Journal of Essential Oil Research

ISSN: 1041-2905 (Print) 2163-8152 (Online) Journal homepage: http://www.tandfonline.com/loi/tjeo20

The use of essential oils as natural antifungal preservatives in bread products

Els Debonne, Filip Van Bockstaele, Simbarashe Samapundo, Mia Eeckhout & Frank Devlieghere

To cite this article: Els Debonne, Filip Van Bockstaele, Simbarashe Samapundo, Mia Eeckhout & Frank Devlieghere (2018): The use of essential oils as natural antifungal preservatives in bread products, Journal of Essential Oil Research, DOI: 10.1080/10412905.2018.1486239

To link to this article: https://doi.org/10.1080/10412905.2018.1486239

Published online: 22 Jun 2018.

JOURNAL OF ESSENTIAL OIL RESEARCH, 2018
https://doi.org/10.1080/10412905.2018.1486239

Taylor & Francis
Taylor & Francis Group

The use of essential oils as natural antifungal preservatives in bread products

Els Debonne[a,b], Filip Van Bockstaele[a], Simbarashe Samapundo[c], Mia Eeckhout[a,b] and Frank Devlieghere[b,c]

[a]Faculty of Bioscience Engineering, Research Unit of Cereal and Feed Technology, Department of Food Technology, Safety and Health, Ghent University, Ghent, Belgium; [b]Laboratory of Applied Mycology (MYCOLAB), Faculty of Bioscience Engineering, Department of Food Technology, Safety and Health, Ghent University, Ghent, Belgium; [c]Laboratory of Food Microbiology and Food Preservation, Faculty of Bioscience Engineering, Department of Food Technology, Safety and Health, Food2Know, Ghent University, Gent, Belgium

ABSTRACT

This review addresses the recent advances of the application of essential oils (EOs) in *in vitro* systems and in bread systems for the reduction of fungal spoilage. Given the number of research articles concerning the use of EOs as potential antifungal food preservatives, it is generally accepted that they must be given further attention for use in specific food matrices. However, despite the numerous articles stating the antifungal effect of EOs, very few report the actual application in bread or other bakery products and the impact addition can have on dough and bread production, physico-chemical, microbiological and sensorial quality. Advances have been made in the area of food preservation, but further research is necessary to fully comprehend the mode of action and to establish actual food applications of EOs in the bread and bakery industry.

ARTICLE HISTORY
Received 25 April 2017
Accepted 30 May 2018

KEYWORDS
Bread; *clean label*; food preservation; bread; essential oil; antifungal screening methods

1. Introduction

Much research has already been conducted in the domain of natural antimicrobial preservatives as a result of increasing signs of negative effects due to the intake of chemical preservatives (1–4) and the changing consumer perception towards food preservatives. Shim et al. (5) reported that consumers were very concerned about the use of food additives, due to difficulties understanding the topic of food preservatives, insufficient education and public relations. The increasing interest in *clean label* food products has heightened the need for natural antimicrobial preservation strategies. In bread and other bakery products, the replacement of chemical preservatives such as propionates and sorbates is of particular interest (6).

Microbiological spoilage of bread is primarily an issue for bread products intended to be stored at room temperature for a longer period than the time needed to be rejected because of staling. Bread products such as packaged sliced toast bread and par-baked bread products packed under modified atmosphere fall under this scope. Conditions in food, such as pH and water activity (a_w), are very important as they determine which type of microbiological spoilage can occur. The a_w of wheat bread is generally higher than 0.96 (7,8) and the pH is higher than 5.5 (8). Rye bread and sourdough fermented bread have respectively a_w-values and pH-values of 0.94–0.97 and 4.4–4.8 (9,10). However, variations can occur due to differences in fermentation and production of bread. Mould growth is by far the most important shelf-life limiting factor of bread products, with *Penicillium* spp. and *Aspergillus* spp. being the most dominant species (11). Next to mould growth, the formation of *rope* as a result of the growth of the spore forming bacterium *Bacillus subtilis* usually present in raw bakery materials can also result in the rejection of bread products. Spores of *Bacillus* can survive the baking process, after which they can potentially cause spoilage of the baked product (8,10,12). Spoilage of bread can also be caused by chalk yeasts (cf. chalk moulds). These are spoilage yeasts which cause chalk mould defects on food (dust-type spots) (13). They are most common on sliced bread and on rye bread. There are approximately 24 types of chalk moulds. *Saccharomycopsis fibuligera* (10,14,15), *Hyphopichia burtonii* (9,10,14), *Zygosaccharomyces bailli* and *Saccharomyces cereviseae* (13) have been reported as dominant species. According to Deschuyffeleer et al. (16), only *S. fibuligera* and *H. burtonii* can be labelled as true chalk yeasts. Spoilage can also be caused by non-chalk yeasts, e.g. *Wickerhamomyces anomalus*. *W. anomalus* and *S. fibuligera* were identified as being responsible for the

CONTACT Els Debonne els.debonne@ugent.be

early spoilage of commercial modified atmosphere packaged (MAP) par-baked breads.

Among the many potential preservation techniques developed in attempting to achieve longer microbiological safe shelf-lives are technological/chemical techniques and strategies whereby natural ingredients are added to the bread recipe (biopreservatives), e.g. the use of essential oils (EOs) with antifungal activity. Smith et al. (8) have presented a thorough review on microbial control measures for bakery products, with a main focus on technological strategies and the use of organic acids to reduce post-baking contamination. However, regardless of the fact that these technological methods show beneficial lethal effects towards microorganisms, few of these techniques are commercially applied on bread. Chemical preservation of bread is mainly done by propionic acid and its salts (12). Besides chemical preservation, packaging and storage can also be used to control the growth of post-baking contaminants. Modified atmosphere packaging (MAP) can help with the reduction or total elimination of chemical preservatives in bread products (16,17). In the packaging industry different ways for creating an optimal condition for a shelf-life extension have been developed (e.g. ethanol emitters and O_2 absorbers) (18). Natural packaging materials can also be used, such as chitosan films (19,20). One of the simplest ways to prolong shelf-life is to store the breads at chilled or freezing temperatures. However, on an industrial level, these solutions require a large chill/frozen storage capacity and chilled/frozen transportation and come with a high additional cost (21). The latter, non-technological/non-chemical, biopreservative strategy can be divided into two subcategories according to their mode of action, namely (i) by addition of an active antimicrobial ingredient (e.g. EOs) or (ii) by addition of an ingredient and consecutive *in situ* production of active compounds. An example of the latter strategy often described for mould inhibition of bread products is the use of lactic acid bacteria through sourdough fermentation. However, the antimicrobial compounds formed by lactic acid bacteria are often produced in concentrations of that are too low to be a stand-alone preservation strategy (22–26). In comparison to lactic acid bacteria, propionic acid bacteria are far less documented despite their favourable antimicrobial effect. Propionic acid is primarily inhibitory to moulds and *Bacillus spp*. (27). A study by Gardner et al. (28) suggests the use of yeast extracts which are previously fermented by propionibacteria instead of non-fermented yeast extracts as the former results in better leavening as well as preservation properties. Yeasts as such can have both beneficial and detrimental effects on foods (29). For example, regardless of the fact that *W. anomalus* is a common spoilage yeast in bakery products (16), it was also shown to have food-flavouring and emulsifying properties (30). More research and evidence is needed on the use of yeasts as biocontrol agents, because a true consensus has not yet been found in literature.

