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REVIEWS

APPLICATION OF ESSENTIAL OILS AS NATURAL PRESERVATIVES IN CHEESEMAKING

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ABSTRACT

INTRODUCTION: **Cheese occupies an important place among dairy products and has been present in the diet of people since ancient times. The production of cheese allows one to concentrate the most valuable ingredients of milk—proteins and fats. From the concentrate, durable products with proven taste qualities and a healthy effect are obtained. Cheeses are products that are easily susceptible to contamination with microorganisms, leading to their spoilage. This fact is the reason for the withdrawal of large amounts of finished products from the market and, above all, a high risk to the health of consumers. Although cheeses have a relatively long shelf life, effective preservation techniques must be used before the products are commercialized. The costs of preservative procedures to prevent or control the surface growth of molds and yeasts in cheese are high. These procedures aim to decrease and inhibit spoilage microorganisms without interfering with the lactic bacteria responsible for the final characteristics of the cheese.**

AIM: **This article aimed to summarize the antimicrobial potential effects of essential oils, obtained from different plant sources, that are used in various types of cheese as natural preservatives.**

MATERIALS AND METHODS: **Data on essential oils used as natural preservatives have been gathered from various scientific publications found through academic search engines, such as Google Scholar, ResearchGate, PubMed, Web of Science, Scopus, and others. Additionally, supplementary sources have been used, including online resources and books from scientific libraries.**

RESULTS: **Essential oils obtained from medicinal plants, herbs, and spices are natural biologically active mixtures. Many of them have a special place in cooking, medicine, pharmacy, cosmetics, and perfumery. The food industry's growth drives the demand for products with high organoleptic qualities. Essential oils, rich in active ingredients like terpenoids, terpenes, coumarins, and flavonoids, show antimicrobial and preservative properties valuable to the industry.**

CONCLUSION: **Essential oils are natural products that have the potential to be a green alternative to synthetic preservatives. Their effects are observed at low concentrations; they are not toxic to humans, and are considered safe in canning.**

Keywords: *essential oils, natural preservatives, cheese, diary products*

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INTRODUCTION

Cheese occupies an important place among dairy products and has been present in the diet of people since ancient times (1). The production of cheese allows one to concentrate the most valuable ingredients of milk, proteins and fats (2). From the concentrate, durable products with proven taste qualities and a healthy effect are obtained. To date, an increase in consumer interest in farm and artisanal products has been reported. They are preferred primarily because of the ecologically clean raw materials from which they are produced, the absence of harmful additional ingredients, and their good taste. However, there is also the perception that the microbial safety control of these products may be understated, although their physicochemical characteristics, such as salinity, acidity, water content, and others, characterize it as natural and beneficial. Cheeses are products that are easily susceptible to contamination by pathogens and microorganisms, leading to their spoilage. This fact is the reason for the withdrawal of large amounts of finished products from the market and, above all, a high risk to the health of consumers. The pathogens *Listeria monocytogenes, Staphylococcus aureus,* and *Salmonella spp*. are identified as the most common cause of deterioration of the microbial safety of cheese (3).

Important reasons for the organoleptic and nutritional properties of cheese are changes in fat and protein content during ripening. Lipolysis is a biochemical process accountable for creating the desired taste of various cheese varieties. Nevertheless, excessive levels of short-chain fatty acids can induce a rancid flavor in aged cheeses (4). As a result of the catabolism of amino acids, fatty acids, and lactic acid, volatile compounds are also formed that greatly affect the taste of cheese (5). Microbial lipases, proteases, and mycotoxins are also responsible for generating unpleasant flavors and discoloration in cheeses, leading to a decrease in quality and microbial safety (6). Economic losses to producers are reported during cheese ripening, followed by a long storage period, especially if spoilage processes also occur (7). Therefore, it is very important to avoid deterioration of the quality of the dairy product at all stages (8).

