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#### **REVIEW**



# Essential oils as natural preservatives for bakery products: Understanding the mechanisms of action, recent findings, and applications

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#### **ABSTRACT**

Bakery products, as an important part of a healthy diet, are characterized by their limited shelf-life. Microbiological spoilage of these products not only affects the quality characteristics and result in the economic loss but also threatens consumer's health. Incorporation of chemical preservatives, as one of the most conventional preserving techniques, lost its popularity due to the increasing consumer's health awareness. Therefore, the bakery industry is seeking alternatives to harmful antimicrobial agents that can be accepted by health-conscious customers. In this regard, essential oils have been previously used as either a part of product ingredient or a part of the packaging system. Therefore, the antimicrobial aspect of essential oils and their ability in delaying the microbiological spoilage of bakery products have been reviewed. Several types of essential oils, including thyme, cinnamon, oregano, and lemongrass, can inhibit the growth of harmful microorganisms in bakery products, resulting in a product with extended shelf-life and enhanced safety. Research revealed that several bioactive compounds are involved in the antimicrobial activity of essential oils. However, some limitations, such as the possible negative effects of essential oils on sensory parameters, may limit their applications, especially in high concentrations. In this case, they can be used in combination with other preservation techniques such as using appropriate packaging materials. Further research regarding the commercial production of the bakery products formulated with essential oils is required in this area.

#### **KEYWORDS**

Plant extracts; bioactive compounds; natural antimicrobial; shelf-life; cereal based; spoilage; fungal

#### 1. Introduction

Bakery products, including bread, cake, muffins, pastries, biscuit, and cookies, play important roles in human health and diet. Among them, bread, as a staple food throughout European societies and many other countries, is believed to be among the first processed food by our ancestors who baked the mixture of grains and water (Shewry and Hey, 2015). The baked products have evolved into an advanced and large-scale industry, which generates billions of dollars in revenue and provides several jobs for thousands of people. According to the statics, sales of bakery products in the United States was over ten million tones with a market value of about 27 billion USD at the beginning of the 21st century (Smith et al., 2004). There is an upward trend in the amount of production and revenue of this industry over past decades highlighting the economic values of baked goods. This continued growth has been spurred by consumer demands for convenient products. Recently, there has been an increasing interest in organic, ethnic, i.e., chemical free products (Rana and Paul, 2017).

Several bakery goods, such as bread and cake, are prone to spoilage when no preservative is added to the product that is stored under ambient conditions. The spoilage of bakery products may occur in forms of physical (moisture migration and staling), chemical (rancidity), and microbiological. While the first and second types of these spoilages mainly affect sensory attributes, the latter may cause foodborne disease outbreaks, which resulted in huge losses in both health and economic aspects (Smith et al., 2004).

In this context, the microbiological spoilage can be also considered as one of the main causes of economic loss in the bakery industry while several types of baked products, such as bread and cake, can be wasted through the activity of undesirable microorganisms. In the United States, as an example, losses due to microbiological spoilage in the production companies and stores are estimated to be about 3% of the products, which means about 90,000,000 kg of products year (Smith et al., 2004). A big percentage might be also affected by microorganisms at the consumer level, i.e. while storing at home. Therefore, the bakery industry tries to control the microbiological spoilage by following several strategies including reformulation of the product and incorporation of some of the allowed preservatives (Saranraj and Geetha, 2012).

Chemical preservatives are among the most convenient procedures to control microbiological spoilage of bakery commodities (Saranraj and Geetha, 2012). The conventional chemical preservatives in baked goods include propionic acid and propionates, sorbic acid, sodium diacetate, potassium sorbate, methylparaben, sodium benzoate, and propylparaben (Smith et al., 2004). A minimum concentration of each preservative is required to inhibit the deteriorative activity of spoilage-causing microorganisms. However, usually maximum allowed levels of incorporation were established by the regulatory bodies. For instance, a maximum concentration of 0.32% for calcium/sodium propionate can be used in bakery goods (Dwivedi et al., 2017). While lower concentrations could be ineffective on some spoilage microorganisms, higher concentrations might not be permitted by some regulatory bodies.

The type and concentration of chemical preservatives allowed for bakery goods are limited mainly due to their potential side effects on human health, i.e., hormonal imbalance, teratogenicity, carcinogenicity, high and acute residual toxicity, and spermatotoxicity (da Cruz Cabral, Pinto and Patriarca, 2013). In this regard, the cytotoxicity of several chemical preservatives was studied by Spindola et al. (2018). In addition, Soni, Carabin, and Burdock (2005) also highlighted the possible estrogenic hazards of parabens i.e., breast cancer (Castelain and Castelain, 2012; Okubo et al., 2001; Byford et al., 2002). Because of the above-mentioned undesirable effects, the recent investigations led to the revocation of registration of some of these chemical compounds (da Cruz Cabral, Pinto and Patriarca, 2013). Furthermore, public concerns regarding the incorporation of chemical additives into food products significantly increased, and it is generally believed that synthetic preservatives negatively affect the consumer's health. The recent findings considering the side effects of synthetic additives along with the increase in public concerns about healthy diet drive the bakery industry to seek for natural, safe and economic feasible alternatives to harmful chemical preservatives.

