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Antifungal performance of essential oils in breadmaking by in situ, in vitro and active packaging evaluation – a review

Desempenho antifúngico de óleos essenciais na panificação por avaliação in situ, in vitro e em embalagens ativas – uma revisão

Desempeño antifúngico de los aceites esenciales en la panificación por evaluación in situ, in vitro y de embalaje activo: una revisión

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Abstract

Due to antifungal properties, the application of essential oils in bread has attracted commercial interest due to its highly perishable. Their limited shelf life is due to their high water activity and is dependent on good manufacturing practices and proper storage and distribution methods. The main spoilage microorganisms in bread are molds. Therefore, the present study aimed to provide an integrative review of scientific literature related to recent studies on the application of essential oils to limit or prevent the development of spoilage fungi in breadmaking. The approach includes the use of essential oils as an additive in the formulation of bread through addition in the dough, as surface protection or in active packaging. Essential oils are composed of bioactive substances with antifungal properties that are suitable for the preservation of bread. High volatility, high costs, and strong odors are characteristics that restrict their applicability. Therefore, it is important to evaluate the dosage, forms of application, different mechanisms of antimicrobial action, and the effects of their individual or combined use with other preservation technologies. In this way, essential oils there may be inhibitory to spoilage microorganisms while maintaining adequate technological and sensorial characteristics in breadmaking.

Keywords: Active packaging; Clean label; Molds; Natural antimicrobials; Plant-based metabolites; Preservation technologies.

Resumo

Devido às propriedades antifúngicas, a aplicação de óleos essenciais em pães tem despertado interesse comercial devido à alta perecibilidade. A vida útil limitada dos produtos de panificação é devido à alta atividade de água e depende de boas práticas de fabricação e métodos adequados de armazenamento e distribuição. Os principais micro-organismos de deterioração no pão são os bolores. Portanto, o presente estudo teve como objetivo fornecer uma revisão integrativa da literatura científica relacionada aos estudos recentes sobre a aplicação de óleos essenciais para limitar ou prevenir o desenvolvimento de fungos deteriorantes na panificação. A abordagem inclui o uso de óleos essenciais como aditivo na formulação de pães por meio de adição na massa, como proteção de superfície ou em embalagens ativas. Os óleos essenciais são compostos por substâncias bioativas com propriedades antifúngicas adequadas para a conservação do pão. A alta volatilidade, os custos elevados e os odores fortes são características que restringem sua aplicabilidade. Portanto, é importante avaliar a dosagem, formas de aplicação, diferentes mecanismos de ação antimicrobiana e os efeitos de seu uso individual ou combinado com outras tecnologias de preservação. Desta forma, os óleos essenciais podem ser inibidores de micro-organismos deteriorantes mantendo características tecnológicas e sensoriais adequadas na panificação.

Palavras-chave: Antimicrobianos naturais; Bolores; Embalagem ativa; Metabolitos de origem vegetal; Rótulo limpo; Tecnologias de preservação.

Resumen
Debido a sus propiedades antifúngicas, la aplicación de aceites esenciales en pan ha atraído interés comercial debido a su alta perecedera. Su vida útil limitada se debe a su alta actividad de agua y depende de buenas prácticas de fabricación y métodos adecuados de almacenamiento y distribución. Los principales microorganismos que deterioran el pan son los mohos. Por lo tanto, el presente estudio tuvo como objetivo proporcionar una revisión integradora de la literatura científica relacionada con estudios recientes sobre la aplicación de aceites esenciales para limitar o prevenir el desarrollo de hongos en la panificación. El enfoque incluye el uso de aceites esenciales como aditivo en la formulación del pan a través de la adición en la masa, como protección superficial o en envases activos. Los aceites esenciales están compuestos por sustancias bioactivas con propiedades antifúngicas adecuadas para la conservación del pan. La alta volatilidad, los altos costos y los olores fuertes son características que restringen su aplicabilidad. Por lo tanto, es importante evaluar las dosis, formas de aplicación, diferentes mecanismos de acción antimicrobiana y los efectos de su uso individual o combinado con otras tecnologías de conservación. De esta manera, los aceites esenciales allí pueden inhibir los microorganismos de deterioro mientras mantienen características tecnológicas y sensoriales adecuadas en la panificación.

Palabras clave: Antimicrobianos naturales; Embalajes activos; Etiqueta limpia; Metabolitos de origen vegetal; Mohos; Tecnologías de conservación.

1. Introduction

Bakery products with high moisture content, mainly bread but also cakes, are extremely susceptible to microbial spoilage due to high water activity (Rahman et al., 2021; Neves et al., 2020). Among the different types of bread, whole wheat bread stands out because of its high perishability due to the presence of bran, which is composed of the external morphological structure of wheat grain, called pericarp. Bran is the most microbiologically contaminated fraction in whole-wheat flours as a result of their direct contact with the external environment during harvesting, transportation, processing, and storage (Santos et al., 2016; Neves et al., 2020).

Several techniques have been studied to reduce the microbiological contamination of wheat grains before the milling process. The most used techniques applied chemical agents (calcium oxide, ozone, calcium hydroxide, sodium bicarbonate, sulfur dioxide, carbon dioxide and potassium carbonate) and sourdough and emerging technologies (γ irradiation, infrared radiation, microwave heating, UV-C radiation, cold plasma, electrolyzed water, pulsed electric field and nanotechnologies techniques) (Tatar et al., 2021; Silveira et al., 2019; Pankaj et al., 2018; Neves et al., 2021). The greatest challenge in carrying out the decontamination of the wheat grains still refers to the large surface area required for the treatment to be effective, the large volumes of grains that need to be decontaminated, the high cost involved and the need to treat effluents generated, in some cases.

The most common way to prevent or control the growth of fungi in food is by using antifungal agents consisting of chemical compounds that prevent or delay fungi development. Food-grade weak organic acids based on propionic, sorbic, benzoic and acetic acids and their salts, which are generally recognized as safe (GRAS) compounds, are alternative forms of preventing the spoilage of bakery products and extending shelf life (Liewen & Marth, 1985).

One recent trend in the food industry that can be applied to this product is the reduction or elimination of the use of chemical preservatives, which are known as clean label products (Santos & Novales, 2012). Natural antimicrobials can originate from plants, animals and microorganisms (Gyawali & Ibrahim, 2014; Silva et al., 2020). Among plant sources, the main compounds that inhibit or delay microorganism growth are essential oils and their components extracted from spices and other aromatic plants (Tanackov & Dimić, 2013; Ju et al., 2020; Reis et al., 2020). However, the antimicrobial compound should be chosen based on its chemical and sensory compatibility with the target food, effectiveness against undesirable microorganisms, and food safety (Settanni & Corsetti, 2008).

Essential oils are phytochemicals (Callaway et al., 2011) formed by volatile compounds, that can be found in all plant organs, including the buds, flowers, leaves, seeds, branches, stems, culms, fruits, roots or bark (periderm). They are usually stored in secretory cells, cavities, canals, glandular trichomes or epidermal cells. The main characteristic of essential oils is their volatility, differing from fixed oils obtained from seeds, which are predominantly composed of non-volatile triacylglycerols (Simões & Spitzer, 2007; Bora et al., 2020; Nascimento et al., 2021).

These metabolites are related to several functions necessary for plant survival and play a vital role in the defense against microorganisms (Siqui et al., 2000). Plants that produce essential oils, in general, are known for their antioxidant capacity (Barba et al., 2014; Fernandes et al., 2018) and antimicrobial effects (Bakhtiary et al., 2018; Mahmoudzadeh et al., 2016), as well as their medicinal, and aromatic properties. Additionally, essential oils are reported to function as analgesics, sedatives, anti-inflammatory drugs, local anesthetics, and spasmolytics (Bakkali et al., 2008; Nedorostova et al., 2008; Rehman et al., 2020; Sil et al., 2020).