The surface growth of yeasts and molds in different types of cheese requires control and its costs are high. Processing of the products aims to reduce and inhibit the growth of spoilage microorganisms without affecting the milk bacteria responsible for the final characteristics of the cheese. Direct heat, light, and oxygen access have been proven to lead to various degradation processes, as a result of which toxic substances are formed and the organoleptic and nutritional properties of cheese are affected (9). Due to the composition, production technology, and others, the approaches for the preservation of cheese, which are applied in storage and marketing, must be specifically studied for each variety of cheese.

Adding various additives directly to foods is one of the oldest and easiest preservation techniques. Currently, cheese canning is done more frequently with chemical or biological additives (10,11). Approved food preservatives in the EU that are used for ripened cheeses are divided into three functional groups. According to cheese production technology, these compounds are added to the milk bath as antimicrobial agents and antioxidants, or as surface protection against unwanted agents. Compounds approved for the addition to ripened cheeses are sorbic acid/sorbates, lysozyme, nisin, natamycin, hexamethylene tetramine, propionic acid/propionates, and nitrates/nitrites (12).

In recent years, a clear trend to purchase products without preservatives or with maximum natural preservatives has emerged in the consumer demand for food products. This fact forced the food industry to seek and start using plant and microbial preservatives in their production instead of the standard artificial ones. Essential oils (EOs) are volatile compounds employed in cosmetics, pharmaceuticals, traditional medicine, flavoring, and food conservation. These diverse compounds have significant antibacterial, antioxidant, antifungal, analgesic, and antiseptic properties, which they achieve through different mechanisms of action.

Nowadays, replacement of synthetic preservatives with natural ones is becoming increasingly preferred, as consumer demand is directed toward healthy foods. Generally recognized as safe (GRAS) food additives are widely used in the food industry (13). Although no specific category has been defined for natural supplements, there is a wide variety of natural antimicrobials, antioxidants, sweeteners, and colors that are the product of microorganisms, plants, and animals. For example, bacteriocins, natamycin, and reuterin are derived from microbial sources. Lactoperoxidase, lysozyme, and lactoferrin are of animal origin, and EOs are of plant origin. All of the above-mentioned compounds can be defined as natural preservatives (14). Essential oils are lipophilic plant products, ordinary yellowish-colored liquids, containing a very rich range of biologically active substances. A small part of their effects are related to their antimicrobial and antioxidant properties (15,16). There are various reports that present the proven antimicrobial properties of EOs. Particularly impressive is the fact that they act against a very wide range of pathogenic microorganisms, including molds and yeasts (17,18,19,20,21). The hydrophobic nature of the components in EOs facilitates their easy passage through the lipophilic bacterial membrane. Entering the cells, they block the mechanisms of molecular transport and enzyme activity, thus inactivating the cell (22,23). Essential oils generally inhibit Gram-positive bacteria more strongly than Gram-negative bacteria due to the structural characteristics of the lipopolysaccharide barrier in the outer membrane of Gram-negative bacteria (24,25).

The Food and Drug Administration (FDA) of the United States and the European Commission (EC) of Europe legally regulate the application of EOs and extracts in food. Essential oils and extracts have been confirmed as GRAS by the FDA and are consequently not subject to quantitative limitations on usage; yet their utilization must align with good manufacturing practices. Technically, they are not considered supplements, as no government approval is required before use (26,27,28). Therefore, their use in foods and cheeses is safe and legal in the USA. As per the regulatory framework in Europe and the European Union, EOs are not classified as additives, and their inclusion in food is prohibited. However, numerous EO components, such as limonene, are acknowledged as flavoring agents. It is crucial to distinguish between flavors and additives, both categorized as food enrichment agents. Concerning extracts, only rosemary extract, designated with the code number E392, is permitted for use as a food additive within the specified maximum quantities across various food categories in the European Union (29,30). However, the trend in the coming years is for more extracts to be approved for use in food. According to previous regulations, neither rosemary extracts nor flavorings can be included in cheeses and cheese products in the European Union. This limits the variety of new products that can be traded (31).

