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Potential application of plant essential oils for food preservation

Bachelor Thesis

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Sustainable Use of Natural Resources

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Declaration

I declare that the Bachelor Thesis Potential application of plant essential oils for food preservation is my own work and all the sources I cited in it are listed in Bibliography.

Prague, 17.7.2020. **Signature**

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Potenciální aplikace rostlinných éterických olejů pro konzervaci potravin

Shrnutí

Tato bakalářská práce se bude zabývat potenciálním použitím éterických olejů jako konzervačních látek, a to formou průzkumu různých již existujících časopisů a knih, které se zaměřují na výsledky aplikace éterických olejů na různé potraviny. V následujících kapitolách budou nejprve rozebrány antimikrobiální a antioxidační vlastnosti éterických olejů, způsoby jejich působení, chemická struktura sloučenin rostlinného původu a důvody, proč představují potenciální novou alternativu k syntetickým konzervačním látkám.

Kapitoly dále pojednávají o použití éterických olejů v určitých skupinách potravin, v této kompilaci budou mořské plody, maso, zelenina, ovoce a pekárenské výrobky. Oblastí zájmu týkající se těchto potravin je účinnost různých éterických olejů, která je vyvozena z výsledků mnoha provedených výzkumů a experimentů. Protože éterické oleje jsou potenciálními konzervačními alternativami, je důležité prozkoumat jejich toxicitu bezpečnost pro komerční použití, jakož i jejich organoleptický dopad, který by ovlivnil jejich přijatelnost pro spotřebitele. Budou uvedeny a popsány některé výrobky obsahující éterické oleje, které splňují vládní předpisy a jsou používány v reálném životě jako konzervační látky, jakož i to, co budoucnost přináší pro éterické oleje a jejich probíhající výzkum.

Klíčová slova: éterické oleje (vonné silice), konzervace potravin, rostlinné sloučeniny, antimikrobiální, antioxidační

Potential application of plant essential oils for food preservation

Summary

This bachelor thesis will review the potential application of essential oils as food preservatives through exploration of different already existing journals and books that covered and discussed the results of application of essential oils on different food items. In the following chapters, firstly the antimicrobial and antioxidant properties of essential oils will be discussed, their methods of action, chemical structure of those plant-derived compounds, and the reasons behind why they have the potential new alternative to synthetic preservatives.

The chapters discussing the application of essential oils in certain group of food in this compilation will be seafood, meat, vegetables and fruits and bakery products. The areas of interest regarding these food items are in the effectiveness of different essential oils, which are concluded from the results of many research and experiments taken. As essential oils are potential preservative alternatives, it is important to investigate their toxicity and safety for commercial use as well as their organoleptic impact which would affect the consumers' acceptability. Some products containing essential oils which have met the governmental regulations and are in use in real life as preservatives will be listed and described as well as what the future brings for essential oils and their on-going research.

Keywords: essential oils, food preservation, plant-derived compounds, antimicrobial, antioxidant

Contents

1 Introduction

Essential oils have been of great interest throughout history because ever since their discovery they were and remained of many countless uses to people as the extraction of oils from aromatic plants is known for their many benefits in everyday life, especially in temperate climates. Essential oils by definition can be described as secondary metabolites which by nature are complex and volatile compounds mostly known for their distinctive scent. Also once extracted, they are found in a liquid form, usually transparent, soluble in organic solvents and a good lipid solvent with a lower density point than the one found in water (Bakkali et al., 2008).

The application of essential oils is of a large spectrum nowadays too as they have been more researched (Bakkali et al., 2008); their use can be found greatly in the food industry as flavour and fragrance improving substances in many familiar consumer goods but it is surpassing their use only as spices and fragrance enhancers and it is gaining additional popularity in the field of biotechnology as individuals discovered that the essential oils can be used as food preservatives due to their antimicrobial and antioxidant properties. If used correctly, with great knowledge and consideration to specifically targeted issues and problems of interest in consumer good preservation, they can be of great assistance (Ríos, 2016). All this knowledge is taken as an example from their natural activity at the beginning of the food production which starts at an agricultural field, their properties in the plants grown are antiviral, antibacterial and antifungal which helps in fighting pests and diseases (Bakkali et al., 2008).

Essential oils get their antimicrobial properties as complex organic compounds containing active antimicrobial structures coming in as a natural protection for plants, which can be divided into four groups named terpenes, terpenoids, phenylpropenes and others. Often concealed among other structures, their active compounds depend on the way of extraction and seasonality. (Hyldgaard et al., 2012)

2 Objectives of work

The aim of this thesis is to evaluate the existing scientific evidence about the effectiveness of essential oils as preservatives in the food industry, more specifically the application methods, toxicity and safety, organoleptic impact and real life use.

3 Literature Overview

3.1 Essential oils and their antimicrobial activity

The antimicrobial activity in essential oils depends on the type and chemical structure of the oil and the targeted area of interest, whether that would be fungi or bacteria, gram-positive or gram-negative bacteria. Their uses as antimicrobial agents are found even in dental medicine, antiseptics, and food additives for livestock (MANABE et al., 1987; Cox et al., 2001; Thacker, 2013, as cited in Burt, 2004).

Considering that the antimicrobial activity needs to remain as high as possible after extraction from the plants, it is important to use the right techniques of extraction. The recommended technique is extraction by a solvent rather than by distillation as essential oils are volatile substances, also coming with a need of special storage meaning dark and dry, air-tight containers. Essential oils can contain more than 60 active components, but of interest are the ones which are good in antimicrobial activity, whether alone or in synergy with other components, or of economic interest. Moreover, their active extracted components vary depending on the geography, seasonality, part of plant, etc. (Burt, 2004).