The incorporation of essential oils is becoming a promising technique in food and pharmaceutical industries due to their antioxidant activities (Barba, Esteve, and Frígola, 2014; Fernandes et al. 2018; Hashemi et al. 2017a; Hashemi et al. 2017b; Hashemi et al., 2015; Smeriglio et al., 2018), anti-inflammatory (Habashy et al. 2018), antiviral, antibacterial and antifungal properties (Bakhtiary et al. 2018; Mahmoudzadeh et al. 2016; Hashemi et al. 2017c; Şahin et al. 2017; Asl et al. 2018; Hashemi et al. 2017c). In this context, their consumption as medicinal plants is considered of great interest over the last years (Eş et al. 2017; Saljoughian et al. 2018). Moreover, several studies have been conducted to explore the effectiveness of these components on extending the shelf-life of baked goods (Debonne et al., 2018; Mani López et al., 2018; Nanasombat et al., 2010; Faccin et al, 2015; Gonçalves et al., 2017). Many of these compounds are regarded as generally recognized as safe (GRAS), which allows the industry to include them in the product formulation (FDA, 2017; Gavahian et al, 2018). These valuable materials can be extracted from plant

material by several methods including traditional and emerging techniques. The antimicrobial activity of essential oils is related to the presence of antimicrobial components such as carvacrol, thymol, p-cymene, and γ-terpinene (Hashemi et al., 2017a). The structure and characteristics of several essential oils are comprehensively reviewed (Beek and Joulain, 2018) and their potential applications in the meat industry were previously discussed (Lorenzo et al., 2018). However, the potential applications of essential oils in the bakery industry, as one of the most important industries, which suffer from the negative impacts of microbiological spoilage and the mechanisms of the shelf-life enhancement of essential oil-enriched products, need to be explored. The present paper was aimed to review the recent findings regarding essential oils incorporation as a natural preservation tool to extend the shelf-life of baked goods through microbial growth inhibition.

#### 2. Essential oils extraction techniques

The essential oils' extraction techniques can be classified as traditional and innovative alternative approaches. Steamand hydro-distillation are known as the most used conventional methods of extraction (Bouaziz et al., 2017; Roohinejad et al., 2017). While these methods need low capital investment, they suffer from low extraction yield and high energy and time consumption. Besides, many studies showed that they might negatively affect the chemical composition of the extract (Gavahian et al., 2013; Asl et al., 2018; Gavahian and Farahnaky, 2018; Gavahian, Chu and Sastry, 2018). On the other hand, ohmic assisted hydrodistillation (Gavahian et al., 2011; Gavahian et al, 2012; Gavahian et al., 2015; Gavahian et al., 2016a,b,c), microwave-assisted hydrodistillation (Gavahian et al., 2015), dual-cooled solvent-free microwave extraction (Wei et al., 2018), ultrasoundassisted supercritical carbon dioxide extraction (Wei et al., 2016), and ultrasound-assisted extraction (Hashemi et al., 2015; Gavahian et al., 2017; Wang et al. 2018) are among the innovative alternative extraction techniques, which aim to reduce the process time and consumed energy as well as enhance the essential oil quality. Previous reports confirmed that the extraction technique and extraction conditions can alter the chemical composition of the extract (Gavahian and Farahnaky, 2018; Gavahian, Chu and Sastry, 2018; Burt, 2004). These effects should be considered according to the application of essential oils (Li, Fabiano-Tixier, and Chemat, 2014). Therefore, selecting of the appropriate technique and optimizing the extraction conditions should be considered for preparing an essential oil with desirable properties (Gavahian and Farahnaky, 2018; Gavahian, Chu and Sastry, 2018).

### 3. Antimicrobial properties of essential oils

Aromatic herbs produce several chemical components with antimicrobial properties. While some are always available in their essential oils, others are formed as a response to physical injury or microbial invasion (Roller, 2003). Several

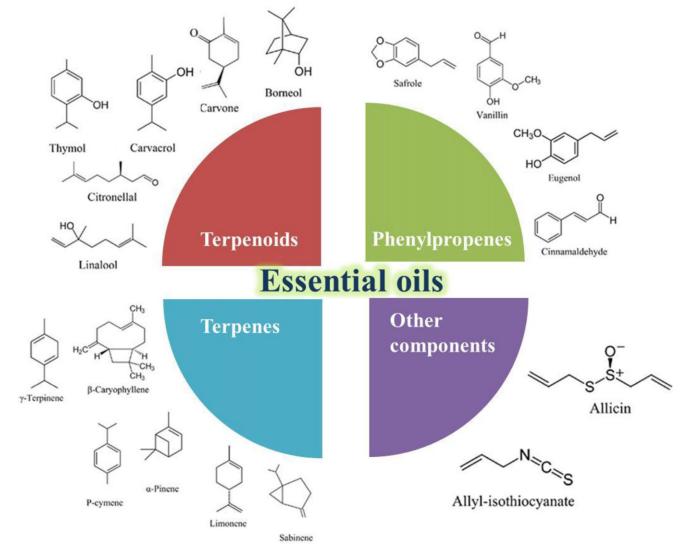


Figure 1. Selected components of essential oils with antimicrobial activity.