AIM

This article aimed to summarize the antimicrobial potential effects of EOs obtained from different plant sources that are used in various types of cheese as natural preservatives.

MATERIALS AND METHODS

Data on EOs used as natural preservatives were obtained through literature publications using different search engines of scientific literature, including Google Scholar, ResearchGate, PubMed, Web of Science, Scopus, etc. Other references to the literature were also used, such as online sources and scientific library books.

RESULTS AND DISCUSSION

Mechanism of Action of Essential Oils

Essential oils are a blend of volatile and fragrant compounds, typically clear to faintly yellow liquids, insoluble in water, yet soluble in organic solvents (32). Almost 3000 different EOs have been identified, and about 300 of them are commercially used in the perfume and fragrance market (22). Their composition is determined by several characteristics and can vary depending on the type of plant, geographical origin, climatic conditions under which it is grown, soil composition, the part of the plant used to extract EOs, and the phase of the vegetative cycle in which the plant is harvested (33,34). They are secreted as secondary metabolites. According to some scientists, they have a protective function against bacteria and fungi and, according to others, they are involved in pollination processes (35). Essential oils are obtained primarily by steam or water distillation, maceration, and supercritical fluid extraction (22,36,37). While EOs are primarily utilized as flavorings in the food sector, their antimicrobial properties render them valuable for prolonging shelf life and exploring new facets of their attributes. This includes investigating minimum inhibitory concentrations (MICs), targeting novel microorganisms, understanding their mechanisms of action, and potential interactions with food matrices and sensory attributes. The antimicrobial efficacy of EOs is entirely contingent upon their active constituents (38). Approximately 90–95% of all EOs contain volatile components, including monoterpene and sesquiterpene hydrocarbons, their oxidized derivatives, in addition to aliphatic alcohols, aldehydes, and esters. The non-energetic part in the composition of EOs includes sterols, fatty acids, hydrocarbons, coumarins, flavonoids, and waxes and represents about 5–10% of the total EO (39). The most active antimicrobial agents in the composition of EOs are four main groups according to their chemical structure: terpenes and terpenoids (cymene, limonene, thymol, carvacrol), phenylpropenes (eugenol, vanillin), and others, such as allicin or isothiocyanates (38).

The main mechanism of action of EOs against various microorganisms is not fully understood and cannot be determined unequivocally. There are several target sites in microorganisms that are hypothesized to be the target of the EO action (32,40). The probable mechanisms of antimicrobial action of EOs are shown in Fig. 1. The antimicrobial activity of EOs is directly related to the hydrophilic or lipophilic nature of their main components, the type of microorganism, and the structural organization of their cell

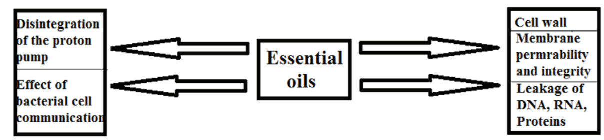

Fig. 1. Probable mechanisms of action of essential oils.

walls. Essential oils have been shown to be more effective against Gram-positive bacteria than against Gram-negative bacteria. The reason for this lies in the more complex cell wall of Gram-negative bacteria (38,41,42). Gram-positive bacteria have a wall containing up to 40 layers of murein and few other components. In Gram-negative bacteria, the murein network is single-layered and over it is a lipid membrane similar to the cell, but much more permeable. It is called the outer membrane. The carbohydrate chains of lipopolysaccharides protrude from it. These, together with the outer membrane itself, pro-

Table 1. Various types of EOs used as natural preseravtives in different types of cheese.