To our knowledge, no review article has yet been published, which collects the most relevant up-to-date literature on the use of EOs as promising natural antimicrobial preservation strategy targeting bread products. This review article therefore constitutes a novel contribution to the literature on natural preservation strategies and provides a clear overview specifically for the bread baking industry.

2. Natural antimicrobial preservation strategies derived from plant components

Plants possess a range of tools to combat pathogen infections, including the formation of substances with antifungal activity. These compounds can be classified in three categories: (cat. 1) phytoanticipins which are preformed antimicrobial components present in plants; (cat. 2) inducible preformed compounds and; (cat. 3) phytoalexins which include induced inhibitory components when the plant is under pathogen attack. These components are low-molecular weight, wide-spectrum secondary antimicrobial metabolites (31) and can be present in fruits, vegetables and plants in the peels, seeds, bark and cereal bran (32). All three categories have potential biopreservative activity in food products. Among category 1, EOs form the largest group and will be discussed in detail throughout this review. Compared to category 1, categories 2 and 3 are far less documented. Till today, there is still very little known on actual applications of these latter compounds in food products (33). Some believe it is due to the fact that rather high concentrations are needed in order to show antimicrobial activity and are therefore not considered as potential antimicrobial compounds. In addition, they are expensive to isolate or to synthesize and would be an expensive alternative to the antifungal preservatives currently being used. Many phytoalexins have been reported to be rather unspecific in their toxicities, meaning that they can also be toxic towards humans (34). Table 1 gives a clear overview of the most relevant studies performed on the antifungal activity of EOs that can be used or has already been tested for use in the bakery industry. The different antifungal screening techniques, target moulds and main results are summarised as well.

2.1. Essential oils (EOs)

Approximately 3 000 EOs have already been discovered. Only 300 of them have commercial importance. They are used in the food industry, as well as in pharmaceutical, agronomic and cosmetic industries (35,36). Knowledge of

Table 1. Overview of preformed resistance components in plants tested for antifungal food preservation (Category 1: phytoanticipins) (Test methods: agar diffusion (Adif), disc diffusion (Ddif), agar dilution (Adil), broth dilution (Bdil), Poisoned Food (PF) and Inverted Petri Plate (IPP)).

	Target moulds	Test method	Result – active component(s)	Refs.
Essential oils				
Bay (*Pimenta racemosa*)	Af, An, Ea, Eh, Er, Eru, Pcr	Adif	*agar/ cake analogue* – / – eugenol (57%)	(52)
Boswellia carterii Birdw	Af	Bdil; PF	MIC 1.75 µL/mL	(57)
Chamomile (*Matricaria chamomilla L.*)	Af	Bdil; Ddif	MIC 10–17.5 µg/mL	(59)
Cinnamon	Af, Ef, Pcm, Pr	Ddif	100% growth reduction of Pr	(51)
Cinnamon leaf	Af, An, Ea, Eh, Er, Eru, Pcr	Adif	*agar/ cake analogue* – / – eugenol (78.5%)	(52)
Cinnamon leaf and bark volatile oils and oleoresins	Af,An, Ao, At, Pci, Pvir	Adif; PF	eugenol (87.3%), (E)-cinnamaldehyde (49.9% in bark oleoresin)	(44)
Cinnamon bark (*Cinnamomum jersenianum Hand.-Mazz*)	Af	PF	MIC 8 µL/mL for 9d	(62)
Clove (*Syzygium aromaticum L.*)	Af, An, Ea, Eh, Er, Eru, Pcr	Adif	*agar/ cake analogue* – / – eugenol (83.9%)	(52)
Clove (*Syzygium aromaticum L.*)	Af, Ef, Pcm, Pr	Ddif	94% growth reduction of Pr	(51)
Clove (*Syzygium aromaticum L.*)	Af, Ap	Adil	*MIC 1500 µL/L*	(54)
Clove (*Syzygium aromaticum L.*)	Af, Pci, Rhn	Adil	MIC (Af, Pci) 25 µL/mL, MIC (Rhn) 50 µL/mL – eugenol (83.02%)	(55)
Coriander (*Coriandrum sativum L.)*	Af	Bdil	MIC 2.0–3.0 µL/mL; *Chickpea seed spraying* – 65.45% protection of seeds during 172 h incubation	(58)
Cumin seed (*Cumimum cyminum L.*)	Af	PF	MIC 0.6 µL/mL – cymene (47.08%); *Inoculation* of wheat and chickpea samples	(45)
Curcuma (*Curcuma aromatica*)	Cf	IPP	MIC 3000 ppm	(48)
Daucus carota	Cf	IPP	MIC 3000 ppm	(48)
Fennel (*Foeniculum vulgare L.*)	Af	Bdil	MIC 10–12.5 µg/mL; *Disc diffusion*	(59)
Garlic (*Allium sativum*)	Af, Ef, Pcm, Pr	Ddif	79% growth reduction of Pr	(51)
Grapefruit (*Citrus paradise L.*)	Af, An, Pch, Pv	PF	MIC (An) 0.94%	(61)
Lemon (*Citrus lemon L.*)	Af, An, Pch, Pv	PF	MIC (An) 0.94%	(61)
Lemon (*Citrus limon Risso*)	Af, An,F, P, Rh	Adif	effective against Af, An and Rh	(49)
Lemon grass (*Cymbopogon citratus*)	Af, An, Ea, Eh, Er, Eru, Pcr	Adif	*agar/ cake analogue* – / – geraniol (50.5%), neral (29.4%)	(52)
Majoram (*Origanum majorana L.*)	Af	Bdil	MIC 2.0–3.0 µL/mL; *Chickpea seed spraying* – 67.86% protection of seeds during 172 h incubation	(58)
Mandarin (*Citrus reticulate L.*)	Af, An, Pch, Pv	PF	MIC (An) 0.94%, growth inhibition of An at respective concentrations 0.47% and 0.71%: 14.7% and 31.9%	(61)
Mustard	Af, Ef, Pcm, Pr	Ddif	100% growth reduction of Pr	(51)
Orange (*Citrus sinensis L.*)	Af, An, Pch, Pv	PF	MIC (An) 0.94%	(61)
Orange (Citrus sinensis)	Af, Afu, P, Rh, M		*Mould counting of bread* – Spraying technique resulted in the highest inhibitory effect on mould growth	(67)
Oregano (Origanum vulgare L.)	Af, Afu, An, Ao Ap, At	Adif	MIC 20–80 µL/mL	(50)
Oregano (*Origanum vulgare*)	Af, Ef, Pcm, Pr	Ddif	50% growth reduction of Pr	(51)
Rosemary (*Rosemary officinalis*) (microencapsulated)	A, P	PF	MIC 50 µL/mL (P), MIC 20 µL/mL (A) – 1.8-cineole (29.0%), camphor (26.6%), alpha-pinene (10.6%)	(63)
Thyme (-)	Af, An, Ea, Eh, Er, Eru, Pcr	Adif	*agar/ cake analogue* – / – thymol (53.9%), p-cimene (25.2%)	(52)
Thyme (*Thymus vulgaris L.*)	Af, Alt, At, Fo	PF	MIC 0.7 µL/mL	(64)
Thyme (*Thymus leptobotrys*)	Pi	Adil	MIC 10% (wt/v)	(56)
Thyme (*Thymus zygis*)	An, Pp	Adil	Bread shelf-life	(42)
Phenolic compounds				
(S)-limonene	An, Fo, Pd, Rhs	PF	EC_{50} 38.04 (An), 120.6 (Fo) and 26.83 mg/L (Pd)	(65)
1,8-cineole	An, Fo, Pd, Rhs	PF	EC_{50} 36.40 (An), 148.4 (Fo) and 51.61 mg/L (Pd)	(65)
1,8-cineole	Af, Ap	PF; Ddif	inhibition from 1.35 µL/20 mL YES or mL headspace Petri plate	(46)
Eugenol	Af, An, F, P,Rh	Adif	MIC < 0.5% (An), MIC 2% (Af), 4% (F, P, Rh)	(49)
Eugenol	Af	Bdil	MIC 0.5 µL/mL	(60)
Geraniol	Cf	IPP	MIC 3000 ppm	(48)
Linalool	Cp	IPP	MIC 3000 ppm	(48)
Linalool	Af	Bdil		(60)
Menthol	Cp	IPP	MIC 2000 ppm	(48)
Menthol	Af	Bdil	MIC 0.9 µL/mL	(60)
Myrcene	Af, An,F, P, Rh	Adif		(49)
Thymol	An, Fo, Pd, Rhs	PF	EC_{50} 23.80 (An), 50.37 (Fo) and 20.14 mg/L (Pd)	(65)
Thymol	Af	Bdil	MIC 0.2 µL/mL	(60)
Glucosinolates				
Allyl isothiocyanate	Af, Ef, Pcm, Pr	Ddif	MIC 2.4 µg/mL gas (rye bread), MIC 1.8–3.5 µg/mL gas (hot-dog bread)	(51)
Allyl isothiocyanate	Af, Alt, An, Fg, Fo, Fs, Pch, Pci	Adif	MIC, respectively, 37, 22, 37, 16, 22, 34, 62, 22 ng/mL	(53)