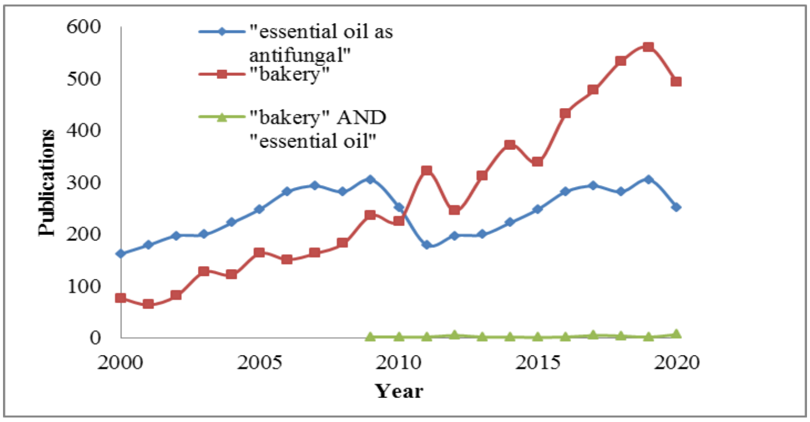
This article analyzes the role of essential oils as inhibitors of fungal species commonly found in bread. In addition, several forms of directly applying this type of product are discussed. The main objective is to provide an overview of the essential oils application by summarizing the main conclusions of recent studies in the food industry.

2. Methodology

This literature review was a theoretical study of an integrative nature, method that permits summarizing knowledge through a systematic and rigorous process (Mendes et al., 2019), being carried out by tracking scientific articles that focus on the use of essential oils as bread preservatives. The scientific databases ScienceDirect (https://www.sciencedirect.com), Scopus (http://www.scopus.com), Scielo – Scientific Electronic Library Online (https://www.scielo.org/), PubMed (MEDLINE) (http://www.ncbi.nlm.nih.gov/pubmed), and CAPES/MEC Journal Portal (http://www.periodicos.capes.gov.br) were used to find articles. Articles in English and Portuguese, published between 2007 and 2022, were selected. The exclusion of those that did not fit the objective of the research or repeated articles was carried out. The selected articles included timeless scientific research, provided that they presented specific content on the use of essential oils as antifungals in breadmaking.

Since the year 2000, only 3 literature reviews were recorded on the application of essential oils in bakery products (www.scopus.com). The total number of publications (Figure 1) shows the innovative character of this article, with few documents about this topic.

Figure 1. Number of publications indexed by Scopus (www.scopus.com) based on search strings related to the use of “essential oils as antifungal”, “bakery”, and “bakery” AND “essential oil” in the title, keywords, and abstract of the publication



Source: Authors (2022).

3. Spoilage in Bread and Antifungal Mechanism of Action of Essential Oils

Bread is made through the mixture of ingredients, followed by molding, proofing, and baking in the oven at high temperatures (Almada-Érix et al., 2022). The economic losses associated with bread are due to high perishability and susceptibility to microbial contamination by several species, mainly fungi. Molds form colonies of filaments that grow and reproduce spores. These spores determine the color of the mold visible on the surface of the loaves and can spread by air current dispersal, when in an appropriate place, to another food, thus forming another colony. During cooling, slicing, and packaging, there may be cross-contamination by air, surfaces, equipment, raw materials, and manipulators. Also, ambient temperatures and high water activity during storage make bread more susceptible to fungi, especially molds. Therefore, moisture inside the package must be avoided by packing after the complete cooling of the product (Jarvis, 2001). However, this prolonged cooling step may be responsible for cross-contamination in bread, being the most common fungi of the genera: *Rhizopus sp*., *Aspergillus sp*., *Penicillium sp*., *Mucor sp*., and *Eurotium sp*. (Saranraj & Geetha, 2012). One of the most common is *Rhizopus stolonifer*, often referred to as the 'bread mold' (Khan et al., 2013).

Rhizopus sp. is a type of mold, which develops in tropical and subtropical regions, in almost every type of food, fresh, moist, or partially dried (Silva, 2008). Morphologically, they present non-septate hyphae, cottony mycelium and produce sporangiophores in nodules, where the rhizomes are found. Usually, their sporangia are very large and black, and hemispherical columella. The major characteristic of *Rhizopus stolonifer* is excessive growth at 25 °C, and can be seen in bread the sporangium that is white at first, then becoming black with maturation (Pitt & Hocking, 1997).

Species of the genus Aspergillus typically present septate conidiophores and base shaped as "L" or "T", commonly called foot cell, which is attached to vegetative hyphae. The conidiophore extends from the foot cell and may extend for a few millimeters until it reaches the vesicle (Klich, 2002). The members of the genus Aspergillus have a green to black coloration, and the *Aspergillus niger* presents a black cottony appearance, with spore heads often clearly visible. Similarly, *Penicillium spp*. can grow on bread surface and form a greenish layer. As described by Seiler (1992), colonies flat, and slow growth are characteristic of this genus. The conidia are spherical, unicellular, hyaline, and may be smooth or rough depending on the species (Cardoso, 2007).

Moreover, hot and humid storage conditions are suitable circumstances for the development of rope, which is a type of spoilage caused by bacteria of the genus *Bacillus spp*. The presence of this microorganism results in a soft, sticky, and fibrous dough that is brown in color. In several countries, the development of rope has become uncommon due to the use of chemical preservatives (calcium propionate), satisfactory hygiene conditions, and appropriate baking methods (Cauvain, 2015).

Fermentative yeasts (*Saccharomyces cerevisiae*) are added to the formulations in the baking process and play an important role in obtaining products with adequate softness and volume. However, undesirable yeasts may develop during storage and be found as white dots on the surface of the bread, which are known as chalk mold. These yeasts (*Pichia burtonii*) are easily confused with filamentous fungi due to the morphological similarity of their colonies (Legan & Voysey, 1991). To delay the development of fungi in bakery products, chemical preservatives such as calcium propionate and potassium sorbate, respectively, are added to the dough and/or applied to the surface of the bread (Saranraj & Geetha, 2012). Weak organic acids, including propionic, benzoic, and sorbic acids are also applied to the packaging material in order to extend the shelf life of this type of product. Undissociated acids penetrate the fungal membrane and acidify the cytoplasm, leading to cell death (Fernandez et al., 2006).

Essential oils can be used as an alternative natural preservative for bread, limiting or preventing the development of harmful fungi and mycotoxin producers in food (Tanackov & Dimić, 2013). The chemical structure of essential oils is composed of basic elements such as carbon, oxygen, and hydrogen and their chemical classification is complex, as they are formed by a mixture of several organic molecules, such as hydrocarbons, alcohols, esters, aldehydes, ketones, phenols, among others. The most common compounds in volatile oils are monoterpenes (90 %) and sesquiterpenes (Bizzo, Rezende & Hovell, 2009). For example, carvacrol (30 %) and thymol (27 %) are the major components of the Origanum compactum essential oil, linalool (68 %) of the *Coriandrum sativum* essential oil, α-phellandrene (36 %) and limonene (31 %) of leaf and carvone (58 %) and limonene (37 %) of seeds from *Anethum graveolens* essential oil, menthol (59%) and menthone (19%) of *Mentha piperita* essential oil (Bakkali et al., 2008). Several aromatic plants and their main compounds with antimicrobial activity reported in the literature are detailed in Table 1.

Table 1. Aromatic plants and/or spices and the main compounds associated with their antifungal activity.

Spice	Scientific name	Main compounds
Cinnamon	*Cinnamomum spp.*	Cinnamaldehyde
Clove	*Eugenia caryophyllus*	Linalol
Clove	*Syzygium aromaticum*	Eugenol
Thyme	*Thymus vulgaris*	Thymol
Laurel	*Laurus nobilis*	1,8-cineol
Oregano	*Origanum vulgare*	Carvacrol e thymol
Salvia	*Salvia officinalis*	Linalyl acetate
Marjoram	*Origanum majorana*	4-terpineol
Coriander	*Coriandrum sativum*	Camphor
Garlic	*Allium sativum*	Allicin
Ginger	*Zingiber oficinale*	α-zingiberene
Orange peel	*Citrus sinensis*	D-limonene
Star anise	*Illicium verum*	Trans-anethole
Peppermint	*Mentha piperita*	Menthol
Lemon grass	*Cymbopogon citratus*	Citral e mircene
Rosemary	*Rosmarinus officinalis*	α-pinene e camphor
Basil	*Ocimum basilicum*	Linalol

Source: Authors (2022).