Type of Essential Oil	**Microorganism Type**	**Type of Cheese**
Mentha longifolia	*S. aureus, L. monocytogenes, E. coli*	Cheese
Mentha spicata	*L. monocytogenes*	Lighvan cheese
Mentha pulegium	*L. monocytogenes*	Iranian white brine cheese
Orignaum vulgare	*E. coli*, *L. monocytogenes*, *S.* Enteritidis, *S. aureus*	Tuma sheep cheese
Orignaum vulgare	*Escherichia coli O157: H7, Listeria monocytogenes*	Feta cheese
Rosmarinus officinalis	*E. coli*	Minas Frescal cheese
Rosmarinus officinalis	*E. coli*	Coalho cheese
Thymus algeriensis	*Penicillium aurantiogriseum*	Soft cheese
Thymus vulgaris	molds and yeasts	Cream cheese
Thyme EO	*Escherichia coli O157: H7, Listeria monocytogenes*	Feta cheese
Black cumin	*Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 8739, Listeria monocytogenes Scott A, Salmonella enteritidis PT4*	Domiati cheese
Cinnamomum zeylanicum	*Aspergillus terreus, Aspergillus ustus, Aspergillus niger, Aspergillus aculeatus, Penicillium brevicompactum, Scopulariopsis brevicaulis*	Wagashi cheese
Ocimum gratissimum	*Aspergillus terreus, Aspergillus ustus, Aspergillus niger, Aspergillus aculeatus, Penicillium brevicompactum, Scopulariopsis brevicaulis*	Wagashi cheese

vide the murein layer with some protection against adverse factors. The lipophilic nature of the hydrocarbon skeleton and, on the other hand, the hydrophilic nature of the functional groups in EOs determine the potential for antimicrobial effects. Phenolic compounds exhibit the most potent antimicrobial effects, succeeded by aldehydes, ketones, alcohols, ethers, and hydrocarbons (42). The efficacy of phenols stems from the acidic properties of the hydroxyl group. These substances regulate cell permeability, hinder enzymes crucial for energy production, and disturb protein transport, ultimately resulting in cellular demise (43).

Essential Oils as Natural Preservatives

Essential oils obtained from medicinal plants and spices are biologically active natural substances. Various EOs used as cheese preservatives are presented in Table 1.

Mentha ssp. occupies a special place in cooking, medicine, pharmacy, and cosmetics. The development of the food industry leads to the desire to produce products with increasingly high organoleptic properties. Shahdadi et al. investigated the antibacterial properties of *M. longifolia* EO and the active component pulegone incorporated into edible chitosan and alginate coatings against *S. aureus, L. monocytogenes*, and *E. coli* in cheese. The authors isolated the EO from a preexisting drug. To refine the chemical compounds of the EO used, the authors performed a gas chromatography-mass spectrometry (GC-MS) analysis. Pulegone (≈ 26%) was found as the main component in the highest percentage, followed by piperitone oxide (≈ 20%) and piperitone (≈ 12%). Cheese samples were prepared according to an established methodology and pH, salt content, and moisture content were tested (44). The results showed that the incorporation of peppermint EO and pulegone in edible coatings significantly reduced bacterial growth during storage. The authors found that the bacterial population decreased significantly and that pulegone was found to have a stronger effect compared to peppermint EO. The samples showed higher antibacterial activity against *E. coli* than the other bacteria used (45).

Moosavy et al. (2013) investigated traditional Lighvan cheese. They found that *Mentha spicata* EO acted against a particular strain of *Listeria*. They described that the EO significantly reduced *L. monocytogenes* growth at EO concentrations of 2 or 2.5% v/w. They found variations in the effect at different ripening temperatures of 4°C or 14°C and salt water concentrations of 12% or 15% over a period of 60 days. The authors described a pattern associated with a reduction in the number of pathogens. Increased EO quantities, higher saltwater concentrations, ripening temperatures, and storage durations resulted in the diminishment of microorganisms. Specific environmental conditions, like temperature and salt content percentage, have been determined to interact synergistically with EOs, enhancing their efficacy (46).