Notes: A: *Aspergillus* spp.; Af: *A. flavus*; Afu: *A. fumigatus*; Ap: *A. parasiticus*; At: *A. terreus*; Ao: *A. ochraceus*; An: *A. niger*; Alt: *Alternaria alternata*; Cf: *Colletotrichum falcatum*; Cp: *Curvularia pallescens*; Ef: *Endomyces fibuliger*; Ea: *Eurotium amstelodami*; Eh: *Eur. herbariorum*; Er: *Eur. repens*; Eru: *Eur. rubrum*; F: *Fusarium* spp.; Fg: *F. graminearum*; Fo: F. oxysporum; Fs: *F. solani*; M: *Mucor* spp.; P: *Penicillium* spp.; Pch: *P. chrysogenum*; Pci: *P. citrinum*; Pcm: *P. commune*; Pcr: *P. corylophilum*; Pd: *P. digitatum*; Pe: *P. expansum*; Pi: *P. italicum*; Pp: *P. paneum*; Pr: *P. roqueforti*; Pv: *P. verrucosum*; Pvir: *P. viridicatum*; Rh: *Rhizopus* spp.; Rhn: *R. nigricans*; Rhs: *Rhizoctonia solani.*

antifungal and bactericidal activity of EOs exists since the Middle Ages (35). Initially, its use was mainly situated in the medicinal world. Nowadays, with the rising awareness for green consumerism, the *clean label* trend and growing negative perception of synthetic preservatives, EOs have regained interest from food producers and scientists.

2.2. Mode of action of EOs

EOs can be classified into four groups of active compounds, including terpenes, terpenoids, phenylpropenes and *others* (37). Due to the lipophilic behaviour of EOs, they can pass through the cell wall and cytoplasmic membrane. Hereby, exerting cytotoxic effects on living cells and affecting the cell membrane and organelles (38). The antimicrobial activity of EOs is influenced by the composition, concentration, structure as well as the functional groups of their constituents, with phenolic groups being most effective (36). EOs can cause extensive lesions of the cell membrane and a reduction of ergosterol, which is a major component present in fungal cell membranes (39). For example, the active components thymol and carvacrol induce cell lysis and alter the cell structure of proliferating cells; cinnamaldehyde is responsible for inhibition of cell division (40,41) which leads to an extension of the shelf-life; and the activity of isothiocyanates, such as those present in mustard, is based on the isothiocyanate group which is highly electrophilic and reacts with oxygen, sulphuric or nitrogen-centred nucleophiles. The general inhibitory action is due to enzyme inhibition and alteration of proteins by oxidative cleavage of disulfide bonds (37).

2.3. In-vitro antifungal assessments of EOs and active components

In-vitro screening of antifungal activity of EOs and its active components are mostly performed by three different techniques: (i) diffusion assay, (ii) dilution assay and (iii) *Poisoned food* assay (Table 1). To screen the antifungal activity of EOs and its components in the vapour phase, an agar diffusion, disc diffusion or *Inverted Petri plate* assay is mostly performed. The antifungal activity of the EO or component is expressed by the zone of inhibition of the fungi surrounding the filter paper or well containing the active component (38). The diffusion assays account for 50% of the antifungal screening methods of EOs. The agar or broth dilution and the Poisoned Food assays are generally used in, respectively, 10, 20 and 30% of the test cases. These assays are based on the principle of preparing dilutions of the EOs (or components) in the agar or broth. Further, the agar plates or broth are inoculated with a fungal spores solution (42) or a disc of fungal mycelium with a certain diameter is placed in the centre of the agar plate in case of the Poisoned Food assay (43–46). Radial growth is recorded in function of incubation time. These dilution methods allow the investigation of multiple organisms at the same time (47).