The mode of action and morphological alterations were not completely elucidated and may vary according to the fungal species and essential oil components. Furthermore, the antimicrobial activity of essential oils also depends on the application scheme and the matrix into which it is put (Rahman et al., 2021). The presence and position of the hydroxyl group in the molecule, the presence of the aromatic nucleus, solubility in fats and spatial orientation affect the antifungal activity. Moreover, oxygen in monoterpenes and their carbonylated compounds enhance the antifungal activity of the components. Phenolic components (carvacrol, thymol, eugenol etc.) exhibit the strongest antifungal and antimycotoxigenic activity, followed by alcohols, aldehydes, ketones, ethers and hydrocarbons (Tanackov & Dimić, 2013).

The main accepted fungal mechanism is regarding the hydrophobicity of essential oils, which leads to an increase in the lipid bilayer permeability of the fungal cell membrane, resulting in cell death, or inhibiting the sporulation and germination of deteriorating molds. In addition, the proposed antifungal effects of essential oils involve a series of reactions in which the integrity of the cell wall and endomembrane system, including the plasma membrane, mitochondria and peroxisomes. In addition, essential oils can cause cytoplasm leakage, cell lysis and death (Grande-Tovar et al., 2018; Kumar et al., 2020; Sil et al., 2020).

Proposed complementary mechanisms involve the interference of essential oils in the enzymatic reactions of wall synthesis, which affect the morphogenesis and growth of hyphae, providing serious damage to the plasma membrane that results from direct injury to the membrane, instead of the metabolic impairment that leads to loss of the secondary membrane.

The inhibition of ergosterol synthesis by essential oils is also reported. Ergosterol is essential for maintaining cell integrity (viability and function) and normal growth of the molds. In addition, essential oils can cause dysfunction of the mitochondrial membrane potential due to changes in the flow of electrons through the electron transport chain, producing an altered level of reactive oxygen species that oxidize and damage lipids, proteins and deoxyribonucleic acid (Tian et al., 2012; Nazzaro et al., 2017; Grande-Tovar et al., 2018).

Sharma and Triphati (2006) studied the antifungal effects of essential oil from *Citrus sinensis* (L.) Osbeck epicarp on growth and morphogenesis of *Aspergillus niger* and concluded that hyphae showed lack of cytoplasm, damage and loss of integrity and rigidity of the cell wall. These observations indicate that the mode of antifungal activity of essential oils is a result of the attack of oil on the cell wall and retraction of cytoplasm in the hyphae, causing the death of the mycelium. Such modifications induced by essential oil may be related to the interference of essential oil components with enzymatic reactions of wall synthesis, which affects fungal morphogenesis and growth. Similarly, Zambonelli et al. (1996) reported fungal growth inhibition associated with degeneration of fungal hyphae after treatment with *Thymus vulgaris* essential oil.

The plasma membrane and the mitochondria of *Aspergillus flavus* were the antifungal targets of dill oil (*Anethum graveolens L.*), whose major components are carvone (41.5 %), limonene (32.6 %), and apiol (16.8 %). The primary lesion in the plasma membrane was verified through the inhibition of ergosterol synthesis, a major sterol component of the fungal cell membrane (Tian et al. 2012). Similarly, Pinto et al. (2013) concluded that the antifungal activity of the essential oil of *Thymus villosus* against *Aspergillus spp*. is probably due, at least in part, to its ability to cause the rapid metabolic arrest and plasma membrane disruption.

4. In Vitro Studies with Essential oils in the Inhibition of Spoilage Fungi of Breadmaking

Essential oils are used in the food industry, especially as flavorings, although they also have antimicrobial and natural antioxidant action, in addition to their use in food conservation. However, essential oils application in food conservation requires detailed knowledge about their properties, i.e., the minimum inhibitory concentration (MIC), microorganisms involved, mode of action, and the effects of the components of the food matrix on their antimicrobial and antioxidant properties (Hyldgaard et al., 2012).

The evaluation of in vitro antifungal activity of essential oils and active components are mainly carried out by disc-diffusion techniques and dilution tests. The results of the tests by the agar diffusion test (the most commonly used) are expressed by the inhibition zone of fungi surrounding the filter paper or well containing the active component (Bagheri et al., 2020). Agar or broth plates are inoculated with a solution of fungal spores or fungal mycelium disc at the center of the plate, with a certain diameter, and radial growth is recorded as a function of incubation time. Dilution assays are generally used to determine the MIC of a given active compound or its mixture (Debonne et al., 2018). Thus, it is possible to verify the effectiveness of inhibition and/or delay in fungal development against essential oils. Studies evaluating the in vitro antifungal activity of essential oils against spoilage fungi that belong to the genera most commonly found in bread are detailed in Table 2.

Table 2. Application of essential oils to inhibit spoilage fungi by microdilution.

Spice	Mold	Minimum inhibitory concentration	Reference
Cinnamon	*Aspergillus flavus*	200 µg.mL^{-1}	Mahmoud, 1994
		1.60 µL.mL^{-1}	Xing et al., 2010
		0.01 µL.mL^{-1}	Freire, 2008
		0.20 µL.mL^{-1}	Gasperini, 2014
	Rhizopus nigricans	6.40 µL.mL^{-1}	Xing et al., 2010
	Penicillium expansum	1.60 µL.mL^{-1}	Xing et al., 2010
	Aspergillus parasiticus	0.25 µL.mL^{-1}	Freire, 2008
Laurel	*Aspergillus niger*	225 µg.mL^{-1}	Santoyo et al., 2006
Star anise	*Aspergillus flavus*	1.0 µL.mL^{-1}	Freire, 2008
	Aspergillus parasiticus	2.0 µL.mL^{-1}	
Marjoram	*Aspergillus parasiticus*	2.0 µL.mL^{-1}	Freire, 2008
Lemon grass	*Aspergillus flavus*	200 µg.mL^{-1}	Gasperini, 2014
Clove	*Aspergillus flavus*	500 µg.mL^{-1}	Gasperini, 2014
Mexican oregano	*Aspergillus sp.*	150 µg.mL^{-1}	Ruiz et al., 2012
	Penicillium sp.	200 µg.mL^{-1}	
Thyme	*Aspergillus flavus*	500 µg.mL^{-1}	Gasperini, 2014

Source: Authors (2022).

Cinnamaldehyde is the major compound of cinnamon essential oil (Ju et al., 2018). It exerts antifungal activity against several species associated with bread including the genera Aspergillus, Penicillium, and Fusarium (Smid & Gorris, 2007). In a concentration of 4500 µg.mL^{-1}, the essential oil of cinnamon completely inhibited *A.flavus, A. parasiticus, and A. ochraceus* growth (Soliman & Badeaa, 2002). While studying the effects of this compound on the growth of A. flavus, Mahmoud (1994) established the MIC at 200 µg.mL^{-1}. Xing et al. (2010), in turn, defined the MIC of cinnamon essential oil against *Rhizopus nigricans*, *A. flavus*, and *Penicillium expansum* as 640 µg.mL^{-1}, 160 µg.mL^{-1}, and 16 µg.mL^{-1}, respectively. Through these results, it is observed that fungi of the genus Penicillium and Aspergillus are more sensitive to cinnamaldehyde, respectively, when compared to the genus Rhizopus.

De Corato et al. (2010) evaluated the antifungal activity of laurel essential oil on *Penicillium digitatum* and reported that the inhibition of mycelial growth after 7 days of incubation was 31, 53, and 71 % for the concentrations of 600, 800, and 1000 µg.mL^{-1}, respectively. This essential oil was also evaluated by the disc diffusion method against Aspergillus niger and presented an average inhibition halo of 19 mm, while the minimum fungicidal concentration (MFC) was 225 µg.mL^{-1} (Santoyo et al., 2006).

Oregano essential oil showed strong inhibitory action against several species of *Aspergillus* spp. At a concentration of 40 and 80 µL.mL^{-1}, the essential oil had fungicidal effects on *A. flavus*, *A. fumigatus*, and *A. niger* by a total inhibition of radial mycelial growth for 14 days of incubation. In addition, the essential oil was able to inhibit spore germination of these fungi at the mentioned concentrations (Carmo et al., 2008).