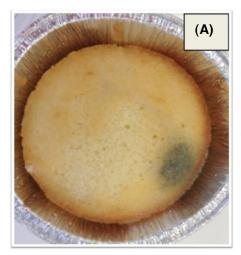
essential oils were shown to be effective on molds, yeast, and bacteria. However, the Gram-positive bacteria are more susceptible than Gram-negative ones due to lack of hydrophilic lipopolysaccharides in their membranes (Nikaido, 2003). Cell wall damage, cytoplasm coagulation, hydrolysis of ATP, increasing cell permeability, and membrane protein destruction are among the suggested mechanisms for the antimicrobial activity of essential oils (Khorshidian et al., 2018). Distinguishing the most active antimicrobial component of essential oils is a complicated task because these natural products are complex mixtures of several components, and their chemical composition, which determines their characteristics and mode of action, can vary depending on the harvest time, ecological condition as well as extraction technique. However, due to a great variety of compounds, their antimicrobial activity cannot be only attributed to a single mechanism of action (Burt, 2004; Hyldgaard et al., 2012). However, to facilitate exploring the antimicrobial effects of essential oils, some researchers connected the antimicrobial activity of the main components to the total activity of the essential oil (Mahmoudzadeh et al., 2016). It is generally believed that some components available in

essential oils have strong antimicrobial activity (Figure 1). Terpenes (i.e.p-cymene and limonene), terpenoids (i.e. thymol and carvacrol), phenylpropenes (i.e. eugenol and vanillin) and some other components such as allicin and isothiocyanates are among the most active antimicrobial components of herbal essential oils (Hyldgaard et al., 2012).

Terpenes are hydrocarbons developed from several isoprene ( $C_5H_8$ ) units. These compounds contain a hydrocarbon backbone that can be rearranged into cyclic structures and form mon- and bi-cyclic structures (Caballero et al., 2003). These chemical families are categorized according to their chain size to several groups including monoterpenes ( $C_{10}H_{16}$ ), sesquiterpene ( $C_{15}H_{24}$ ), diterpenes ( $C_{20}H_{32}$ ), and triterpenes ( $C_{30}H_{40}$ ). Limonene, p-cymene, sabinene, terpinene, and pinene are among the common terpenes in many essential oils.

Due to the lack of a group with high inherent antimicrobial activity in terpenes, they are not efficient antimicrobial agents when applied individually. For instance, the low concentrations of p-cymene (85700  $\mu g/mL)$  did not present any antimicrobial activity against some Gram-negative microorganisms (Bagamboula et al., 2004). Moreover, p-Cymene





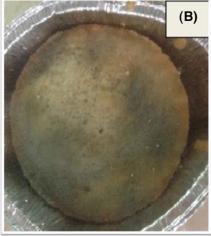




Figure 2. Effect of thyme essential oil on microbiological stability of cake: control sample after 15 (A) and 30 days of storage (B) and (C) the essential oil containing sample after 30 days of storage (Gonçalves et al., 2017).

and γ-terpinene were also ineffective as fungicides against Saccharomyces cerevisiae (Rao et al., 2010). Similar results were reported for limonene,  $\alpha$ -terpinene,  $\alpha$ -pinene, β-pinene, carene, and sabinene (Dorman and Deans, 2000). In addition, limonene, p-cymene, α-pinene, β-pinene, β-myrcene, β-caryophyllene, and γ-terpinene were also shown to be ineffective against Bacillus cereus, Escherichia coli O157:H7, and Staphylococcus aureus (Koutsoudaki et al., 2005).

Terpenoids as biochemically modified terpenes gained extra oxygen molecules, and their methyl groups are moved or removed (Caballero et al., 2003). Alcohols, aldehydes, esters, ethers, ketones, phenols, and epoxides are different types of terpenoids. Famous terpenoids compounds include citronellal, linalool, linalyl, thymol, carvacrol, acetate, piperitone, menthol, and geraniol. The presence of hydroxyl group can enhance the antimicrobial activity of these components. For instance, the antimicrobial activity of the carvacrol methyl ether and p-cymene, which are carvacrol derivatives, were shown to be less than carvacrol (Dorman and Deans, 2000; Ultee et al., 2002; Ben Arfa et al., 2006). Exchanging the hydroxyl group of carvacrol with methyl ether alter its hydrophobicity and the way that it interacts with the cell membrane which affects its antimicrobial activity (Veldhuizen et al., 2006). Carvacrol's antimicrobial activity is comparable to that of 2-amino-p-cymene, which illustrates the importance of hydroxyl group (Veldhuizen et al., 2006). The antimicrobial properties of essential oils have been shown to be closely correlated with their phenolic contents (Aligiannis et al., 2001; Rhayour et al., 2003). These compounds have been shown to be active against a wide range of microorganisms (Dorman and Deans, 2000). Bassolé et al. (2010) investigated the antimicrobial activity of some essential oil components, including linalool, carvacrol, thymol, and menthol, against Enterobacter aerogenes, Listeria monocytogenes, E. coli, and Pseudomonas aeruginosa and reported that carvacrol (MIC of 300 µg/mL) and thymol (MIC of 800 µg/mL) offered the highest antimicrobial activity.