2.3.1. Diffusion assays

The *Inverted Petri plate* assay was performed by Singh et al. (48) for the evaluation of antifungal activity of EOs of curcuma (*Curcuma aromatica*) and *Daucus carota*, and monoterpenoids geraniol, linalool and menthol on pathogenic fungi *Colletotrichum falcatum* and *Curvularia pallescens*. The minimal inhibitory concentration (MIC) ranged between 2000 and 3000 ppm. Leite de Souza et al. (49) described the antifungal activity of phytochemicals citral, eugenol and myrcene through an agar diffusion assay. *Origanum vulgare* L. (Laminaceae) EO showed strong anti-*Aspergillus* activity in an *in vitro* study performed by Carmo et al. (50). The main active constituent of the genus *Origanum* is carvacrol (isothymol) (87%). The EO derived from *Origanum* and its aromatic monoterpene constituents (carvacrol and thymol) have been proven to have good *in vitro* antifungal activity. Nielsen et al. (51) investigated the antifungal effect of several EOs through a disc diffusion assay and found that both cinnamon and mustard EO were able to result in a 100% reduction of growth of *P. roqueforti* with only 1 µL EO used to inoculate the Petri dish system. The main antifungal active component in cinnamon EO is eugenol (52) and in mustard EO allyl isothiocyanate (51,53).

2.3.2. Dilution assays

Clove oil (*Syzygium aromaticum* L.) showed good antifungal activity against *Aspergillus flavus* and *A. parasiticus* (MIC 15 µL/mL) (54); *A. flavus* and *Penicillium citrinum* (MIC 25 µl/mL) and *Rhizopus nigricans* (MIC 50 µL/mL) through an agar dilution assay (55). Thyme oil (*Thymus leptobotrys*) completely inhibited growth of *P. italicum* (citrus blue mould) at a concentration of 10% (wt/v) (56). The agar dilution technique is not often used for the screening of antifungal activity of EOs. Main reason is because of the hydrophobicity of EOs and currently having no suitable solution for this problem. However, in order to screen the antifungal activity of EO as bakery ingredients, it is essential to incorporate the oil in the food matrix or in a model food matrix. Moreover, the antifungal activity of EOs will be higher in diffusion assays compared to dilution assays. Broth dilution is also a widely used technique for a fast screening of the antifungal activity of EOs (or components). EOs of *Boswellia carterii Birdw*, chamomile, coriander, fennel, and majoram showed MIC values towards *A. flavus* ranging from 1.75 to 17.5 µg/mL broth (57–59). Mishra et al. (60) investigated

the antifungal activity of several phenolic compounds against *A. flavus* and found the following order of antifungal effect: thymol > eugenol > menthol > linalool. Viuda-Martos et al. (61) determined via a *Poisoned food* assay with *A. niger* that the MIC of EOs of citrus fruit (e.g. grapefruit, lemon and mandarin) was equal to 0.94%. The MIC of cinnamon bark (*Cinnamomum jerseniacum Hand.-Mazz*) was determined 8 µL/mL for *A. flavus* (62), MIC of encapsulated rosemary 20 µL/mL for *Aspergillus* spp. and 50 µL/mL for *Penicillium* spp. (63) and for thyme (*Thymus vulgaris* L.) 0.7 µL/mL for al tested fungal species including *A. flavus* (64). Incubation times for these dilution assays ranged from seven to nine days and the incubation temperature from 25 to 30 °C. Results of Marei et al. (65) suggest that thymol exerts more antifungal activity on *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium digitatum* compared to other phenolic compounds such as (S)-limonene and 1,8-cineole. The MIC of thymol ranged from 20.14 to 23.80 mg/L for *A. niger* and *P. digitatum*. For limonene, the MIC ranged from 26.83 to 38.08 mg/L and for 1,8-cineole from 36.40 to 51.61 mg/L. *Fusarium oxysporum* showed higher resistance against all three tested compounds.

2.4. Mode of application of EOs in bakery products

Often, the importance of choosing the most suitable *in vitro* assay for antifungal screening of EOs is underestimated. In most cases *in vitro* diffusion assays are performed to determine the minimal inhibitory concentration. However, these data are mainly of interest when the EOs are intended to be used as volatiles in the food packaging atmosphere. For essential oil spray applications, data of both the *in vitro* diffusion and dilution tests can be used. With sprays, both the volatile behaviour as well as the effect of contact is important. In case, EOs will be used in a food matrix, such as in bread dough, an *in vitro* dilution screening assay is important to enable the prediction of the antifungal activity of EOs when dispersed in a medium, ideally with adjusted pH, a_w, composition of the media and incubation temperature similar to the intended end product (42). Dispersal of the EOs in a liquid or solid medium will decrease the antifungal effect compared to diffusion assays by reducing the release of antifungal active volatile components.

2.4.1. Application of EOs as volatiles in the headspace of packaged bakery products

Volatiles of EOs of bay, cinnamon leaf, clove, lemon grass and thyme totally inhibited all fungal species tested in a study of Guynot et al. (52) (e.g. *Eurotium amstelodami, Aspergilus flavus, A. niger* and *Penicillium corylophilum*) in wheat flour based agar irrespective of the a_w level. The activity of the EO volatiles was far more limited in sponge cake, leading to the hypothesis that the activity of the EO volatiles was substrate dependent and that the activity interferes with food matrix components. The main component present in bay, cinnamon leaf and clove was eugenol and in lemon grass and thyme respectively geraniol (50.5%) and thymol (53.9%) (52). Nielsen et al. (51) reported that high levels of CO_2 in active food packaging (cf. modified atmosphere packaging) did not completely retard the growth of spoilage fungi. Therefore, the use of volatile EOs was investigated as an alternative. EOs from mustard, cinnamon, garlic, clove, oregano and vanilla were evaluated in this study. Mustard EO showed the strongest antifungal activity. However, cinnamon, garlic and clove also proved to have useful antifungal activities. EOs from oregano and vanilla had respectively a weak or no effect on bread. The effect of mustard EO volatiles on the sensory properties was determined to be much greater on wheat bread than on rye bread, meaning that an off-flavour is more notable on wheat bread. The activity of mustard EO can be assigned to allyl isothiocyanate (AITC). Saladino et al. (66) investigated that only AITC active packaging was able to reduce growth of *P. parasiticus* on sliced loaf bread and not isothiocyanates of benzyl and phenyl. The active concentration of AITC was 5 µL/L.

2.4.2. Application of essential oil sprays

Essential oil of *Citrus sinensis* peel (orange) is known to have antimicrobial activity. In particular, spraying of Malta citrus peel EO on bread slices proved to be a promising preservation technique. Although citrus peel EO was able to reduce microbial growth albeit not significantly proven, it affected the sensory characteristics of the bread, including crust character, colour of crumb and crust, taste, texture and aroma (67). The antimicrobial efficacy of EO vapour and its molecules is higher compared to when the EO or components are dispersed in a food system. This is mainly due to the greater affinity of EO volatiles towards the hydrophobic cell membrane when not dispersed in a solution or food matrix (68). It is worthwhile noting that the use of essential oil volatiles or sprays is documented frequently in the fruit and plant industry for the extension of the post-harvest shelf-life (69,70). For further investigation on the antifungal efficacy of EO sprays, reports on the agar diffusion assays can be used as pre-screening assays.