Fungal inhibition of Mexican oregano essential oil (*Lippia berlandieri*) was evaluated at concentrations ranging from 50 to 200 µg.mL^{-1}. There was a linear reduction in the specific growth rate and maximum growth in the stationary phase of fungi of the genus *Rhizopus* sp. as the concentration of the essential oil increased. Moreover, Ruiz et al. (2012) found that the concentrations of 150 and 200 µg.mL^{-1}, respectively, inhibited *Aspergillus sp*. and *Penicillium sp*. growth.

The essential oils of star anise showed inhibitory effects against *Aspergillus flavus* and *Aspergillus parasiticus*, as the growth and/or mycelial inhibition of fungal cultures was observed. The MIC for the mycelial growth of *A. flavus* was 1 µL.mL^{-1}. However, the MIC for *A. parasiticus* was 2.0 µL.mL^{-1} (Freire, 2008).

The antifungal activity of basil (*Ocinum basilicum L.*) oil was evaluated and the inhibition was 93.65 % for *Eurotium herbariorum* (Císarová et al., 2018). Pereira et al. (2006) tested the MIC against *Fusarium sp.*, *Aspergillus ochraceus*, *Aspergillus flavus*, and *Aspergillus niger* and found the value of 1500 µg.mL^{-1}.

Nguefack et al. (2004) investigated the inhibitory effects of lemongrass essential oil against three food-damaging fungi and mycotoxin producers (*Fusarium moniliforme, Aspergillus flavus*, and *Aspergillus fumigatus*). The essential oil prevented conidia germination and the growth of the three fungi at 800, 1000, and 1200 µg.mL^{-1}, respectively. In this case, fungi of the genus Aspergillus showed greater resistance to essential oil.

The in vitro antifungal activity of thyme essential oil (*Thymus zygis*) showed promising potential against *Aspergillus niger* and *Penicillium paneum* fungal strains while using the macro-dilution method with different pH ranges (4.8, 5.0, 5.5, and 6.0), water activity (0.95 and 0.97), and temperatures (22 and 30 °C). The diameter of the colonies was not significantly affected by pH and water activity, however, mycelial growth reduced as the concentration of thyme essential oil increased (0, 0.2, 0.5, and 1 µL.mL^{-1}) (Debonne et al., 2018). The thyme essential oil showed the MIC of 800, 1000, and 1200 µg.mL^{-1} for *Fusarium moniliforme*, *Aspergillus flavus*, and *Aspergillus fumigatus*, respectively (Nguefack et al., 2004). The essential oil of thyme (4500 µg.mL^{-1}) completely inhibited *A.flavus, A. parasiticus,* and *A. ochraceus* growth (Soliman & Badeaa, 2002).

In general, the minimum concentrations necessary to inhibit the fungi vary between 100 and 1500 µg.mL^{-1}, with higher concentrations (4500 µg.mL^{-1}) causing complete inhibition (Soliman & Badeaa, 2002). Aspergillus were the most studied and have shown to be more resistant to lemongrass, thyme, cinnamon essential oils, when compared to *Fusarium* and *Penicillium fungi*, respectively (Nguefack et al., 2004; Xing et al., 2010).

5. Essential Oils in Bread Analyzed in Situ

Some studies have shown that the addition of essential oils in bread inhibits the growth of spoilage microorganisms (Table 3). Rehman et al. (2007) conducted a study to determine the effects of orange peel essential oils on microbial growth and sensory characteristics of bread. Treatments included the application in the dough, sprinkling on the surface of the whole bread, slices, and packing material. The essential oils significantly affected sensory characteristics such as symmetry, the color of the crumbs and crust, flavor, texture, and aroma, in addition to inhibiting or delaying microbial growth. The maximum inhibitory effect was achieved by spraying the essential oils on all slices of the bread, which was more effective against fungal spoilage.

Table 3. Application of GRAS (Generally recognized as safe) essential oils and alcoholic extracts (AE) of aromatic plants in breadmaking

Product	**Mold**	**Spice**	**Effective concentration**	**Reference**
Bagels	*Penicillium expansum*	Oregano	150 µg.g^{-1} of emulsion with pectin	Salgado-Nava et al., 2020
Bread	*Aspergillus flavus*	Thyme	31.25 µL.L^{-1} of air	Císarová et al., 2020
Bread	*Aspergillus niger*	Cinnamon	100 µg.mL^{-1}	Sun et al., 2020
Spanish bread	*Rhizopus stolonifer*	Cinnamon Mustard	200 µg.mL^{-1} 50 µg.mL^{-1}	Clemente, Aznar & Nerín, 2019
Active bread packing	*A. niger,* *P. chrysogenum* *Rhizopus spp*	Salvia Marjoram	30 µL.mL^{-1} of gas phase	Krisch et al., 2013
Bread	*Aspergillus flavus,* *Penicillium spp.,* *Rhizopus and Mucor*	Orange peel	1.0 µL.mL^{-1}	Rehman et al., 2007
Salt bread	*Penicillium roqueforti* *Aspergillus ochraceus* *Rhizopus tolonifer*	Thyme Ginger (AE)	100 µL.mL^{-1}	Araújo, 2009
Sweet bread	*Penicillium roqueforti,* *Aspergillus ochraceus,* *Rhizopus stolonifer*	Clove Cinnamon (AE)	100 µL.mL^{-1}	Araújo, 2009
Hot dog bread	*Penicillium spp.* *Aspergillus flavus* *Endomyces fibuliger*	Mustard	3.5 mg.mL^{-1} of gas phase	Nielsen & Rios, 2000

Source: Authors (2022).

Rosa et al. (2020) studied the application of zein nanocapsules loaded with *Origanum vulgare L*. and *Thymus vulgaris* as a biopreservative in bread through in situ evaluation. The nanocapsules produced presented high thermal resistance to baking processes and extended the shelf life of this product by a period of 21 days with no colonies of molds and yeasts. While regular packaging showed visible growth of *A. niger* after 3 days of incubation, bread slices treated with cinnamaldehyde at 500 µg/mL exhibited 3–4 days of shelf-life improvement (Sun et al., 2020). Rodriguez, Nerin and Battle (2008) observed a strong correlation between the concentration of cinnamaldehyde in bread and the inhibition of fungal growth. After 3 days of storage, almost complete inhibition was obtained with 6 % cinnamon essential oil. However, sensory changes were undesirably significant. According to the authors, the solution may be the use of combined treatments, for example, essential oil with other preservatives, active packaging or modified atmosphere, thus requiring further investigation.

A combination of cinnamon and mustard essential oils (100:8) was suggested to be used at an industrial scale after the antifungal effect against R. stolonifer presented positive results in the acceptability and shelf-life tests. The combined essential oils showed mostly additive and synergistic and the application in Spanish bread has proved to be viable (Clemente, Aznar & Nerín, 2019). Mustard essential oil alone showed the biggest antifungal effect but acceptability tests were negative. The combined use with cinnamon masked the mustard flavor, benefiting the product sensorially.

Valková et al. (2021) evaluated the mycelial growth inhibition (MGI) of essential oils of rosemary (REO), lavender (LEO), and mint (MEO) on molds of the genus Penicillium. In situ analysis on the bread model showed that 125 µL/L of REO exhibited the lowest MGI of *P. citrinum*, and 500 µL/L of MEO caused the highest MGI of *P. crustosum*. The authors concluded that the analyzed essential oils have promising potential for use as innovative agents in the storage of bakery products to extend their shelf-life.

According to Preedy (2016), despite the proven preservative properties of essential oil, there are still limitations to their wider use in the food industry. In this context, several factors have become notable obstacles, including high extraction cost, diversity of chemical composition according to environmental conditions, low extraction yield regarding the needs of large volumes necessary to meet the demand of the food industry, and the intense sensory properties (flavor and aroma) that lead to rejection by consumers.

To make the use of essential oils viable for industrial application, the amount needed to perform antifungal activity should be evaluated, at a low cost and without undesirable sensory changes. Therefore, it may be interesting to use it in combination with other essential oils or chemical preservatives, such as weak organic acids.