On the other hand, phenylpropanoids have an aromatic phenol group and the three-carbon propene tail of cinnamic acid. The most famous phenylpropenes include vanillin, safrole eugenol, isoeugenol, and cinnamaldehyde. It was shown that the free hydroxyl groups play a crucial rule in the antibacterial activities of these compounds (Laekeman et al., 1990). Their antimicrobial activities could be also related to the type and number of substituents on their aromatic ring (Pauli and Kubeczka, 2010). A study conducted by Dorman and Deans (2000) confirmed the antibacterial activity of eugenol against several bacteria. Although understanding the mode of action of each essential oil component can give an idea about the antimicrobial activity of essential oils, the mixture of these components might show the synergistic effect. This aspect of the essential oils needs fundamental investigations in the future to be fully understood.

# 4. Applications of essential oils as bio-preservatives in bakery commodities

The compositions of essential oils, rich in terpenes, terpenoids, phenylpropenes, and other antimicrobial components offer a protective character against spoiling microorganisms, which are a concern in bakery products (Smith et al., 2004). However, it is crucial to investigate the essential oils properties, specific modes of action and effects on product components and matrix before using them as natural preservatives in baked products (Hyldgaard et al., 2012). Therefore, some essential oils with high-concentrations of antimicrobial components, such as thymol and carvacrol, may effectively enhance the bakery product's shelf-life while some might be considered as a less effective preservative. Some investigations have been carried out regarding the feasibility of replacing the synthetic preservatives with natural essential oils in bakery products such as bread and cake (Debonne et al., 2018; Mani López et al., 2018; Nanasombat et al., 2010; Faccin et al, 2015; Gonçalves et al., 2017). They can be used directly as an ingredient of bakery product formulation (Debonne et al., 2018; Faccin et al., 2015) or be incorporated in the packaging material (Lopes et al., 2014; Passarinho et al., 2014) to enhance the shelf-life of the product.

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Bakery product/ culture media	Incorporated essential oil	Investigated microorganisms	Key findings	Reference
wheat flour agar medium	Clove (Syzygium spp) Basil (Ocimum spp) Neem (Azadirachta sp.), Ajwain (Trachyspermum sp.), cimamon	Penicillium oxalicum, Aspergillus flavus	Clove essential oil was the only effective preservative against the studied microorganisms. Synergistic antifungal effects of clove escential oil and coveral chemical preservatives was observed	Mishra et al., 2014
Cup cake	Orange peel	Aspergillus spp, Fusarium spp., Penicillium spp.	Incorporation of 0.3% essential oil did not alter the Sensory properties of the product. The orange peel essential oil showed inhibitory effect against the studed microorganism	Ahmed et al., 2009
Butter cake	Coriander (Coriandrum sativum L.)	¥N.	Encoporation of coriander essential oil enhanced the shelf life of the cake. Increasing the essential oil concentration from 0.05% to 0.15% enhanced the antifungal activity. This essential oil also improved the oxidative stability of the product	Darughe et al., 2012
Cake	Clove	Coliforms, molds, yeasts	Clove essential oil retarded the cake oxidation. Increasing the essential oil concentration from 400 to 800 ppm enhanced its antimicrobial activity but negatively affected the sensory characteristics of the product	lbrahium et al., 2013
Cake	thyme ( <i>Thymus vulgaris</i> )	Candida albicans, Enterococcus faecium, Enterococcus hirae, Escherichia coli , Salmonella choleraesuis, Staphylococcus aureus , Salmonella typhimurium, Pseudomonas aeruginosa, and Asperrillus niger	Both free and encapsulated thyme essential oils presented high in vitro antioxidant and antimicrobial activities. Most of the studied microorganisms were inhibited by this essential oil during a one month storage time. MIC value of the encapsulated essential oil was lower than that of free essential oil. The shelf life of the product was enhanced by including thyme essential oil in the cake formulation.	Gonçalves et al., 2017
Cake	Chamomile ( <i>Matricaria chamomilla</i> L.)	yeast and mold	Chamomile essential oil retarded the mold growth in cake samples but not as good as sodium sorbate. The essential oil was more effective at higher concentrations. chamomile essential oil enhanced the oxidative stability of the product. Including the essential oil in the cake formulation decreased the sensory scores. Increasing the essential oil concentration decreased the sensory scores.	
Bread Wheat Bread	Aqueous Yerba Mate ( <i>Ilex paraguarien-sis</i> ) Extract Aqueous garlic (Allium sati-	Fungi	Formulating the bread with Yerba Mate extract enhanced the shelf life probably because of the phenolic compounds of the herbal extract which have antimicrobial activity.	Faccin et al, 2015
bread model system	Varin extract Mexican oregano ( <i>Lippia berlan-dieri</i> Schauer)	Aspergillus spp	Increasing the essential oil concentration enhanced the shelf-life by better inhibition of fungal activities Vatriation in the ration of thymol and carvacrol content of the essential oil had no effect on the finnal property inhalication.	Ávila Sosa Sánchez et al., 2015
Wheat and Rye Bread	Mustard essential oil	P. commune, P. solitum, A. flavvus, E. fibuliger, P. roqueforti, P. corylophilum, E. repens, E. fibuliger	Formulating the bread with essential oil along with modified atmosphere packaging enhanced the shelf-life of the wheat and rye breads. A. flavus and Eurotium repens were the most resistant species. In the mustard essential oil containing wheat and rye breads, respectively.	Suhr & Nielsen, 2005
Wheat bread	Cinnamomum verum ), pummelo (Citrus maxima ), kaffir lime ( Citrus hvrrix ) peels	Rhizopus stolonifer, Aspergillus fl avus	Cinnamon essential oil had the hoighest inhibitory effect on the studied microorganisms.	Nanasombat et al., 2010
Bread	Lemongrass (Cymbopogon citratus)	Penicillium expansum	750 ppm of essential oil inhibited <i>P. expansum</i> for 3 weeks at 20°C. The inhibitory effect enhanced with increasing the essential oil concentration Essential oil didi not affected the sensory attributes if the bread at the studied concentrations.	Mani López et al., 2018
Par-baked wheat and sourdough breads	thyme ( <i>Thymus zygis</i> )	Aspergillus niger, Penicillium paneum	Despite the promising in-vitro antimicrobial activity of thyme essential oil, this essential oil did not improve the shelf-life of par-baked bread. Thyme essential oil negatively affected the color values, volume, and flavor of the par-baked breads	Debonne et al., 2018
NA: not available.				