Sadeghi et al. applied *Mentha pulegium* EO at concentrations of 7.5, 15, or 30 μL/mL against *L. monocytogenes* inoculated at 103 cfu/mL in Iranian white brine cheese for a period of 60 days. The results showed that, in the control samples without EO, on day 7 the pathogen growth increased, but gradually decreased within the 60-day period. In the samples tested, the maximum growth lasted 14 days, after which the number of bacteria decreased logarithmically. The highest antibacterial effect was reported at a concentration of 0.03%, although a lower concentration of 0.015% was considered optimal for consumers (48).

Garofalo et al. used oregano EO to process the Tuma fresh sheep cheese obtained by pressed cheese technology. They reported that the addition of oregano EO did not affect the ash, fat, or protein content of the product, but increased the antioxidant capacity of experimental cheeses by 43%. To investigate the ability of oregano EO to be used as a natural preservative, an artificial contamination test was conducted on the samples and the results showed a significant reduction of the main pathogens in cheese with oregano EO included (48).

Another study investigated the activity of oregano EO in cheese inoculated with *E. coli O157:H7* and *L. monocytogenes*. The concentration of the EO used for its reported activity was 0.1 mL and 0.2 ml, respectively, per 100 g of feta cheese. The study was conducted by monitoring the cheese's storage time in modified atmosphere packaging containing 50% CO_2 and 50% N_2 at 4°C. Carvacrol and thymol were identified as the primary constituents of the EO. In the control feta cheese, which was inoculated with pathogens and stored under the aforementioned conditions, the results revealed that the strains survived for up to 32 and 28 days for *E. coli* and *L. monocytogenes*, respectively. However, in feta cheese treated with oregano EO at the lower dosage, both bacteria endured for a shorter storage period, surviving up to 22 and 18 days, respectively. At the higher EO dosage, bacteria persisted for only 16 or 14 days, respectively (49).

Fernandes et al. examined the impact of encapsulated rosemary EO and various additional components on the shelf life of Minas Frescal cheese. The EO was incorporated into the cheese both individually and in microencapsulated formats at concentrations of 0.5%, and then compared with a control cheese devoid of rosemary EO. The results showed that the level of aerobic mesophilic pathogen was lower in the samples with microencapsulated rosemary EO than in the other samples. Encapsulated rosemary EO decreased pathogenic microorganisms by over 90% after 3 days of storage and by less than 90% after 15 days of storage, compared to the control. The microencapsulation procedure maintains the chemical composition of the rosemary EO and aids in regulating the acidity of the cheese. The findings suggest that rosemary EO holds promise as a potential natural preservative in cheese. Microencapsulated rosemary EO successfully controlled the proliferation of mesophilic bacteria in Minas Frescal cheese by retarding the growth of microorganisms during storage, thus extending the shelf life of the product (50).

Ribeiro et al. inoculated Coalho cheese with a multidrug resistant strain of *E. coli* to study its growth after adding rosemary EO to the cheese. They determined that the minimum inhibitory EO concentration against the test strain was 20% (v/v). Two cheese samples were inoculated with the *E. coli* strain EC16. A test sample was enriched with 20% REO (v/v) and a control sample was without the addition of rosemary EO. Both samples were of the same size and were kept cool. During the seven-day cooling period, the number of *E. coli* in each sample was determined regularly. A 100% reduction was observed in the first 24 hours in the test sample. The results showed that rosemary EO has inhibitory activity against *E. coli* in a food matrix. This makes it suitable for the control of pathogenic microorganisms, but its use as a food preservative should not degrade the sensory characteristics of the product (51).