6. Essential Oils in Active Packaging for Breadmaking

Active packaging aims to extend the shelf life of the food through the interaction between the product and the protective microenvironment created in the packaging stage (Dainelli et al., 2008; Gonçalves et al., 2017; Adel et al., 2019; Jiang et al., 2020). According to Long, Joly and Dantigny (2016) and De Azeredo (2013), the use of films and edible coatings incorporating essential oils is a promising technique to preserve food quality and increase shelf life. The same authors also mentioned that the main applications of films or modified atmosphere packaging containing essential oils for fungal control are related to the genera *Penicillium sp*. and *Aspergillus sp*., which are commonly found in breadmaking.

The incorporation of essential oils affects the continuity of the polymer matrix, leading to changes in the specific interactions of polymer-essential oil components. Generally, the film structure is weakened by the addition of essential oils, while the water barrier properties are improved and transparency is reduced. Antioxidant and/or antimicrobial properties of essential oils can be transferred to the packaging, being the chemical composition and specific interactions with the polymer determinant for its effectiveness as an active ingredient (Atares & Chiralt, 2016; Maisanaba et al., 2016; Assadpour & Jafari, 2019; Oliveira et al., 2020; Gholamian et al., 2021).

Gutierrez et al. (2009) evaluated the effects of microperforated polypropylene films added to cinnamon essential oil (*Cinnamomum zeylanicum*) on the conservation and sensory characteristics of a commercial bakery product (composed of sugar, egg yolks, almonds, walnuts, raisins, and other ingredients). The authors reported an increase in the shelf life of 3 to 10 days with maximum quality and safety, according to their sensory and microbiological evaluation. Furthermore, the authors described that the film produced can be easily disseminated with applicability in commercial systems in the short term.

Mustard essential oil was applied in a modified atmosphere system of rye and hot dog bread in order to inhibit spoilage fungi (*Penicillium spp*, *Aspergillus flavus*, and *Endomyces fibuliger*). The MIC for the active component, which was allyl isothiocyanate (AITC), was determined and the values estimated at 1800 to 3500 $\mu g.mL^{-1}$ of the gas phase, with more satisfactory results for rye bread compared to the hot dog bread. *Aspergillus flavus* was more resistant, while *Penicillium roqueforti* was more sensitive. However, when the gas phase contained at least 3500 $\mu g.mL^{-1}$, AITC was fungicidal for all fungi tested. The results indicated that the necessary storage time of rye bread may be achieved by active packaging with AITC. Still, active packaging with mustard essential oil for hot dog buns requires the added effect of other preservation factors to prevent mold growth (Nielsen & Rios, 2000).

Aiming to minimize the environmental impact caused by conventional polymers, Pontes (2013) produced and characterized biodegradable films of methylcellulose added from oil/water-type nanoemulsions of clove, oregano, and cinnamon essential oil. The application was performed on bread slices for them to be packed into the films and their microbiological, physic-chemical, and sensory quality studied. With the use of 2 % of essential oil, the incorporation of nanoemulsions into the films allowed the antimicrobial action on *Aspergillus niger* and *Penicillium spp*. in vitro, which was effective in reducing the numbers of yeasts and molds in slices of bread for 15 days of storage. The parameters of pH, total titratable acidity, and instrumental color of the bread samples were not affected by the incorporation of the nanoemulsions of the EOs. However, the sensory use of antimicrobial packaging negatively interfered in the flavor of the bread loaves.

Otoni et al. (2014) applied clove and oregano essential oil in free form and nanoparticles and added them to the formulation of antimicrobial methylcellulose films. The counts of molds and yeasts in sliced bread packed in these films were significantly reduced and the reduction in droplet size improved antimicrobial properties. Due to increased bioavailability, less preservative content might be used and still deliver the same antimicrobial efficiency if encapsulated in smaller particles.

Krisch et al. (2013) used the disk diffusion method with 30 μL of essential oil (steam phase), simulating an active packaging for bread. The application of sage and marjoram essential oils in a closed environment showed good antifungal activity against spoilage fungi of bread (*A. niger*, *P. chrysogenum*, and *Rhizopus spp*). Changes in colony size and growth rate were evaluated as markers to conclude that the growth of all the molds investigated was significantly reduced in the slices of bread treated in this system. However, the sensory analysis showed that the effective concentration of essential oils is very high, resulting in unacceptable changes in flavor and aroma. These undesirable sensory changes suggest the use of combined technologies to mitigate this effect and make the product palatable and safe.

7. Conclusion and Future Perspectives

Essential oils have a potential for application as natural preservatives in bread making as a way to inhibit the growth of spoilage microorganisms, especially molds. Their application on the surface of bread or in the packaging material is more viable compared to their application as an additive in the formulation of the product. In addition, the application of essential oil in the form of micro or nanoencapsulation or sachets can be promising. The main limiting factor, which presents itself as a major disadvantage, is the significant sensory alteration in food.

To enable the use of essential oils in breadmaking it is suggested that future research evaluate the use combined conservation technologies and emerging technologies with natural and chemical substances, in order to reduce the necessary proportions and enable the production of these foods on a commercial scale.

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References

Adel, A. M., Ibrahim, A. A., El-Shafei, A. M., & Al-Shemy, M. T. (2019). Inclusion complex of clove oil with chitosan/β-cyclodextrin citrate/oxidized nanocellulose biocomposite for active food packaging. *Food Packaging and Shelf Life*, 20, 100307. https://doi.org/10.1016/j.fpsl.2019.100307.

Almada-Érix, C. N., Almada, C. N., Pedrosa, G. T. S., Biachi, J. P., Bonatto, M. S., Schmiele, M., Nabeshima, E. H., Clerici, M. T. P. S., Magnani, M., & Sant'Ana, A. S. (2022). Bread as probiotic carriers: Resistance of *Bacillus coagulans* GBI-30 6086 spores through processing steps. *Food Research International*, 155, 111040. https://doi.org/10.1016/j.foodres.2022.111040.

Araújo, R. C. Z. (2009). Embalagens ativas com ervas aromáticas e condimentares na conservação de pães artesanais. 88p. Dissertação (Mestrado em Ciência de Alimentos). Universidade Federal de Lavras.

Assadpour, E., & Jafari, S. (2019). A systematic review on nanoencapsulation of food bioactive ingredients and nutraceutical by various nanocarriers. *Critical Reviews in Food Science and Nutrition*, 81, 209-219. https://doi.org/10.1080/10408398.2018.1484687

Atares, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in Food Science and Technology*, 48, 51-62. https://doi.org/10.1016/j.tifs.2015.12.001

Bagheri, L., Khodaei, N., Salmieri, S., Karboune, S., & Lacroix, M. (2020). Correlation between chemical composition and antimicrobial properties of essential oils against most common food pathogens and spoilers: In-vitro efficacy and predictive modelling. *Microbial Pathogenesis*, 2020, 104212. https://doi.org/10.1016/j.micpath.2020.104212

Bakhtiary, F., Sayevand, H. R., Khaneghah, A. M., Haslberger, A. G., & Hosseini, H. (2018). Antibacterial efficacy of essential oils and sodium nitrite in vacuum processed beef fillet. Applied Food Biotechnology, 5,1-10. https://doi.org/10.22037/afb.v5i1.17118

Bakkali, F. S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology*, 46, 446-475. https://doi.org/10.1016/j.fct.2007.09.106.

Barba, F. J., Esteve, M. J., & Frígola, A. (2014). Bioactive components from leaf vegetable products. In Atta-ur-Rahman (Ed.), Studies in natural products chemistry (pp. 321-346). Oxford: Elsevier. https://doi.org/10.1016/B978-0-444-63294-4.00011-5.

Bizzo, H. R, Rezende, C. M., Hovell, A. M. C. (2009). Brazilian essential oils: general view, developments and perspectives. *Química Nova*, 31, 588-594. https://doi.org/10.1590/S0100-40422009000300005.

Bora, H., Kamle, M., Mahato, D. K., Tiwari, P., & Kumar, P. (2020). Citrus essential oils (CEOs) and their applications in food: An overview. Plants, 9, 357. https://doi.org/10.3390/plants9030357.