# 4.1. Essential oils as a preservative ingredient for baked products

There are limited published data considering the uses of essential oils as an antimicrobial agent in bakery goods. However, the growing interest in the application of multifunctional natural ingredients in bakery products also resulted in several investigations on essential oils as natural preservatives. The number of published papers over the last years is increasing, and researchers assessed the effect of several herbal extracts on the shelf-life of bakery products such as several types of bread and cakes. Table 1 summarizes the recent studies on the application of essential oils as natural preservatives in bakery products.

Mishra et al. (2014) isolated two fungal strains, namely Penicillium oxalicum and Aspergillus flavus, from spoiled bread and cake samples and investigated their response to clove, Basil, Neem, Ajwain (carom seeds), and cinnamon essential oils. While the extracted essential oils from Syzygium spp. (clove) showed the inhibition zone of 45 mm and 15 mm against Penicillium oxalicum and Aspergillus flavus, respectively. They also found that combining this essential oil with other chemical preservatives, including acetic, benzoic citric and lactic acid, offered synergistic effects in antifungal activity. The author also claimed that the incorporation of essential oils could enhance the flavor and aroma of the baked goods (Mishra et al., 2014).

The extracted essential oil orange peel showed an inhibitory effect on several microorganisms including Aspergillus niger and Penicillium spp., in a cupcake prepared from wheat flour (Ahmed et al., 2009). According to the results obtained by the authors, the addition of 0.3% of orange peel essential oil did not affect the sensory evaluation, and the enriched cupcake with orange peel essential oil was proposed as a naturally preserved product. In a similar study, Darughe et al. (2012) reported that the incorporation of the extracted essential oil (0.15%) from coriander in a butter formulation reduced the fungi population during 60-day storage at ambient conditions. The main components of this essential oil were camphor, cyclohexanol acetate, limonene, and α-pinene, which could be responsible for the antimicrobial activity of this extract.

The feasibility of the application of clove essential oil as a natural preservative in the cake formulation was investigated by Ibrahium et al. (2013). The antimicrobial activity of clove essential oil in concentrations of 0, 400, 600, and 800 ppm in cakes was determined by the counts of total bacteria, coliform bacteria, molds, and yeasts during 28 days at room temperature. The cakes contained essential oil had better oxidative stability than the control samples (without essential oil added). The microbiological evaluation showed that higher concentrations of the clove essential oil (800 ppm and 600 ppm instead of 400 ppm) are more effective in inhibiting the growth of tested microorganisms. On the other hand, the sensory evaluation revealed that the sample containing 800 ppm of essential oil showed the lowest acceptability score among other formulations. Based on the findings of that study, the negative impacts of essential oils incorporation on sensory attributes of the product should be also taken into account.

