the stomach, that undermines the effectiveness of natural antimicrobial agents, the lipid content and protein present in food (Bisson et al., 2016). The present substances can wrap the surface of the microorganism, forming a physical barrier that prevents contact of the bioactive compound with the microorganism thus reducing the efficacy of the natural compound (Gouvea et al., 2017). Researches have shown that the addition of lipids in high concentrations reduced the inhibitory activity against *Listeria monocytogenes*, through the evaluation of lipid interference on the effectiveness of the essential oil antimicrobial activity simulating the food matrix (Chouhan et al., 2017). Similarly, antimicrobial activities of cinnamon and clove essential oils were lower in milk samples with higher fat content than in skim milk samples, which indicates the importance of possible interaction of essential oils with the composition of the raw milk (Cava et al., 2007).

Having in mind that cheese contains considerable concentration of proteins and lipids, the concentrations of natural compounds to be added to achieve the wanted amount of microbiological inhibition must be greater than those tested *in vitro* experiments (Robinson, 2015). To inhibit the growth of pathogenic microorganisms in cheese, the level of tested active compounds on the cheese used *in vitro* was effectively higher. Antimicrobial activity of *Thymus vulgaris* L. oil and of *Origanum vulgare* L. oil against *Staphylococcus aureus* both *in vitro* and on

fresh cheese was investigated to determine whether the use of essential oils can modify the microbiological or chemical-physical properties of the cheese. The antimicrobial activity against *S. aureus in vitro* was assessed by preparation of the aromatogram, minimum inhibitory concentration test, and minimum bactericidal concentration assessment. Raw sheep milk was experimentally contaminated with a strain of *S. aureus* ATCC 25922 and was used to produce three types of fresh cheese: without essential oils, with thyme and oregano essential oils both at a concentration of 1:1000. The samples were analyzed on the day of production, after three and seven days. The results obtained from the tests showed that the concentration of *S. aureus* and the counts of lactic flora remained unchanged for all types of cheese. Even the chemical-physical parameters were constant. The results of inhibition tests on the cheese disagree with those relating to the *in vitro* tests. Most likely this is due to the ability of essential oils to disperse in the lipids the food: the higher the fat content is, the lower the oil fraction will be able to exert the antimicrobial activity (Liu et al., 2017). The antibacterial activity of the essential oils of oregano and thyme added at doses of 0.1 or 0.2 and 0.1 mL 100 g^{-1} respectively, to feta cheese inoculated with Escherichia coli O157:H7 or *Listeria monocytogenes* was investigated during cheese storage under modified atmosphere packaging (MAP) of 50 % CO_2 and 50 % N_2 at 4 °C (Govaris et al., 2011). The compositional analysis showed that the predominant phenols were carvacrol and thymol for both essential oils. In control feta cheese inoculated with the pathogens and stored under MAP, results showed that *E. coli* O157:H7 and *L. monocytogenes* strains survived up to 32 and 28 days of storage. However, in feta cheese treated with oregano essential oils at the dose of 0.1 mL 100 g^{-1}, *E. coli* O157:H7 or *L. monocytogenes* survived up to 22 and 18 days, respectively, whereas at the dose of 0.2 mL 100 g^{-1} up to16 or 14 days, respectively. Feta cheese treated with thyme essential oil at 0.1 mL 100 g^{-1} showed populations of *E. coli* O157:H7 or *L. monocytogenes* not significantly different than those of feta cheese treated with oregano at 0.1 mL 100 g^{-1}. Although both essential oils exhibited equal antibacterial activity against both pathogens, the populations of *L. monocytogenes* decreased faster than those of *E. coli* O157:H7 during the refrigerated storage, indicating a stronger antibacterial activity of both essential oils against the former pathogen (Govaris et al., 2011). The antifungal activity of nanoemulsions encapsulating essential oil of oregano (*Origanum vulgare*), both *in vitro* and after application in cheese was evaluated. *Cladosporium* sp., *Fusarium* sp., and *Penicillium* sp. genera were isolated from cheese samples and used to evaluate antifungal activity (Bedoya-Serna et al., 2018). Minimal inhibitory concentrations (MIC) of non-encapsulated and encapsulated oregano essential oil were determined, and they were influenced by the encapsulation of the essential oil depending on the type of fungus. The antifungal activity of the nano encapsulated oregano essential oil in cheese slices showed no evidence of an effect of the MICs, when applied in the matrix. It was concluded that nano encapsulated oregano essential oil presented an inhibitory effect against the three genera of fungi evaluated. If environmental parameters, such as storage temperature and water activity, were controlled, the inhibitory effect of nanoemulsions of oregano oil could possibly be greatly improved, and they could be presented as a potential alternative for the preservation of cheese against fungal contamination (Bedoya-Serna et al., 2018). To assess the efficiency of plant essential oils as natural food preservatives in cheese was investigated. Samples of cheese were stored at 10 °C for about 6 days. The cell loads of spoilage and useful microorganisms were monitored to calculate the microbial acceptability limit. Results show that some tested compounds were not acceptable by the panel from a sensorial point of view. Most compounds did not affect the microbial acceptability limit value to a great extent, and only a few such as lemon, sage, and thyme markedly prolonged the microbial acceptability limit of the investigated fresh cheese. Moreover, the above active agents exerted an inhibitory effect on the microorganisms responsible for spoilage without affecting the dairy microflora (Fazlara et al., 2008).