Callaway, T. R., Carroll, J. A., Arthington, J. D., Edrington, T. S., &erson, R. C., and Ricke, S. C. (2011). Citrus products and their use against bacteria: potential health and cost benefits. In R. Watson, J. L. Gerald, & V. R. Preedy (Eds.), Nutrients, dietary supplements, and nutriceuticals: Cost analysis versus clinical benefits (pp. 277-286). New York: Humana Press. https://doi.org/10.1007/978-1-60761-308-4.

Cardoso, P. G et al. (2007). Diferenciação morfológica e molecular de fungos produtores de pectinases: *Penicillium expansum* e *Penicillium griseoroseum*. *Brazilian Journal of Microbiology*, 38, p. 71-77.

Carmo, E. S., Oliveira, E. L., & Souza, E. L. (2008). The potential of *Origanum vulgare* (Lamiaceae) essential oil in inhibiting the growth of some food-related Aspergillus species. *Brazilian Journal of Microbiology*, 39, 362-367. http://dx.doi.org/10.1590/S1517-83822008000200030

Cauvain, S. P. (2015). Technology of breadmaking. (3rd ed.). New York: Springer International Publishing. https://doi.org/10.1007/978-3-319-14687-4

Císarová, M., Hleba, L., Medo, J., Tančinová, D., Mašková, Z., Čuboň, J., Kováčik, A., Foltinová, D., Božik, M., & Klouček, P. (2020). The in vitro and in situ effect of selected essential oils in vapour phase against bread spoilage toxicogenic aspergilli. *Food Control*, 110, 107007. https://doi.org/10.1016/j.foodcont.2019.107007

Císarová, M., Hleba, L., Tančinová, D., Florková, M., Foltinová, D., Charousová, I., Vrbová, K., Božik, M., & Klouček, P. (2018). Inhibitory effect of essential oils from some Lamiaceae species on growth of Eurotium spp. isolated from bread. *Journal Microbiology Biotechnology Food Science*, 8, 857-862. https://doi.org/10.15414/jmbfs.2018.8.2.857-862.

Clemente, I., Aznar, M., & Nerín, C. (2019). Synergistic properties of mustard and cinnamon essential oils for the inactivation of foodborne moulds in vitro and on Spanish bread. *International Journal of Food Microbiology*, 298, 44–50. https://doi.org/10.1016/j.ijfoodmicro.2019.03.012.

Dainelli, D., Gontard, N., Spyropoulos, D., Beuken, E. Z., & Tobback, P. (2008). Active and intelligent food packaging: legal aspects and safety concerns. *Trends in Food Science and Technology*, 19, S103-S112. https://doi.org/10.1016/j.tifs.2008.09.011.

De Azeredo, H. M. (2013). Antimicrobial nanostructures in food packaging. *Trends in Food Science and Technology*, 30, 56-69. https://doi.org/10.1016/j.tifs.2012.11.006.

De Corato, U., Maccioni, O., Trupo, M., & Di Sanzo, G. (2010). Use of essential oil of Laurus nobilis obtained by means of a supercritical carbon dioxide technique against postharvest spoilage fungi. *Crop Protection*, 29, 142-147. https://doi.org/10.1016/j.cropro.2009.10.012.

Debonne, E., Bockstaele, F.V., Leyn, I., Devlieghere, F., & Eeckhout, M. (2018). Validation of in-vitro antifungal activity of thyme essential oil on Aspergillus niger and Penicillium paneum through application in par-baked wheat and sourdough bread. *LWT - Food Science and Technology*, 87, 368-378. https://doi.org/10.1016/j.lwt.2017.09.007.

Fernandes, R. P. P., Trindade, M. A., Lorenzo, J. M., & Melo, M. P. (2018). Assessment of the stability of sheep sausages with the addition of different concentrations of *Origanum vulgare* extract during storage. *Meat Science*, 137, 244-257. https://doi.org/10.1016/j.meatsci.2017.11.018.

Fernandez, F., Vodorotz, Y., Courtney, P., Pascall, M. A. (2006). Extended shelf-life of soy bread using modified atmosphere packaging. *Journal of Food Protection*, 69, 693-698. https://doi.org/10.4315/0362-028x-69.3.693.

Freire, J. M. (2008). Óleos essenciais de canela, manjerona e anis estrelado: caracterização química e atividade biológica sobre S. aureus, E. coli e A. flavus e A. parasiticus. 68 p. Dissertação (Mestrado em Agroquímica) – Universidade Federal de Lavras: Lavras.

Gasperini, A. M. (2014). Efeito de óleos essenciais sobre o crescimento e produção de aflatoxinas por *Aspergillus flavus*. Dissertação (Mestrado em Ciência de Alimentos) – Universidade Estadual de Campinas: Campinas.

Gholamian, S., Nourani, M., & Bakhshi, N. (2021). Formation and characterization of calcium alginate hydrogel beads filled with cumin seeds essential oil. *Food Chemistry*, 338, 128143. https://doi.org/10.1016/j.foodchem.2020.128143.

Gonçalves, N. D., Pena, F. L., Sartoratto, A., Derlamelina, C. B., Duarte, M. C. T., Antunes, A. E. C., & Prata, A. S. (2017). Encapsulated thyme (*Thymus vulgaris*) essential oil used as a natural preservative in bakery product. *Food Research International*, 96, 154-160. https://doi.org/10.1016/j.foodres.2017.03.006.

Grande-Tovar, C. D., Chaves-Lopez, C., Serio, A., Rossi, C., & Paparella, A. (2018). Chitosan coatings enriched with essential oils: Effects on fungi involved in fruit decay and mechanisms of action. *Trends in Food Science and Technology,* 78, 61-71. https://doi.org/10.1016/j.tifs.2018.05.019.

Gutiérrez, L., Sánchez, C., Batlle, R., & Nerín, C. (2009). New antimicrobial active package for bakery products. *Trends in Food Science and Technology*, 20, 92-99. https://doi.org/10.1016/j.tifs.2008.11.003.

Gyawali, R., Ibrahim, S. A. (2014). Natural products as antimicrobial agents. *Food Control*, 46, 412–429. https://doi.org/10.1016/j.foodcont.2014.05.047.

Hyldgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology*, 3, 12. https://doi.org/10.3389/fmicb.2012.00012.

Jarvis, B. (2001). Mould spoilage of food. *Process Biochemistry*, 7, 11-14.

Jiang, J., Lan, W., Sameen, D. E., Ahmed, S., Qin, W., Zhang, Q., Chen, H., Dai, J., He, L., & Liu, Y. (2020). Preparation and characterization of grass carp collagen-chitosan-lemon essential oil composite films for application as food packaging. *International Journal of Biological Macromolecules*, 160, 340-351. https://doi.org/10.1016/j.ijbiomac.2020.05.202.

Ju, J., Xie, Y., Yu, H., Guo, Y., Cheng, Y., Qian, H., & Yao, W. (2020). A novel method to prolong bread shelf life: Sachets containing essential oils components. *LWT - Food Science and Technology*, 131, 109744. https://doi.org/10.1016/j.lwt.2020.109744.

Ju, J., Xu, X., Xie, Y., Guo, Y., Cheng, Y., Qian, H., & Yao, W. (2018). Inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods. *Food Chemistry*, 240, 850-855. https://doi.org/10.1016/j.foodchem.2017.07.120.

Khan, S., Hashmi, S., & Saleem, Q. (2013). Microbial spoilage of bakery products and its control by preservatives. *Shodhankan*, 2, 169-177.

Klich, M. A. (2002). Identification of Common Aspergillus species. Amsterdam: Centraalbureau voor Schimmelautures, 116 p.

Krisch, J., Rentskenhand, T., Horváth, G., Vágvölgyi, C. (2013). Activity of essential oils in vapor phase against bread spoilage fungi. *Acta Biologica Szegediensis*, 57, 9-12.

Kumar, U., Kumari, P., Sinha, D., Yadav, M., Singh, D., & Singh, B. K. (2020). Antimicrobial activity of essential oils against plant pathogenic fungi: A review. *International Journal of Inclusive Development*, 6, 37-44. https://doi.org/10.30954/2454-4132.1.2020.7.