2.4.3. Direct use of EOs in bread

To date, very few articles have reported the direct use of EO in dough systems. This is assumed to result from the fact that potential antifungal activity of EOs is partly lost during the heat treatment of baking and therefore under evaluated in literature. Teodoro et al. (63) investigated the antifungal efficacy of microencapsulated rosemary EO in fresh dough.

Rosemary EO particles were made by spray-drying using modified starch and maltodextrin as coating materials. The hypothesis is that many of the antimicrobial active components present in EOs can undergo oxidization and volatilization during storage and baking, which can reduce the stability and efficacy of the EOs. Another hypothesis raised is the fact that when encapsulated, the release of active constituents would be more gradual resulting in a better shelf-life extension and protection over the intended shelf-life period. The efficacy of the microencapsulated rosemary EO was proven by the fact that a longer mould free shelf-life was obtained when the microencapsulated rosemary EO was used. The results also illustrated that *Aspergillus* species were slightly more sensitive to the action of rosemary EO. The use of edible films containing nanoemulsions of clove bud (*Syzygium aromaticum*) and oregano (*Origanum vulgare*) EOs on sliced bread was investigated by Otoni et al. (71). Results showed reduced yeast and mould counts during 15 days of storage. The most suitable pre-screening techniques for the use of EOs as ingredients are the dilution assays, including the agar/broth dilution assays and the Poisoned food assays.

2.4.4. Hurdle technology

Next to the aforementioned modes of application of EOs, a fourth category must be considered. This includes the use of EOs in combination with other preservation strategies (cf. *hurdle technology*). These combinations can possibly identify novel strategies for achieving good antifungal activity in bakery products. The impact of other antifungal *hurdles* such as pH, water activity, temperature, matrix composition and packaging in combination with EOs (42,72,73) and respective potential synergistic mechanisms must be given further attention. Moreover, increasing the number of preservation *hurdles* together with the use of EOs can reduce potential negative organoleptic or physico-chemical characteristics induced by these EOs as the final concentration can be lowered. However, there are not sufficient reports in literature on the use of EOs in combination with other *hurdles* specifically for the bakery industry and therefore this subject must be considered as important for further analysis.

2.5. Legislation and safety of EOs

In the USA, several EOs and their components have a GRAS status (Generally Recognized As Safe) regardless of its intended use as flavouring or preservative substance. However, although most EOs are considered safe, these substances are only safe when used in commonly used amounts. Moreover, ingestion of these natural components is not always recommended regardless of its GRAS status. Every use of EOs and its components must be done with caution. In Europe, the GRAS status does not apply and the legislation for EOs depends on their intended use, like in this case as food additive for their antifungal activity and not primary as food-flavouring substance. The food additive legislation is based on a restrictive positive list and therefore the use of EOs as food additive in bakery products is not legally allowed. However, this is often bypassed in the food industry by declaring its primary use as food-flavouring substance. In fact, European Regulation (EU) No. 1334/2008 defines EOs as flavouring preparations and the active components are called flavouring substances. In case the latter are derived naturally they can be stated as natural-flavouring substances. Not all flavourings need evaluation and approval by the European Commission which means the positive list is not exclusive. The positive list includes only those flavourings for which evaluation and approval were necessary. Use of other flavourings has to meet the requirements of Regulation 1334/2008.

2.6. Future prospects and challenges

Different EOs have strong antimicrobial and antioxidant potential. Therefore, their use may play a significant role in overcoming storage losses and in enhancing food shelf-life. This review has shown that there are two groups of methods most currently used for the determination of antifungal activity of EOs (diffusion and dilution assays). Both have their advantages and must be taken into consideration when one wishes to proceed to a real food application. Due to small variations in the assays reported in literature (e.g. incubation time, temperature, interpretation of results, reporting, etc.), it is difficult to compare findings from different authors. The most frequently applied methods are the diffusion assays whereby the activity in the vapour phase is investigated. This is mainly due to the fact that the antimicrobial efficacy of EO vapour and its volatile components is higher when not dissolved in a hydrophilic medium (68). Therefore, *in vivo* trials are mostly performed with the use of volatiles in active packaging or with EO sprays (51,67,72). However, when application of EO in bread dough or other bakery products is intended for, a dilution screening assay would be more suitable.

Despite the benefits of the use of EOs in the food industry, it still has some challenges to face. First, the main challenge for the use of EOs in food is that the single components present in EOs are often not strong enough to inhibit microbial growth. EOs or its constituents can cause negative organoleptic effects when added at the levels required to achieve preservation (37). Second, the antimicrobial efficacy of an essential oil not only depends on the active compounds present in the EO but also on the application method (74) and the matrix in which they are

applied (75). Thyme, clove and cinnamon leaf were most effective on a rye bread-based agar medium as they contain larger phenolic structures. Mustard and lemon grass on the other hand, were most effective when they were applied as volatiles in the headspace of packaged rye bread. Third, when applied on the surface via spraying, it has to be taken into account that many EOs' active components are unstable and can undergo oxidation or volatilization. Teodoro et al. (63) reported the importance of microencapsulation as a solution for their instability. Fourth, the impact of EOs on dough structure when added as a food additive during bread production is still questionable as some are able to break disulfide bridges which are very crucial for the formation of dough structure. The effects of EOs or their components on dough properties need further exploration before they can be used in dough systems (76). A number of research articles concerning the *in vitro* antifungal activity of EOs and its components have been published. However, due to differences in the antifungal screening techniques and reporting of the data, it is very difficult to compare publications. Studies in real bread systems are less frequently published compared to plant research and are a true necessity in the field of essential oil application in foods in the context of *clean label* technology.

3. Conclusion

In this review, we have provided an overview of the use of EOs and their main antifungal components for food preservation, both in *in vitro* systems as in bread systems, with attention to the methods used to determine the antifungal activity. Due to the fact that EOs contain volatile components with potentially strong antifungal activity the choice of antifungal *in vitro* screening assay is of importance and must be food-application oriented. Moreover, *in vivo* trials in different food matrices are crucial to clearly eliminate effects of matrix interference and off-flavours and to see the true impact of EOs and its components on the production of bakery products. In order to overcome these problems, different preservation strategies (*hurdle technology*) can be combined to reduce the active concentrations of the volatile components and to stimulate potential synergistic preservation mechanisms.

Disclosure statement

No potential conflict of interest was reported by the authors.