The antibacterial and antifungal activities of free and encapsulated thyme essential oil in the cake were verified by Gonçalves et al. (2017). Both forms of this essential oil showed inhibitory activities against a variety of microorganisms including Candida albicans, Enterococcus, faecium, Enterococcus, Escherichia coli, Salmonella choleraesuis, Staphylococcus aureus. Salmonella typhimurium, Pseudomonas aeruginosa, and Aspergillus niger. Moreover, encapsulation enhanced the antimicrobial activity of thyme essential oil, and the MIC value of the essential oil decreased after the encapsulation process. The authors suggested that protective micro-environment promoted by the particle wall. The microparticles incorporated in cake formulation reduced the volatilization of the encapsulated essential oil and enhanced the product shelf-life. The results showed that the sample that contained essential oil was microbiologically safe for thirty days under ambient conditions, while the control samples were spoiled by microorganisms and deteriorated in two weeks.

On the other hand, Khaki et al. (2012) evaluated the antimicrobial and antioxidant effects of chamomile essential oil at the concentrations of 0.05%, 0.1% and 0.15% added in the cake during 2.5 months and compared the results with those from a control and chemically preserved samples. According to the result, increasing the essential oil concentration from 0.05% to 0.15% enhanced the stability against microbial activities and oxidation reactions. However, the chemical preservatives were more effective than the studied concentrations of the chamomile essential oils. Moreover, the incorporation of the essential oil negatively affected the sensory properties and the higher concentrations of the essential oil resulted in a product with a lower overall quality score.

An investigation on formulating a bread model system with Lippia berlandieri Schauer (Mexican oregano) essential oil, as a natural preservative, verified the antifungal activity of this herbal product (Ávila Sosa Sánchez et al., 2015). According to the authors, including this essential oil in the bread formulation reduced the Aspergillus growth within twenty days of storage. This study also revealed that when essential oils were combined with other preservation techniques, such as water activity (aw) reduction, a promising result could be obtained concerning inhibiting fungal growth. Although high concentrations of essential oil may negatively affect the sensory characteristics of products, investigations can suggest a minimum required concentration, which can effectively inhibit the fungal growth without any negative effect on the sensory quality of food (Avila Sosa Sánchez et al., 2015). It should be also noted that different consumers have different preferences and a comprehensive sensory study is required to investigate the effect of different essential oils in different bakery products in different regions of the world. Likewise, Ya-hui et al. (2011) showed that a mixture of several essential oils together with cold storage prolonged the shelf-life of Chinese steamed bread (Wang et al., 2018).

Suhr and Nielsen (2005) showed that studied the inhibitory effects of mustard essential oil against common spoilage fungi of wheat bread, i.e., Penicillium commune, P. solitum, P. polonicum, and Aspergillus flavus, and of rye bread, i.e. P. roqueforti, P. corylophilum, and Eurotium repens, and Endomyces fibuliger in a wheat and rye bread, respectively. They combined this innovative preservation technique with modified atmosphere packaging system and reported that the most mustard essential oil-resistant microorganisms on wheat and rye bread were A. flavus and Eurotium repens, respectively. A combination of essential oil incorporation and modified atmosphere packaging was the optimal preservation technique, and total inhibition was achieved with  $2\,\mu L$  essential oil/rye bread slice and 2-3  $\mu L$  essential/wheat bread. In addition, nature and surface area of the product influences the effectiveness of active packaging with mustard essential oil. The modified atmosphere packaging along with mustard essential oil reduced the critical demand for low residual oxygen and inhibited all the spoilage microorganisms in massive inoculation loads for the desired period of one month. Moreover, a higher dose of essential oil was required for wheat bread in comparison with rye bread to inhibit E. fibuliger growth with possible sensory quality implications (Suhr and Nielsen, 2005).

In another study, Nanasombat et al. (2010) evaluated the antimicrobial activities of four essential oils, namely pummelo (Citrus maxima), peels, and kaffir lime (Citrus hytrix) peels, and cinnamon (Cinnamomum verum) barks, and reported that the latter has the highest inhibitory activity against Rhizopus stolonifer (MIC of 20 g/mL) and Aspergillus flavus (MIC of 80 g/m). This essential oil along with reduced water activity showed a synergistic effect on the inhibition of these two microorganism growths on a bread model agar. However, only 10 µg/mL cinnamon essential oil was required to inhibit R. stolonifer at aw of 0.90, and 20 µg/mL of this essential oil was required against this microorganism at higher water activities, i.e., aw values of 0.93 and 0.97. Storage of the wheat bread, containing 0.16%, 0.32%, 0.65%, and 1.30% ground cinnamon/dough weight, at the relative humidity of 75% showed a slower fungal growth as compared to that of stored at the relative humidity of 90%. In addition, increasing the essential oil concentration enhanced its antifungal activity, i.e., 1.3% cinnamon showed the greatest inhibitory effect on the studied molds.