Influence of botanicals and essential oils on pathogenic microorganisms in cheese

Photogenic microorganisms such as *L. monocytogenes*, *S. aureus*, *E. coli*, and *Salmonella* spp. present a major problem in the dairy industry and in dairy products. Thus the inhibition of pathogenic microorganisms are commonly reported in cheeses by essential oils (Table 1), and for that reasons they have been widely investigated over the years (Cancino-Padilla et al., 2017).

The effects of *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils in combination on the counts of a mesophilic starter co-culture and *E. coli* O157:H7 in cheese was investigated (Diniz-Silva et al., 2019). Results of these investigations show that essential oils did not decrease the counts of *Lactococcus* spp. while a decrease in counts of *E. coli* O157:H7 in the storage was noticed. Also, research showed that *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils did not affect the physicochemical parameters. These results show the prevalent terpenes of *Origanum vulgare* L. and *Rosmarinus officinalis* L. essential oils in fresh cheese during refrigerated storage and suggest their incorporation during manufacture as a strategy to preserve this product, with particular effects on the survival of *E. coli* O157: H7 (Diniz-Silva et al., 2019).

Gram-positive psychotropic bacteria such as *L. monocytogenes* have the ability to form biofilms that may persist for years on the surface of equipment and handling devices (Colagiorgi et al., 2017). *L. monocytogenes* is more severe in risk groups such as elderly people, pregnant women, and immunodeficiency. The use of essential oils and botanicals

TABLE 1. Inhibitory effects of selected essential oils on different cheeses verities

Cheeses	Essential oils	Target bacteria inhibition	Reference
Feta	*Origanum vulgare*	*Listeria monocytogenes* *Escherichia coli*	Govaris et al. (2011)
Domiati	*Nigella sativa*	*Salmonela enteritides* *Escherichia coli*	Hassanien et al. (2014)
Brie	*Thymus vulgaris*	*Staphylococcus aureus* *Yersinia enterocolitica*	Zantar et al. (2014)
Feta Roquefort Manchego Ricotta	*Rosmarinus officinalis*	*Clostridium* spp.	Moro et al. (2015)
Cheddar	*Origanum vulgare* *Allium sativum* *Rosmarinus officinalis* *Salvia officinalis*	*Listeria monocytogenes*	Tayel et al. (2015)
Lighvan	*Mentha pulegium*	*Listeria monocytogenes*	Sadeghi et al. (2016)
Curde	*Thymus mastichina*	*Pseudomonas* sp. *Staphylococcus* sp.	Carvalho et al. (2018)
Béja Sicilian	*Pimpinella saxifraga*	*Escherichia coli* *Pseudomonas aeruginosa* *Salmonella typhimurium* *Listeria monocytogenes* *Micrococcus luteus* *Bacillus cereus*	Ksouda et al. (2019)

has provided anti-Listeria activity in cheeses. Reduction in a population of *L. monocytogenes* after 18 days of storage of Feta cheese with the addition of essential oils from oregano and thyme was also reported (Colagiorgi et al., 2017). Results for thyme essential oil is similar, being effective in the inhibition of the two strains of *L. monocytogenes*, which may be due to similar concentrations of the antimicrobial components present in both oils (Mith et al., 2014). Besides essential oils, botanical such as garlic had been reported to poses the inhibition of *L. monocytogenes*, during the storage of Cheddar cheese, at room temperature (Gouvea et al., 2017).