Notes on contributors

Els Debonne is a PhD student and teaching assistant at the Department of Food Technology, Safety and Health at Ghent University. In 2014, she obtained the degree of MSc in Bioscience Engineering: Food Science and Nutrition. After that she started her PhD in 2014 on the topic of exploring natural preservation strategies for bread products. Her research interest lay in the domain of food preservation and shelf-life, antifungal research and food technology.

Filip Van Bockstaele is a post-doctoral assistant at Ghent University and has obtained a PhD in 2011 in applied Biological Sciences. As an assistant he participates in education, research and services within the research Department of Food Technology, Safety and Health at Ghent University. The courses under his supervision are related to engineering technology, unit operations in the food industry, food technology and rheology and texture analysis. His research and services are linked to the Laboratory of Cereal Technology focusing on quality of cereal products.

Simbarashe Samapundo is an experienced food microbiologist. He obtained a PhD in Applied Biological Sciences in 2006 at Ghent University, MSc in Food Safety Management in 2012 at University of Central Lancashire and a M.B.A. in 2013 at University of Wales. Currently he is working at Ghent University as Post-doctoral scientist and lecturer on the microbiological aspects of post-harvesting handling, processing and preservation.

Mia Eeckhout is a professor in food and feed technology at Ghent University. She has a PhD in Agricultural sciences and is head of the unit Cereal and animal feed technology of the department of Food Technology, Safety and Health at the Faculty of Bioscience Engineering at Ghent University. In 2001, she coordinated a project (as result of the dioxin crisis) on Traceability in the feed production chain, financed by the federal government in cooperation with BEMEFA (Belgian Feed manufacturers) and the Dutch government. Since 2004 she was occupied with the coordination of self-checking guides for Belgian Industrial Milling – Trade in cereals and agri-products – Bread and pastry production.

Frank Devlieghere is a professor in food microbiology and food preservation at Ghent University. He has a PhD in applied biological sciences on the topic predictive modelling of the microbial quality of modified atmosphere packaged cooked meat products. He is the chairman of the center of excellence Food2Know and the co-founder of Pack4Food. He is the director of the Laboratory of Food Microbiology and Food Preservation of Ghent University. He is also a consultant for several food companies and their suppliers helping them in product and process innovations. His key areas are food packaging, microbial spoilage and safety, new food preservation methods and predictive modelling.

ORCID

Els Debonne http://orcid.org/0000-0002-3883-1323
Filip Van Bockstaele http://orcid.org/0000-0001-6878-249X
Simbarashe Samapundo http://orcid.org/0000-0001-6543-5206
Mia Eeckhout http://orcid.org/0000-0002-8537-5084
Frank Devlieghere http://orcid.org/0000-0003-3754-1225

References

1. S. Dengate and A. Ruben, *Controlled trial of cumulative behavioural effects of a common bread preservative*. Journal of Paediatrics and Child Health, **38**, 373–376 (2002).

2. D.V. Parke and D.F.V. Lewis, *Safety aspects of food preservatives*. Food Additives and Contaminants, **9**(5), 561–577 (1992).
3. K.N. Priftis, D.B. Panagiotakos, G. Antonogeorgos, M. Papadopoulos, M. Charisi, E. Lagona and M.B. Anthracopoulos, Factors associated with asthma symptoms in schoolchildren from Greece: the physical activity, nutrition and allergies in children examined in Athens (PANACEA) study. Journal of Asthma, **44**(7), 521–527 (2007).
4. J. Ring, K. Brockow and H. Behrendt, *Adverse reactions to foods*. Journal of Chromatography B: Biomedical Sciences and Applications, **756**(1–2), 3–10 (2001).
5. S.-M. Shim, S.H. Seo, Y. Lee, G.I. Moon, M.S. Kim and J.H. park, *consumers' knowledge and safety perceptions of food additives: evaluation on the effectiveness of transmitting information on preservatives*. Food Control, **22**(7), 1054–1060 (2011).
6. S. Samapundo, F. Devlieghere, A. Vroman and M. Eeckhout, *Antifungal properties of fermentates and their potential to replace sorbate and propionate in pound cake*. International Journal of Food Microbiology, **237**, 157–163 (2016).
7. D.P. Puhr and B.L. D'Appolonia, *Effect of baking absorption on bread yield, crumb moisture, and crumb water activity*. Cereal Chemistry, **69**(5), 582–586 (1992).
8. J.P. Smith, D.P. Daifas, W. El-Khoury, J. Koukoutsis and A. El-Khoury, Shelf life and safety concerns of bakery products – a review. Critical Reviews in Food Science and Nutrition, **44**, 19–55 (2004).
9. P. Saranraj and M. Geetha, *Microbial spoilage of bakery products and its control by preservatives*. International Journal of Pharmaceutical & biological archives, **3**, 38–48 (2011).
10. K.I. Suhr and P.V. Nielsen, *Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activities and pH values*. International Journal of Food Microbiology, **95**(1), 67–78 (2004).
11. J.D. Legan, *Mould spoilage of bread: the problem and some solutions*. International Biodeterioration & Biodegradation, **32**, 33–53 (1993).
12. F. Devlieghere, J. Debevere, L. Jacksens, M. Uyttendaele and A. Vermeulen, Levensmiddelenmicrobiologie en – conservering, p. 260. Kleine Pathoekeweg 3, 8000. die Keure, Brugge (2011).
13. V. Nikolajeva, D. Klava and D. Kunkulberga, Evaluation of spoilage yeasts causing chalk mould defects on rye bread. In 73rd Scientific Conference of the University of Latvia, Environmental and Experimental Biology, Latvia (2015). p. 50.
14. O. Filtenborg, J.C. Frisvad and U. Thrane, *Moulds in food spoilage*. International Journal of Food Microbiology, **33**, 85–102 (1996).
15. K.I. Suhr and P.V. Nielsen, Inhibition of fungal growth on wheat and rye bread by modified atmosphere packaging and active packaging using volatile mustard essential oil. Journal of Food Science, **70**(1), M37–M44 (2005).
16. N. Deschuyffeleer, K. Audenaert, S. Samapundo, S. Ameye, M. Eeckhout and F. Devlieghere, *Identification and characterization of yeasts causing chalk mould defects on par-baked bread*. Food Microbiology, **28**(5), 1019–1027 (2011).
17. N. Degirmencioglu, D. Göcmen, A.N. Inkaya, E. Aydin, M. Guldas and S. Gonenc, *Influence of modified atmosphere packaging and potassium sorbate on microbiological characteristics of sliced bread*. Journal of Food Science and Technology, **48**(2), 236–241 (2011).
18. S. Muizniece-Brasava, L. Dukalska, I. Murniece, I. Dabina-Bicka, E. Kozlinskis, S. Sarvi, R. Santars and A. Silvjane, *Active packaging influence on shelf life extension of sliced wheat bread*. International Scholarly and scientifc research and innovation, **6**(7), 939–945 (2012).
19. F. Devlieghere, A. Vermeulen and J. Debevere, *Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables*. Food Microbiology, **21**(6), 703–714 (2004).
20. P.K. Dutta, S. Tripathi, G.K. Mehrotra and J. Dutta, *Perspectives for chitosan based antimicrobial films in food applications*. Food Chemistry, **114**(4), 1173–1182 (2009).
21. M.E. Bárcenas and C.M. Rosell, *Effect of frozen storage time on the bread crumb and aging of par-baked bread*. Food Chemistry, **95**, 438–445 (2006).
22. A. Corsetti and L. Settanni, *Lactobacilli in sourdough fermentation*. Food Research International, **40**(5), 539–558 (2007).
23. P. Lavermicocca, F. Valerio and A. Visconti, Antifungal activity of phenyllactic acid against molds isolated from bakery products. Applied and Environmental Microbiology, **69**(1), 634–640 (2003).
24. K. Ström, J. Sjögren, A. Broberg and J. Schnürer, *Lactobacillus plantarum MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo (L-Phe-trans-4-OH-L-Pro) and 3-phenyllactic acid*. Applied and Environmental Microbiology, **68**(9), 4322–4327 (2002).
25. B.A. Black, E. Zannini, J.M. Curtis and M.G. Gänzle, *Antifungal hydroxy fatty acids produced during sourdough fermentation: microbial and enzymatic pathways, and antifungal activity in bread*. Applied and Environmental Microbiology, **79**, 1866–1873 (2013).
26. J. Schnürer and J. Magnusson, *Antifungal lactic acid bacteria as biopreservatives*. Trends in Food Science & Technology, **16**(1–3), 70–78 (2005).
27. T.H. Suomalainen and A.M. Mäyrä-Makinen, *Propionic acid bacteria as protective cultures in fermented milks and breads*. Le Lait, **79**, 165–174 (1999).
28. N. Gardner, C.P. Champagne and P. Gélinas, *Effect of yeast extracts containing propionic acid on bread dough fermentation and bread properties*. Journal of Food Science, **67**(5), 1855–1858 (2001).
29. E.K. Mo and C.K. Sung, *Production of white pan bread leavened by Pichia anomala SKM-T*. Food Science and Biotechnology, **23**(2), 431–437 (2014).
30. V. Passoth, E. Fredlund, U.Ä. Druvefors and J. Schnürer, *Biotechnology, physiology and genetics of the yeast Pichia anomala*. FEMS Yeast Research, **6**(1), 3–13 (2006).
31. G.W. Gould, *Industry perspectives on the use of natural antimicrobials and inhibitors for food applications*. Journal of Food Protection, 59, 82–86 (1996).
32. A.F.S. Sanchez Maldonado, A. Schieber and M.G. Gänzle, *Plant defence mechanisms and enzymatic transformation products and their potential applications in food preservation: advantages and limitations*. Trends in Food Science & Technology, **46**(1), 49–59 (2015).