On the other hand, Mani López et al. (2018) assessed the antimicrobial effect of the vapor phase of microwave-assisted steam distilled Cymbopogon citratus (lemongrass) essential oil (at the concentrations of 125-4,000 ppm) on Penicillium expansum in bread samples, which were inoculated with 10<sup>6</sup> spores/mL. According to these authors, 750 ppm essential oil successfully inhibited the fungal activity for three weeks at 20 °C. They also reported that increasing essential oil concentrations enhanced the inhibitory effect. Moreover, incorporation of 500-10,000 ppm essential oil did not negatively affect the sensory evaluation results.

Sikkhamondhol et al. (2009) evaluated the sensorial attributes of bread containing turmeric (Curcuma longa) essential oil. This essential oil successfully reduced the

growth rate of yeast and molds. According to the findings, although the antimicrobial and antioxidant activities of this essential oil could be beneficial for the consumers, increasing the essential oil concentration from 0 to 0.3% decreased the overall sensory score from 7.2 to 4.8.

Debonne et al. (2018) investigated the antifungal activity of Thymus zygis (thyme) essential oil against Aspergillus niger and Penicillium paneum in vitro and in vivo, i.e., in sourdough bread, par-baked bread, and par-baked wheat. Despite the promising in-vitro antimicrobial activity of this essential oil, no shelf-life extension was observed for par-baked bread. These authors hypothesized that nonhomogeneously dispersion of the thyme essential oil in the bread dough resulted in a non-significant effect of essential oil in par-baked bread. In addition, color values, volume, and flavor of the par-baked bread were inversely affected by the thyme essential oil (Debonne et al., 2018).

Finally, Sunil and Desai (2018) evaluated the antifungal activity of orange (Citrus aurantium) essential oils against common bread spoilage molds, i.e., Penicillium chrysogenum, Aspergillus niger, and Rhizopus spp. on whole wheat, brown and multigrain bread slices. The growth rate of all studied molds was affected by orange essential oil. Penicillium chrysogenum and Aspergillus niger showed higher sensitivity to orange essential oil as compared to Rhizopus.

#### 4.2. Essential oil as an additive in protective packaging

Packaging is an important technique for enhancing the shelf-life of bakery products. While conventional packaging provides adequate amounts of oxygen for spoilage aerobic microorganisms, modified atmosphere packaging replaces the oxygen with carbon dioxide or other gas mixtures (Ghidelli and Pérez-Gago, 2018). Recent studies showed that active packaging provides better protection for bakery products through the application of oxygen absorbers and other beneficial components such as antimicrobial agents (Ghidelli and Pérez-Gago, 2018; Galić et al., 2009). These components can be incorporated as a packaging layer/film in the packaging system or be placed as sachets inside the package, .i.e. beside the food product. The importance of packaging on the shelf-life of bakery products was comprehensively reviewed elsewhere (Galić et al., 2009). Therefore, this section only focuses on the shelf-life extension of bakery products using essential oil as an antimicrobial agent in the packaging system. In this regard, Otoni et al. (2014) reported antifungal activity of nano-emulsions of oregano and clove bud essential oils in methylcellulose/metalized polypropylene packages. In addition, Balaguer et al. (2013) reported in-vitro antifungal activity of gliadin films containing cinnamaldehyde (the major compound of cinnamon essential oil) against A. niger and P. expansum. They also inoculated P. expansum conidial suspension (10<sup>6</sup> spores/mL) into the slices of wheat bread and evaluated the effect of this innovative antifungal film. According to the authors, essential oil containing film (5% cinnamon aldehyde) inhibited the fungal growth for one month at 23 °C. Likewise, cellulose-derived polymers containing 5% cinnamaldehyde



Table 2. The list of some essential oils as potential preservatives in bakery products.

Common name	scientific name	Major components	Tested microorganism	References
Garlic creeper	Adenocalymma alliaceum	diallyl disulfide, diallyl trisulfide	A. niger, A. flavus, C. cladospor- ioides, Mucor sp., Dreschlera sp. and F. roseum	Shukla et al. (2008)
.Chamomile	Anthemis nobilis	1.8-Cineol, isobutyl angelate, isoamyl angelate, 2- methylbutyl isobutyrate	A. candidus, A. niger, Penicillium sp., and F. culmorum	Magro et al. (2006)
Caraway	Carum carvi	Carvone, limonene	F. oxysporum, F. verticillioides, P. expansum, P. brevicompactum, A. flavusand A. fumigatus	Zabka et al. (2009)
Mexican tea	Chenopodium ambrosioides	cis-piperitone oxide, p-cymene, isoascaridole,α-terpinene	A. flavus, A. fumigatus, Botryodiplodia theobromae, F. oxysporum, P. debaryanum and S. rolfsii	Kumar et al. (2007); Soares et al., 2017
Cinnamon	Cinnamomum zeylanicum	cinnamaldehyde, limonene, copaene, naphtha- lene, heptane	A. niger, A. flavus, F. moniliforme, F. graminearum, Fusarium spp, P. citrinumand P. viridicatum	Singh et al. (2007), Saleem et al (2015)
Citronella grass	Cymbopogon nardus	Citronellal, d-limonene	F. oxysporum, F. verticillioides, P. expansum, P. brevicompactum, A. flavusand A. fumigatus	Zabka et al. (2009); Gavahian et al. (2018)
Cymbopogon spp	Lemongrass	Geranial, neral, Myrcene	Fusarium spp, Colletotrichum	Velluti et al. (2004); Oliveira et al., 2018
Eucalyptus globulus	Eucalyptus	1,8-cineole, camphene, α-terpineol, geranial	A. flavus and A. parasiticus	Vilela et al. (2009); Tomazoni et al. (2017)
Lavandula stoechas	Lavender	Fenchone, Camphor, Eucalyptol, Menthone terpineol	P. infestans, B. cinerea, L. monocy- togenes and S. aureus	Soylu et al. (2010); Bouyahya et al. (2017)
Origanum vulgare	Oregano	$\gamma$ -terpinene, Thymol, Carvacrol	A. flavus and A. parasiticus	Gómez et al., 2018 De Mastro et al., 2017
Syzygium aromaticum	Clove	Eugenol, E-caryophyllene	Fusarium spp.	Velluti et al. (2004); Sharma et al., 2017
Thymus vulgaris	Thyme	Thymol, carvacrol, p-Cymene	F. oxysporum, F. verticillioides, P. expansum, P. brevicompactum, A. flavus, A. alternate and A. fumigatus	Zabka et al. (2009), Feng and Zheng (2007) Gavahian et al (2012)