Legan, J. D., Voysey, P. A. (1991). Yeast spoilage of bakery products and ingredients. *Journal of Applied Bacteriology*, 70, 361-371. https://doi.org/10.1111/j.1365-2672.1991.tb02950.x.

Liewen, M. B., & Marth, E. H. (1985). Growth and inhibition of microorganisms in the presence of sorbic acid: A Review. *Journal Food Protection*, 48, 364-375. http://dx.doi.org/10.4315/0362-028X-48.4.364.

Long, N. N. V., Joly, C., & Dantigny, P. (2016). Active packaging with antifungal activities. *International Journal of Food Microbiology*, 220, 73–90. https://doi.org/10.1016/j.ijfoodmicro.2016.01.001.

Mahmoud, A. E. (1994). Antifungal action and antiaflatoxigenic properties of some essential oil constituents. *Letters in Applied Microbiology*, 19, 110–113. http://dx.doi.org/10.1111/j.1472-765x.1994.tb00918.x.

Mahmoudzadeh, M., Hosseini, H., Nasrollahzadeh, J., Khaneghah, A. M., Rismanchi, M., Chaves, R. D., Shahraz, F., Azizkhani, M., Mahmoudzadeh, L., & Haslberger, A. G. (2016). Antibacterial activity of *Carum copticum* essential oil against *Escherichia coli* O157:H7 in meat: Stx genes expression. *Current Microbiology*, 73, 265-72. http://dx.doi.org/10.1007/s00284-016-1048-2.

Maisanaba, S., Llana-Ruiz-Cabello, M., Gutiérrez-Praena, D., Pichardo, S., Puerto, M., Prieto, A. I., Jos, A., & Cameán, A. M. (2016). New advances in active packaging incorporated with essential oils or their main components for food preservation. *Journal Food Reviews International*, 33, 447-515. https://doi.org/10.1080/87559129.2016.1175010.

Mendes, K. D. S., Pereira Silveira, R. C. de C., & Galvão, C. M. (2019). Use of the bibliographic reference manager in the selection of primary studies in integrative reviews. *Texto e Contexto Enfermagem*, 28, 1–13. https://doi.org/10.1590/1980-265x-tce-2017-0204.

Nascimento, G. K. S., Rocha, L. O. F., & Schmiele, M. (2021). Potential of enhancing agents in replacing chemical additives in bread processing. In: D. T. Santos, R. A. Castillo-Torres and G. B. F Carvalho (Eds), The Food Industry: Perceptions, Practices and Future Prospects (pp. 13-43). New York: Nova Science Publisher. https://doi.org/10.52305/QFSM8429.

Nazzaro, F. I. D., Fratianni, F., Coppola, R., & De Feo, V. (2017). Essential oils and antifungal activity. *Pharmaceuticals*, 10, 86. https://doi.org/10.3390/ph10040086

Nedorostova, L., Kloucek, P., Kokoska, L., & Stolcova, M. (2008). Comparison of antimicrobial properties of essential oils in vapour and liquid phase against foodbourne pathogens. *Planta Medica*, 74, PI6. http:// dx.doi.org/10.1055/s-0028-1084914.

Neves, N. A., Gomes, P. T. G., Carmo, E. M. R., Silva, B. S., Amaral, T. N., & Schmiele, M. (2020). Sourdough and jaboticaba (*Plinia cauliflora*) for improvement on pan bread characteristics. *Research, Society and Development*, 9, e90691110552. https://doi.org/10.33448/rsd-v9i11.10552.

Neves, N. A., Gomes, P. T. G., Morais, L. A., Silveira, M. P., & Schmiele, M. (2021). Produção e avaliação das características tecnológicas e sensoriais de pães obtidos a partir de fermentação natural e acréscimo de cagaita (*Eugenia dysenterica* DC.). *Revista Brasileira de Agrotecnologia*, 11, 39-48. https://doi.org/10.18378/REBAGRO.V12I2.8749.

Neves, N. A., Gomes, P. T. G., & Schmiele, M. (2020). An exploratory study about the preparation and evaluation of sourdough breads with araticum pulp (*Annona crassiflora* Mart.). *Research, Society and Development*, 9, e956998036. https://doi.org/10.33448/rsd-v9i9.8036.

Nguefack, J., Leth, V., Amvam, P. H., & Mathur, S. B. (2004). Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. *International Journal of Food Microbiology*, 94, 329-334. https://doi.org/10.1016/j.ijfoodmicro.2004.02.017.

Nielsen, P. V., & Rios, R. (2000). Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. *International Journal of Food Microbiology*, 60, 219–229. https://doi.org/10.1016/S0168-1605(00)00343-3.

Oliveira, M. A., Gonzaga, M. L. C., Bastos, M. R. S., Magalhães, H. C. R., Benevides, S. D., Furtado, R. F., Zambelli, R. A., & Garruti, D. S. (2020). Packaging with cashew gum/gelatin/essential oil for bread: Release potential of the citral. *Food Packaging and Shelf Life*, 23, 100431. https://doi.org/10.1016/j.fpsl.2019.100431.

Otoni, C. G., Pontes, S. F. O., Medeiros, E. A. A., & Soares, N. F. F. (2014). Edible films from methylcellulose and nanoemulsions of clove bud (*Syzygium aromaticum*) and oregano (*Origanum vulgare*) essential oils as shelf life extenders for sliced bread. *Journal of Agricultural and Food Chemistry*, 62, 5214-5219. https://doi.org/10.1021/jf501055f.

Pankaj, S. K., Shi, H., & Keener, K. M. (2018). A review of novel physical and chemical decontamination technologies for aflatoxin in food. *Trends in Food Science and Technology*, 71, 73-83. https://doi.org/10.1016/j.tifs.2017.11.007.

Pereira, M. C., Vilela, G. R., Costa, L. M. A. S., Silva, R. F., Fernandes, A. F., Fonseca, E. W. N., and Picolli, R. H. (2006). Inhibition fungi growth through of utilization essential oils of spice. *Ciência e Agrotecnologia*, 30, 731-738. https://doi.org/10.1590/S1413-70542006000400020.

Pinto, E., Gonc, M. J., Hrimpeng, K., Pinto, J., & Vaz, S. (2013). Antifungal activity of the essential oil of *Thymus villosus* sub sp. lusitanicus against *Candida*, *Cryptococcus*, *Aspergillus* and dermatophyte species. *Industrial Crops and Products*, 51, 93–99.

Pitt, J. I. & Hocking, A. D. (1997). Fungi and food spoilage. 2. ed. London: Blackie Academic and Professional.

Pontes, S. F. O. (2013). Desenvolvimento de nanoemulsões de óleos essenciais incorporadas em filme de metilcelulose para uso em alimentos. Tese (Doutorado). Universidade Federal de Viçosa.

Preedy, V. R. (2016). Essential Oils in Food Preservation, Flavor and Safety. London, Academic Press. https://doi.org/10.1016/C2012-0-06581-7.

Rahman, M., Islam, R., Hasan, S., Zzaman, W., Rana, R., Ahmed, S., Roy, M., Sayem, A., Matin, A., Raposo, A., et al. (2022). A comprehensive review on bio-preservation of bread: an approach to adopt wholesome strategies. *Foods*, 11, 319. https://doi.org/10.3390/ foods11030319

Rehman, A., Jafari, S. M., Aadil, R. M., Assadpour, E., Randhawa, M. A., Mahmood, S. (2020). Development of active food packaging via incorporation of biopolymeric nanocarriers containing essential oils. *Trends in Food Science and Technology*, 101, 106-121. https://doi.org/10.1016/j.tifs.2020.05.001.

Rehman, S., Hussain, S., Nawaz, H., Ahmad, M, M., Murtaza, M, A., & Rizvi, A, J. (2007). Inhibitory effect of citrus peel essential oils on the microbial growth of bread. *Pakistan Journal of Nutrition*, 6, 558-561. https://doi.org/10.3923/pjn.2007.558.561.

Reis, J. B., Figueiredo, L. A., Castorani, G. M., Veiga, S. M. O. M. (2020). Evaluation of antimicrobial activity of essential oils against food pathogens. *Brazilian Journal of Health Review*, 3, 342-363. https://doi.org/10.34119/bjhrv3n1-025.