33. C.E.C.C. Ejike, M. Gong and C.C. Udenigwe, *Phytoalexins from the Poaceae: biosynthesis, function and prospects in food preservation*. Food Research International, **52**(1), 167–177 (2013).
34. R.J. Grayer and T. Kokubun, *Plant-fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants*. Phytochemistry, **56**, 253–263 (2001).
35. F. Bakkali, S. Averbeck, D. Averbeck and M. Idaomar, Biological effects of essential oils – a review. Food and Chemical Toxicology, **46**(2), 446–475 (2008).
36. S. Burt, *Essential oils: their antibacterial properties and potential applications in foods – a review*. International Journal of Food Microbiology, **94**(3), 223–253 (2004).
37. M. Hyldgaard, T. Myind and R.L. Meyer, *Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components*. Frontiers in Microbiology, **3**(12), 1–24 (2012).
38. Y.X. Seow, C.R. Yeo, H.L. Chung and H.G. Yuk, *Plant Essential Oils as Active Antimicrobial Agents*. Critical Reviews in Food Science and Nutrition, **54**(5), 625–644 (2014).
39. E. Pinto, L. Vale-Silva, C. Cavaleiro and L. Salgueiro, *Antigunal activity of the clove essential oil from Syzyium aromaticum on Candida, Aspergillus and dermatophyte species*. Journal of Medicinal Microbiology, **58**, 1454–1462 (2009).
40. P. Domadia, S. Swarup, A. Bhunia, J. Sivaraman and D. Dasgupta, *Inhibition of bacterial cell division protein FtsZ by cinnamaldehyde*. Biochemical Pharmacology, **74**, 831–840 (2007).
41. Y. Taguchi, Y. Hasumi, S. Abe and Y. Nishiyama, *The effect of cinnamaldehyde on the growth and the morphology of Candida albicans*. Medical Molecular Morphology, **46**, 8–13 (2013).
42. E. Debonne, F. Van Bockstaele, I. De Leyn, F. Devlieghere and M. Eeckhout, *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 (2018).
43. P. Singh, R. Shukla, B. Prakash, A. Kumar, S. Singh, P.K. Mishra and N.K. Dubey, *Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of Citrus maxima Burm. and Citrus sinensis (L.) Osbeck essential oils and their cyclic monoterpene, dl-limonene*. Food and Chemical Toxicology, **48**(6), 1734–1740 (2010).
44. G. Singh, S. Maurya and C.A. Catalan, *A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents*. Food and Chemical Toxicology, **45**(9), 1650–1661 (2007).
45. A. Kedia, B. Prakash, P.K. Mishra and N.K. Dubey, *Antifungal and antiaflatoxigenic properties of Cuminum cyminum (L.) seed essential oil and its efficacy as a preservative in stored commodities*. International Journal of Food Microbiology, **168–169**, 1–7 (2014).
46. G.R. Vilela, G.S. de Almeida, M.A.B.R. D'Arce, M.H.D. Moraes, J.O. Brito, M.F.D.G. da Silva, S.C. Silva, S.M. de Stefano Piedade, M.A. Calori-Domingues and E.M. da Gloria, *Activity of essential oil and its major compound, 1,8-cineole, from Eucalyptus globulus Labill., against the storage fungi Aspergillus flavus Link and Aspergillus parasiticus Speare*. Journal of Stored Products Research, **45**(2), 108–111 (2009).
47. F. Reyes-Jurado, A. Franco-Vega, N. Ramírez-Corona, E. Palou and A. López-Malo, *Essential oils: antimicrobial activities, extraction methods, and their modeling*. Food Engineering Reviews, **7**(3), 275–297 (2015).
48. G. Singh, O.P. Singh, G.P. Rao and S.R. Sharma, *The vapour action of essential oils and monoterpenoids against pathogenic fungi*. Sugar Technology, **4**(1–2), 69–71 (2002).
49. E. Leite de Souza, E.D.O. Lima, K.R.D.L. Freire and C.P.D. Sousa, Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. Brazilian Archives of Biology and Technology, **48**(2), 245–250 (2005).
50. E.S. Carmo and E. de Oliveira Lima and E. Leite de Souza, *The potential of Origanum vulgare L. (Lamiaceae) essential oil in inhibiting the growth of some food-related Aspergillus species*. Brazilian Journal of Microbiology, **39**, 362–367 (2008).
51. P.V. Nielsen and R. Rios, *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 (2000).
52. M.E. Guynot, A.J. Ramos, L. Seto, P. Purroy, V. Sanchis and S. Marin, *Antifungal activity of volatile compounds generated by essential oils against fungi commonly causing deterioration of bakery products*. Journal of Applied Microbiology, **94**, 893–899 (2003).
53. K. Isshiki, K. Tokuoka, R. Mori and S. Chiba, *Preliminary examination of allyl isothiocyanate vapor for food preservation*. Bioscience, Biotechnology and Biochemistry, **56**(9), 1476–1477 (1992).
54. M.A. Passone, N.S. Girardi, C.A. Ferrand and M. Etcheverry, *In vitro evaluation of five essential oils as botanical fungitoxicants for the protection of stored peanuts from Aspergillus flavus and A. parasiticus contamination*. International Biodeterioration & Biodegradation, **70**, 82–88 (2012).
55. Y. Xing, Q. Xu, X. Li, Z. Che and J. Yun, *Antifungal activities of clove oil against Rhizopus nigricans, Aspergillus flavus and Penicillium cintrinum in vitro and in wounded fruit test*. Journal of Food Safety, **32**(1), 84–93 (2012).
56. L. Askarne, I. Talibi, H. Boubaker, E.H. Boudyach, F. Msanda, B. Saadi, M.A. Serghini and A. Ait Ben Aoumar, *In vitro and in vivo antifungal activity of several Moroccan plants against Penicillium italicum, the causal agent of citrus blue mold*. Crop Protection, **40**, 53–58 (2012).
57. B. Prakash, P.K. Mishra, A. Kedia and N.K. Dubey, *Antifungal, antiaflatoxin and antioxidant potential of chemically characterized Boswellia carterii Birdw essential oil and its in vivo practical applicability in preservation of Piper nigrum L. fruits*. LWT - Food Science and Technology, **56**(2), 240–247 (2014).
58. B. Prakash, P. Singh, A. Kedia and N.K. Dubey, *Assessment of some essential oils as food preservatives based on antifungal, antiaflatoxin, antioxidant activities and in vivo efficacy in food system*. Food Research International, **49**(1), 201–208 (2012).
59. M.H.H. Roby, M.A. Sarhan, K.A.H. Selim and K.I. Khalel, *Antioxidant and antimicrobial activities of essential oil and*