Bukwicki et al. explored the antimicrobial attributes of *Thymus algeriensis* EO as a preservative in soft cheese and devised a novel method to evaluate the preservative's efficacy. They observed no contamination of cheese with *P. aurantiogriseum* after 30 days of storage at 4°C with the addition of 25 μL of EO. The antimicrobial effectiveness of thyme EO was assessed against 8 bacteria and 8 fungi. The findings demonstrated inhibitory effects of the EO against the tested bacteria at concentrations ranging from 0.03 to 0.09 mg/mL, with bactericidal effects observed at 0.05 to 0.15 mg/mL. Regarding antifungal activity, MICs ranged from 0.01 to 0.04 mg/mL, and minimum fungicidal concentrations ranged from 0.01 to 0.04 mg/mL. Furthermore, the EO exhibited significant antiradical activity, achieving half-maximal inhibitory activity at 0.132 mg/mL. Gas chromatographic analysis was also performed to determine the composition of thyme EO, which identified carvacrol as the main compound, representing ≈81% of the total components, followed by p-cymene ≈8% (52).

Thymus vulgaris EO exhibits potent antimicrobial and antioxidant properties, thereby enhancing and prolonging the shelf life of food items. To assess its impact on the physicochemical, microbial, and sensory attributes of cream cheese, various proportions of thyme EO were incorporated. The cheese samples were evaluated during the first, fourth, and seventh weeks of storage. Statistically, the outcomes revealed that different EO concentrations significantly influenced acidity, dry matter, adhesiveness, IC50, and peroxide levels in the cheese. With an increase in EO percentage, acidity, adhesiveness, peroxide, and IC50 levels decreased. However, alterations in EO concentration did not yield significant effects on cheese pH, fat, firmness, or protein content. Concerning coliforms, the addition of thyme EO showed no noteworthy impact, although notable reductions in molds and yeast were observed. Essential oil addition did not affect cheese color and texture, but it did significantly influence taste and sensory attributes. Higher EO concentrations diminished the sensory characteristics of cheese, with the optimal concentration determined to be 0.005% (53).

Black cumin seed oil was evaluated against several pathogenic bacteria *(S. aureus ATCC 6538, E. coli ATCC 8739, L. monocytogenes Scott A, and S. enteritidis PT4)* in Domiati cheese during refrigerated storage. The study documented chemical, physical, and sensory alterations in the cheese throughout storage. Pasteurized milk was pre-inoculated with a mixed bacterial culture prior to curdling. The antimicrobial efficacy of the EO was assessed both in vitro and in situ in regard to the growth of *S. aureus, E. coli, L. monocytogenes,* and *S. enteritidis* in both medium and cheese samples. Addition of EO to cheese samples at concentrations of 0.1% or 0.2% w/w significantly diminished the population of inoculated pathogens after 21 days of storage. Moreover, black cumin EO was found to regulate titratable acidity development, mitigate changes in ripening indices and aroma components, and preserve the essential physicochemical and sensory attributes of the cheese (54).

The current investigation explored the impact of basil EO at varying concentrations on the chemical composition and sensory attributes of full-fat and low-fat Tallaga cheese. The two variants of buffalo milk cheese were divided into four equal portions. Basil oil was excluded from the control sample, while the other batches contained respective concentrations of 0.05%, 0.10%, and 0.15%. The cheese samples were then stored at 6 ± 1°C for 30 days, with chemical and sensory assessments conducted at 0, 10, 20, and 30 days. Gas chromatography-mass spectrometry analysis of the EO utilizing a flame ionization detector revealed that *O. basilicum* EO primarily comprised methylchavicol (≈ 72%), followed by cineole (≈ 12%), linalool (≈ 12%), methylenephenol (≈ 3%), and caryophyllene (≈ 1%). The storage duration significantly influenced the chemical composition of the cheese samples. Concerning sensory attributes, it was observed that the cheese infused with basil exhibited a heightened taste compared to plain cheese over the storage period. However, no noteworthy disparities were detected in the appearance or chemical composition of the cheese between plain and basil-infused variants (55).