Another gram-positive bacteria *S. aureus* is mainly reported in cheeses produced under poor hygiene conditions. This pathogen produces enterotoxin that can endanger the consumers' health, hence this has also been investigated in several studies with essential oils (Gouvea et al., 2017). With the usage of essential oils of hot peppers in Egyptian traditional cheeses reduction of *S. aureus* can be detected with high significance. A similar tendency in reducing the *S. aureus* in fresh curd cheese has the thyme essential oil (Swamy et al., 2016). Opposite to Gram-positive bacteria, Gram-negative bacteria such as *E. coli* 0157:H7 and *Salmonella* spp. have been seen to be more resistant to applied essential oils. This fact comes from the studies that have proven the lower antimicrobial efficacy of essential oils in Gram-negative bacteria, because of their cell wall structure increased resistance (Chouhan et al., 2017).

Essential oil of black cumin showed very strong inhibitory effect against *S. enteritides* and *E. coli*, while the same effect was not observed regarding the population of *L. monocytogenes* and *S. aureus* (Hassanien et al., 2014).

The effects of *Zataria multiflora* essential oil alone and in combination with probiotic bacterium *Lactobacillus acidophilus* PTCC 1643 on the growth and stability of *E. coli* O157 in cheese were investigated during storage time (Mehdizadeh et al., 2018). Various concentrations of the *Zataria multiflora* essential oil alone and in combination with *L. acidophilus* significantly decreased the *E. coli* O157 enumerations. Also pH decline, the number of *E. coli* O157 also decreased in cheese samples. The synergistic effects on the stability of *E. coli* O157 was not observed in different treatments of *Zataria multiflora* essential oil and *L. acidophilus*. The addition of *Zataria multiflora* essential oil and probiotics in samples showed a synergistic trend on the reduction of *E. coli* and significantly improved sensorial properties (Mehdizadeh et al., 2018).

Influence of botanicals and essential oils on the sensory properties of the cheese

Herbs, spices, medicinal plants and aromatic plants come from different parts of the plants are used to impart an aroma and taste to food (Puvača et al., 2013). Some of them have therapeutic properties such as antioxidative, anti-inflammatory, antidiabetic, antihypertensive and antimicrobial activities as it was previously described (Puvača et al., 2018). Therefore, fortification of dairy foods with botanicals and essential oils could help to provide functional dairy products with nutritional and medicinal values. Also, botanicals are used to improve the appearance and attractiveness of fortified foods for consumers and to increase the sale of those botanicals. Only the highest quality botanicals and their essential oils can be added to dairy products to combat contaminating microorganisms (El-Sayed and Youssef, 2019).

Today, consumers are aware of the relationship between their eating habits and nutritional status. Consequently, they look for dairy products that are added to natural products rather than synthetic chemical compounds (Granato et al., 2018). Some companies have created dairy products with the partial or total replacement of those synthetic additives by natural herbal extracts not only because of their antioxidant and antimicrobial properties but also because of the sensory aspects they confer into products. The dairy sector has invested in this segment of healthy foods and yogurts, fermented milk, cheeses, and other milk-based formulations have been added with herbal extracts to attract the consumer's attention and propel the sales of these foods (Granato et al., 2018).

The addition of botanicals and essential oils as flavouring substances in some varieties of cheeses is already a common practice worldwide. One

of the most popular cheeses of Turkey is produced with the addition of 25 different medicinal and aromatic plants (Gouvea et al., 2017). However, added essential oils and different herbs and spices often have a strong flavour even when used in very low amounts, which can also cause a high sensory impact with the possible rejection of the dairy products, which can present a limiting factor in the use of herbs and their essential oils in the dairy industry (Lucera et al., 2012). From customer or buyer point of view visually and sensory acceptance of the product is extremely important because just having a high antimicrobial effect is not enough (Miltgen et al., 2016). Among the botanicals and essentially added to cheeses, oregano, black pepper, garlic, lemongrass, rosemary, and thyme, resulted in good sensory acceptance (Josipović et al., 2015).