(Lopes et al., 2014) and polypropylene with ethyl acetate (as the solvent base) coated with nitrocellulose containing the cinnamon essential oil (Gutiérrez et al., 2011), have been shown to have antifungal activity in bakery products.

It should be noted that the sensory properties of the bakery products can be affected by essential oils even when they are used as a component of active packaging. For example, although antimicrobial sachets containing 5% oregano essential oil inhibited the growth of the fungal on sliced pieces of bread, the essential oil aroma reduced the overall acceptance of the product (Passarinho et al., 2014). Similarly, the taste and odor of the sliced bread were detected as strange and unacceptable while the mold activity was controlled by the vapor of sage and marjoram essentials oils in a packaging system (Krisch et al., 2013).

# 5. Considerations for using essential oils in bakery products

One of the important considerations for using essential oils as antimicrobial agents in bakery products is the legal dose that can be utilized. Several countries have allowance limit or the list of permitted essential oils for each bakery product. However, these regulations depend on the region and product type and are subject to updates and modification. Fortunately, FDA listed several essential oils in the GRAS list, which can be used in the bread, and other bakery products in the United States (Es et al., 2017; Gavahian, Chu, Khaneghah, Barba and Misra, 2018).

Another important aspect to consider while using essential oils as natural preservatives in bakery products is the possibility of the antagonistic and synergistic responses, which depends on various parameters including their composition and product characterizations (Burt, 2004). Moreover, according to previous studies the addition of essential oils into the food materials, especially in high concentration, can negatively affect the sensory characteristic of the product (Gavahian, et al., 2013). Therefore, further investigations are required to address the potential unpleasant aroma of essential oils, as natural preservatives, in bakery products. The combination of this innovative alternative method with other preservation techniques, such as hygienic design, controlling aw, and using appropriate packaging system, may reduce the MIC of essential and result in a product with higher acceptability. Moreover, searching for the antimicrobial essential oil with pleasant aroma for different bakery products in different regions of the word needs further studies.

There are still several essential oils with antimicrobial, especially antifungal, characteristics, which can be investigated as an alternative to chemical preservatives in bread, cake, and other baked products. For example, the effectiveness of Caraway (Carum carvi) against F. oxysporum, F. verticillioides, P. expansum, P. brevicompactum, A. flavus and A. fumigatus was confirmed by Zabka et al. (2009). The Mexican tea (Chenopodium ambrosioides) can inhibit A. flavus, A. fumigatus, Botryodiplodia theobromae, F. oxysporum, P. debaryanum and S. rolfsii. Similar inhibitory



effects against spoiling microorganisms were reported for Eucalyptus (Vilela et al. (2009), Oregano (Gómez et al., 2018), Thyme (Zabka et al. (2009) and Clove (Velluti et al. (2004) (Table 2). Future studies may seek for the appropriate essential oils or their mixtures to enhance the microbiological shelf-life of bakery products with no adverse effect on sensory parameters.

#### 6. Conclusions and future perspectives

This review confirms the possibility of using several essential oils as substitutes for chemical antimicrobial agents to enhance the microbiological shelf-life of the bakery products. The incorporation of these natural compounds into the product formulation or packaging materials not only can inhibit the fungal growth but also can enhance the oxidation stability. In addition, the health effects of several essential oils have been shown which may encourage consumers to use essential oil enriched products. However, the potential negative impact of these compounds on sensory quality, such as aroma and taste, of the product needs to be investigated before their commercial application. The combination of essential oils and other preservation techniques, such as appropriate storage condition, the hygienic design of production lines, and using appropriate packaging material, are among the potential solutions to this drawback.

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