Rodriguez, A., Nerin, C., & Battle, R. (2008). New cinnamon-based active paper packaging against *Rhizopus stolonifer* food spoilage. *Journal of Agricultural and Food Chemistry*, 56, 6364-6369. https://doi.org/10.1021/jf800699q.

Rosa, C. G., Melo, A. P. Z., Sganzerla, W. G., Machado, M. H., Nunes, M. R., Maciel, M. V. O. B, Bertoldi, F. C., & Barreto, P. L. M. (2020). Application in situ of zein nanocapsules loaded with *Origanum vulgare Linneus* and *Thymus vulgaris* as a preservative in bread. *Food Hydrocolloids*, 99, 105339. https://doi.org/10.1016/j.foodhyd.2019.105339.

Ruiz, M. C. P., Sanchez, R. A. S., Ramos, S. V., Munoz, J. V. T., & Moorillón, G. V. N. (2012). Antifungal effect of Mexican oregano (*Lippia berlandieri* Schauer) essential oil on a wheat flour-based medium. *Journal of Food Science*, 77, 441-445. https://doi.org/10.1111/j.1750-3841.2012.02821.x.

Salgado-Nava, A. A., Hernández-Nava, R., López-Malo, A., & Jiménez-Munguía, M. T. (2020). Antimicrobial activity of encapsulated Mexican oregano (*Lippia berlandieri* Schauer) essential oil applied on bagels. *Frontiers in Sustainable Food Systems*, 4, 537091. https://doi.org/10.3389/fsufs.2020.537091.

Santos, F.S., Novales, M. G. (2012). Essential oils from aromatic herbs as antimicrobial agents. *Current Opinion in Biotechnology*, 23, 136–141. https://doi.org/10.1016/j.copbio.2011.08.005.

Santos, J. L. P. Bernardi, A., O., Morassi, L. L. P., Silva, B. S., Copetti, M. V., & Sant'Ana, A. (2016). Incidence, populations and diversity of fungi from raw materials, final products and air of processing environment of multigrain whole meal bread. *Food Research International*, 87, 103-108. https://doi.org/10.1016/j.foodres.2016.07.002.

Santoyo, S. R., Llorıa, L., Jaime, E., Ibanez, F. J., & Senor, G. (2006). Supercritical fluid extraction of antioxidant and antimicrobial compounds from Laurus nobilis: Chemical and functional characterization. *European Food Research Technology*, 222, 565-571. https://doi.org/10.1007/s00217-005-0027-9.

Saranraj, P., & Geetha, M. (2012). Microbial spoilage of bakery products and its control by preservatives. *International Journal of Pharmaceutical and Biological Archives*, 3(1), 38 48.

Seiler, D. (1992). Basic guide to product spoilage and hygiene. Part 1: Mould growth on bread. Chorleywood Digest, 124, CCFRA, Chipping Campden, UK, p.23.

Settanni, L., & Corsetti, A. (2008). Application of bacteriocins in vegetable food biopreservation. *International Journal of Food Microbiology*, 121, 123-138. https://doi.org/10.1016/j.ijfoodmicro.2007.09.001.

Sharma, N., & Tripathi, A. (2006). Effects of *Citrus sinensis* (L.) Osbeck epecarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiological Research*. 163, 337-344.

Sharma, P., Ahuja, A., Dilsad Izrayeel, A. M., Samyn, P., & Rastogi, V. K. (2022). Physicochemical and thermal characterization of poly (3-hydroxybutyrate-co-4-hydroxybutyrate) films incorporating thyme essential oil for active packaging of white bread. *Food Control*, 133(November 2021). https://doi.org/10.1016/j.foodcont.2021.108688

Sil, A., Pramanik, K., Samantaray, P., Mondal, F. M., & Yadav, V. (2020). Essential oils: A boon towards eco-friendly management of phytopathogenic fungi. *Journal of Entomology and Zoology Studies*, 8, 1884-1891.

Silva, F. C. (2008). In vitro and in vivo effect of condiment essential oils on fungi that occur in postharvest on strawberry and papaya fruits. Masters dissertation. Lavras: UFLA, 85 p.

Silva, S. J. M., Spencer, P. V. D., Pinto, N. A. V. D., & Schmiele, M. (2020). Antimicrobial performance on molds isolated from muffin and antioxidant capacity of raw and roasted coffee oils (*Coffea arabica* L.). *Research, Society and Development*, 9, e9289109285. https://doi.org/10.33448/rsd-v9i10.9285.

Silveira, M. P., Neves, N. A., Silveira, J. V. W., & Schmiele, M. (2019). Nanotechnology applied to cereal grains and cereal-based products and its food safety. In: G. Molina, Inamuddin, F. M. Pelissari, and A. M. Asiri (Eds), Food Applications of Nanotechnology (pp. 211-223). Boca Raton: CRC Press.

Simões, C. M. O., & Spitzer, V. (2007). Óleos voláteis. In: C. M. O. Simões, E. P. Schenkel, G. Gosmann, J. C. P. Mello, L. A. Mentz, & P. R. Petrovick (Eds), Farmacognosia: da planta ao medicamento (pp. 467-496). Porto Alegre/Florianópolis: UFRGS/UFSC.

Siqui, A. C., Sampaio, A. L. F., Sousa, M. C., Henriques, M. G. M. O., & Ramos, M. F. S. (2000). Óleos essenciais - potencial antiinflamatório. *Biotecnologia, Ciência e Desenvolvimento*, 16, 38-43.

Smid, E. J., & Gorris, L. G. M. (2007). Natural antimicrobials for food preservation, p. 285-308. M. S. Rahman (ed.), *Handbook of food preservation* (2nd ed.) (pp. 237-258). New York: CRC Press.

Soliman, K. M., & Badeaa, R. I. (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food and Chemical Toxicology*, 40, 1669–1675. https://doi.org/10.1016/S0278-6915(02)00120-5.

Sun, Q., Li, J., Sun, Y., Chen, Q., Zhang, L., & Le, T. (2020). The antifungal effects of cinnamaldehyde against *Aspergillus niger* and its application in bread preservation. Food Chemistry, 317, 126405. https://doi.org/10.1016/j.foodchem.2020.126405.

Tanackov, D. K.; Dimić, G. R.(2013). Antifungal activity of essential oils in the control of food-borne fungi growth and mycotoxin biosynthesis in food. In: A. Méndez-Vilas (Ed), Microbial pathogens and strategies for combating them: science, technology and education (pp. 838-849). Badajoz: Formatex Research Center.

Tatar, Y., Fadavi, A., & Koohsari, H. (2021). Mitigation of *Aspergillus flavus* and its aflatoxins in wheat grains by gamma irradiation and calcium oxide. *Journal of Crop Protection*, 10, 261-270.

Tian, J., Ban, X., Zeng, H., He, J., Chen, Y, & Wang, Y. (2012). The mechanism of antifungal action of essential oil from dill (*Anethum graveolens L.*) on *Aspergillus flavus*. PLoS One, 7, e30147. https://doi.org/10.1371/journal.pone.0030147.

Valková, V., Ďúranová, H., Galovičová, L., Vukovic, N. L., Vukic, M., & Kačániová, M. (2021). In vitro antimicrobial activity of lavender, mint, and rosemary essential oils and the effect of their vapours on growth of penicillium spp. In a bread model system. *Molecules*, 26(13). https://doi.org/10.3390/molecules26133859

Xing, Y., Xihong, L., Qinglian, X., Yun, J., & Lu, Y. (2010). Antifungal activities of cinnamon oil against *Rhizopus nigricans*, *Aspergillus flavus* and *Penicillium expansum* in vitro and in vivo fruit test. *International Journal of Food Science and Technology*, 45, 1837–1842. https://doi.org/10.1111/j.1365-2621.2010.02342.x.

Zambonelli, A., Zechini d'Aulerio, A., Bianchi, A., Albasini, A. (1996). Effects of essential oil on phytopathogenic fungi. *Journal of Phytopathology*, 144, 491–494. https://doi.org/10.1111/j.1439-0434.1996.tb00330.x.