A combination of different extracts and essential oils has been investigated with the aim to minimize the possible negative effects of botanicals and their essential oils on the flavour of the product which can result in a good antimicrobial effect at lower individual concentrations (Swamy et al., 2016). In some cases, synergistic effects can be expressed when several combined compounds might exert stronger antimicrobial activity than when added separately. According to some research combination of essential oils from oregano and thyme is more effective in the antimicrobial activity against pathogenic *Bacillus cereus*, *L. monocytogenes*, and *Pseudomonas aeruginosa* than when each of the essential oils is used separately (Swamy et al., 2016). Application of technologies such as ultrasound, high pressure, and electric pulses damages the cell membrane, thus facilitating cell penetration and action of antimicrobials, so the combined use of botanical as natural compounds with the application of these contemporary technologies can also allow the use of lower concentrations of essential oils in the production of dairy products (Lopez-Romero et al., 2015). Preparing and producing a healthy new dairy product that attracts consumers is an important issue (Milanović et al., 2012). Assessing the effects of supplementation of milk with an essential oil to improve the quality cheese is the main target of dairy industry. Basil essential oil in the preparation of cheese has shown good microbiological, chemical and sensory properties, while added basil oil inhibited mold and yeast growth in cheese. The pH values decreased during the storage period and the addition of essential oil improved the sensory properties of treated cheese (Abbas et al., 2018).

Conclusions

The presented results have shown that the use of natural preservatives could increase shelf life of cheese and minimize the risks to the consumer's health related with cheese consumption. Botanical and essential oils have proved to be natural preservatives with significant inhibitory activity against major pathogens and spoilage microorganisms in cheeses, but the further in vitro investigation in the spotlight of their mechanism of action, proximate, physical and sensory characteristics is more than necessary. It is also very important to perform investigations related to combinations and possible interactions among different essential oils. Furthermore, consumer acceptance of sensory properties of such cheese should not be underestimated.

Acknowledgements

This paper is a part of the project III 46012 which is financed by Ministry for Education, science and technological development of the Republic of Serbia.

Antimikrobna učinkovitost ljekovitog bilja i njihov utjecaj na kvalitetu sireva

Sažetak

Mlijeko i mliječni proizvodi najstarija su i najčešće konzumirana hrana širom svijeta. Ističu se kao izvor visokokvalitetnih proteina i najvažniji izvor bioaktivnih peptida. Mliječni proteini imaju visoku nutritivnu vrijednost i izvanredna ljekovita svojstva. Poznati su kao potencijalni sastojci funkcionalne hrane koja

promiče zdravlje, a mliječna industrija već je komercijalizirala mnoge mliječne proteine i proizvode na bazi peptida koji se mogu konzumirati kao dio redovite dnevne prehrane. Osim toga, sir je vrlo osjetljiv na kontaminaciju patogenim i mikroorganizmima kvarenja, što rezultira smanjenjem njegovog roka trajanja i uzrokuje ozbiljne rizike za zdravlje potrošača. Danas se prehrambena industrija sve više specijalizira, a metode obrade se stalno razvijaju kako bi zadovoljile potrebe i zahtjeve potrošača. Potrošači zahtijevaju proizvode koji su sigurni i po mogućnosti bez sintetskih aditiva, što je dovelo do potrebe za traženjem prirodnih alternativa. Ljekovito bilje i njihovi ekstrakti zanimljiva su prirodna alternativa za očuvanje i poboljšanje kvalitete sira. Neke su tvari pokazale dobre učinke protiv većine uzročnika kontaminacije sira, kao što su *Listeria monocytogenes*, *Staphylococcus aureus* i *Salmonella* spp. Ipak, nije sasvim jasno utječe li dodavanje ljekovitog bilja na promjene karakteristika sira. Cilj ovog rada bio je prikazati učinak dodanog ljekovitog bilja, začina i eteričnih ulja na sireve u funkciji zaštite i kvarenja uzrokovanog patogenima i mikroorganizmima, kao i njihovog utjecaja na kvalitetu sira.

Ključne riječi: **mlijeko, sir, ljekovito bilje, eterično ulje, kvaliteta**

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