The primary aim of Abbas et al. was to assess the impact of milk enriched with EOs on enhancing the quality of ultrafiltered soft buffalo cheese. Traditional preparation methods were employed for UF soft buffalo cheese, comprising control samples (C) devoid of supplementation, alongside three samples supplemented with extracted basil EO at concentrations of 0.025%, 0.050%, and 0.075% v/v. Microbiological, chemical, and sensory characteristics of the resultant samples were evaluated at various intervals (fresh, 15, 30, and 60 days). Findings indicated that a higher concentration of EO (0.075%) facilitated yeast growth, followed by 0.050% and 0.025%. Concurrently, EO inhibited mold and yeast proliferation across all soft UF cheese samples. pH values declined throughout the storage period due to the sourdough starter's activity. Incorporating EO enhanced the sensory attributes of UF soft buffalo cheese compared to the control. In summary, the addition of basil EO to UF soft buffalo cheese bolstered sourdough growth, suppressed mold and yeast proliferation, and enhanced the sensory characteristics of the end product (56).

Essential oils offer a natural substitute for additives or pasteurization in thwarting microbial spoilage across various food substrates. Licon et. al explored the antimicrobial efficacy of *Melissa officinalis, O. basilicum*, and *T. vulgaris* EOs against three distinct microorganisms: *E. coli, C. tyrobutyricum*, and *P. verrucosum*. They utilized pressed sheep's cheese derived from milk enriched with EO (250 mg/kg) as a model. Findings revealed that samples containing *T. vulgaris* EO notably diminished the population of *C. tyrobutyricum* and completely suppressed the growth of *P. verrucosum*, all while preserving the indigenous microflora present in the cheese. However, the inhibitory effect of *M. officinalis* EO on fermenting lactic acid bacteria rendered it unsuitable for this particular matrix (57).

Minas Frescal cheese is a high-humidity product in which several microorganisms can easily spread. Essential oils are natural products that can be used to improve the antimicrobial resistance of dairy products. This study aims to analyze the antimicrobial activity of the oregano (*Origanum vulgare*) and rosemary (*Rosmarinus officinalis*) EOs against *E. coli* and *S. aureus* in Minas Frescal cheese. The MIC for oregano EO was found to be 0.25% and against *S. Aureus*—1%, which also coincides with the minimum bactericidal concentration for burdock oil. In the case of rosemary EO, the MIC against *E. coli* was determined to be 8%. However, no antimicrobial activity of rosemary oil was observed against *S. aureus*.

As for the physicochemical attributes of the cheese, no alterations were detected. Utilizing EOs as antimicrobial agents holds promise for industrial applications, although careful consideration must be given to the specific microorganism, type of oil, and sensory properties of the product (58).

The fungicidal properties of *C. zeylanicum* and *O. gratissimum* EOs were investigated against six mold isolates of Wagashi cheese. Findings revealed that *O. gratissimum* EO exhibited fungistatic activity against all species, with MIC values ranging from 400 to 1000 mg/L. It also demonstrated minimum fungicidal concentration (MFC) against *A. terreus* 1000 mg/L) and *S. brevicaulis* (MFC 600 mg/L), which were the most susceptible strains. Conversely, *C. zeylanicum* displayed inhibitory effects solely against *A. terreus, S. brevicaulis*, and *P. brevicompactum*, with *A. aculeatus* being the most resilient strain to this EO. The heightened efficacy of *O. gratissimum* EO against all tested strains could be attributed to the presence of its key constituent, thymol (59).

CONCLUSION

Essential oils are natural products with the potential to serve as a sustainable, green alternative to synthetic preservatives. Their antimicrobial effects are evident even at low concentrations, they are non-toxic to humans, and are generally regarded as safe for use in food preservation, including canning. However, challenges such as poor water solubility, volatility, and potential impacts on sensory qualities (taste, aroma) remain. Nonetheless, this literature review clearly demonstrates that EOs can be effectively applied in the preservation of cheese, dairy, and other food products.

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