extracts of fennel (Foeniculum vulgare L.) and chamomile (Matricaria chamomilla L.). Industrial Crops and Products, **44**, 437–445 (2013).
60. P.K. Mishra, P. Singh, B. Prakash, A. Kedia, N.K. Dubey and C.S. Chanotiya, *Assessing essential oil components as plant-based preservatives against fungi that deteriorate herbal raw materials*. International Biodeterioration & Biodegradation, **80**, 16–21 (2013).
61. M. Viuda-Martos, Y. Ruiz-Navajas, J. Fernández-López and J. Pérez-Álvarez, *Antifungal activity of lemon (Citrus lemon L.), mandarin (Citrus reticulata L.), grapefruit (Citrus paradisi L.) and orange (Citrus sinensis L.) essential oils*. Food Control, **19**(12), 1130–1138 (2008).
62. J. Tian, B. Huang, X. Luo, H. Zeng, X. Ban, J. He and Y. Wang, *The control of Aspergillus flavus with Cinnamomum jensenianum Hand.-Mazz essential oil and its potential use as a food preservative*. Food Chemistry, **130**(3), 520–527 (2012).
63. R.A.R. Teodoro, R.V. de Barros Fernandes, D.A. Botrel, S.V. Borges and A.U. de Souza, *Characterization of microencapsulated rosemary essential oil and its antimicrobial effect on fresh dough*. Food and Bioprocess Technology, **7**(9), 2560–2569 (2014).
64. A. Kumar, R. Shukla, P. Singh, C.S. Prasad and N.K. Dubey, *Assessment of Thymus vulgaris L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities*. Innovative Food Science & Emerging Technologies, **9**(4), 575–580 (2008).
65. G.I.K. Marei, M.A. Abdel Rasoul, and S.A.M. Abdelgaleil, *Comparative antifungal activities and biochemical effects of monoterpenes on plant pathogenic fungi*. Pesticide Biochemistry and Physiology, **103**(1), 56–61 (2012).
66. F. Saladino, J.M. Quiles, F.B. Luciano, J. Mañes, M. Fernández-Franzón and G. Meca, *Shelf life improvement of the loaf bread using allyl, phenyl and benzyl isothiocyanates against Aspergillus parasiticus*. LWT - Food Science and Technology, **78**, 208–214 (2017).
67. S.H. Salim-ur-Rehman and H. Nawaz, Inhibitory effect of citrus peel essential oils on the microbial growth of bread. Pakistan Journal of Nutrition, **6**(6), 558–561 (2007).
68. A.K. Tyagi, A. Malik, D. Gottardi and M.E. Guerzoni, *Essential oil vapour and negative air ions: A novel tool for food preservation*. Trends in Food Science & Technology, **26**, 99–113 (2012).
69. M. Serrano, D. Martinez-Romero, S. Castillo, F. Guillén and D. Valero, *The use of natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage*. Innovative Food Science & Emerging Technologies, **6**(1), 115–123 (2005).
70. N.G. Tzortzakis, *Maintaining postharvest quality of fresh produce with volatile compounds*. Innovative Food Science & Emerging Technologies, **8**(1), 111–116 (2007).
71. C.G. Otoni, S.F. Pontes, E.A. Medeiros and N.D.F. Soares, *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 (2014).
72. M.E. Guynot, V. Sanchis, A.J. Ramos and S. Marin, Mold-free shelf-life extension of bakery products by active packaging. Journal of Food Science, **68**(8), 2547–2552 (2003).
73. N. Matan, H. Rimkeeree, A.J. Mawson, P. Chompreeda, V. Haruthaithanasan and M. Parker, *Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions*. International Journal of Food Microbiology, **107**(2), 180–185 (2006).
74. K.I. Suhr and P.V. Nielsen, *Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi*. Journal of Applied Microbiology, **94**(4), 665–674 (2003).
75. J. Gutierrez, C. Barry-Ryan and P. Bourke, *The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients*. International Journal of Food Microbiology, **124**(1), 91–97 (2008).
76. M.M. Özcan, *Effect of some essential oils on rheological properties of wheat flour dough*. International Journal of Food Science and Nutrition, **60**(2), 176–181 (2009).