Considering tests on microbial activity of essential oils for evaluation of their activity as possible food preservatives, there has been no standardized test found yet as Burt (2004) has concluded from (Davidson et al., 1989). Burt (2004) also references (Hammer et al., 1999) that there is still a need for such a test, but at the moment researchers are using different common tests for antimicrobial activities and adapting them for testing essential oils, an example of that is how they have adapted the NCCLS (National Committee for Clinical Laboratory Standards) methods for antibacterial susceptibility testing The standardized test is hard to obtain and data is hard to evaluate because many factors are affecting the outcomes, such as pH of the media, the volume of inoculum, culture medium, growth phase, temperature, incubation period, size of the inoculum, emulsifiers and the most used ones to compare studies – minimum inhibitory concentration in which minimum essential oil concentration used showed the result in the size of the inoculum and minimum bactericidal concentration in which concentration of the essential oil resulted in 99.9% of inoculum disappearance.

Just some of the simplest tests that (Burt, 2004) found in research by collecting methods used in experiments used to screen antibacterial activity are to start the observation of the antimicrobial activity of essential oils and to exclude certain selections of essential oils or their comparisons are the disk diffusion method where the disk is submerged in the targeted essential oil of interest and placed on a medium made of agar, or agar well test where bacterial isolates are observed, or the method of dilution in agar medium with different volumes of inoculum smeared and observed over time.

The tests for microbial activity in the food industry, in the sense of food preservation, are different than the ones made only for research purposes, which are usually of a smaller scale, because the results of such specific experiments with food items showed that for the majority of food items required large concentrations of essential oils as to achieve the same successful effects as those of a smaller scale, due to one of the reasons being those that food items containing additional nutritional structures which may help in regeneration of bacteria and microbes overcoming the essential oil efforts to kill (Gill et al., 2002 as cited in Burt, 2004). In addition, some other influences are intrinsic and extrinsic properties which food items contain which are relevant to bacterial sensitivity such as the content of proteins, fat or water, pH, other preservatives, antioxidants, temperature, method of packaging, microorganisms present etc. The pH showed to be of great influence, essential oil's antimicrobial activity rises as the pH decreases because the hydrophobicity of essential oils rises to make it easier to target bacteria through rapturing lipids found in the cell membrane and mitochondria if in general there is also a lower content of fat found in a food item, once raptured leakage of ions and organelles occurs which is a matter of great difference in leading to the disappearance of unwanted cells. On the other hand, carbohydrates showed little effect on the effectiveness of the essential oils, but therefore water and salt, or the combination of two, showed that they facilitate the antimicrobial action in a sense that they lead the essential oil active compounds more effectively to the targeted site containing undesirable bacteria in a food item (SHELEF, 1984; Tassou et al., 1995; Skandamis & Nychas, 2000; Tsigarida et al., 2000; Juven et al., 1994; AURELI et al., 1992, as cited in Burt, 2004).

The mechanisms in which essential oils target the bacterial cells may be consequentially a series of different mechanism s, which depends on the number active compounds of the essential oils, which are either found immediately in plants or synthesized once triggered by specific enzymes if needed to fight against pathogens, and their chemical structure and due to that the expression “essential oil versatility” exists (Holley & Patel, 2005; Burt, 2004, as cited in Nazzaro et al., 2013).

Nazzaro et al., (2013) name some of these mechanisms are essential oils properties like hydrophobicity which disrupts the bacterial membrane and increases the permeability which leads to cell organelles spilling out from the cell wall degradation as in gram-negative bacteria, damage on the cytoplasmic membrane and its proteins, coagulation of the cytoplasm, lower movement of protons and lower ATP pool (Trumpower & Gennis, 1994; Lambert et al., 2001; Helander et al., 1998; Ultee & Smid, 2001; Burt, 2004).

Considering the type of bacteria, gram-negative bacteria are less susceptible to the antimicrobial activity of essential oils due to the possession of an outer membrane surrounding the outer wall which gives them extra protection against hydrophobic structures like essential oils, the outer membrane contains proteins and lipopolysaccharides whose O–chains do not allow essential oils to penetrate the membrane. Although, some studies have found that this is not necessarily true due to the fact that seasonality and amount of essential oils can be of the same effect on both gram-positive and gram-negative bacteria if those determinants improve the effectiveness of the oil extracted and some of the active compounds can penetrate through pores in gram-negative bacteria (Vaara, 1992; Tiwari et al., 2009, as cited in Nazzaro et al., 2013).

When talking about antagonism and synergism, it has been proven that a mix of essential oils does not work better if just their major active components are mixed as a final antimicrobial solution, rather essential oils work better if left with their other minor compounds meaning they are mostly synergetic and work the best when their different active compounds are combined (Gill et al., 2002 as cited by Burt, 2004). Moreover, alongside essential oils, there are other potential synergists which work well once combined like low water activity, low pH and low oxygen tension, higher pressure, and medium heat. A common additive in food preservation is sodium chloride, once considering the use of essential oils in food preservation it is good to look at the effects of other additives in combination with both of their active effects on preservation. The example of sodium chloride as a synergist is when certain active compounds of essential oils have higher permeability to enter the bacterial cells once sodium chloride actively assures higher permeability of the membrane (Lucera et al., 2012; Sánchez-González et al., 2011). There are some examples also of sodium chloride being an antagonist in combination with some essential oils where it inhibited their effective antimicrobial activity (Bassolé & Juliani, 2012).

3.2 Application of essential oils in seafood preservation

Seafood products are a big part of a human diet, especially in Asian, Mediterranean, and many other cultures. Its nutritional value is getting increasingly recognized and the consumption of seafood has risen over the past few decades alongside their production, FAO reported an increase of 11.1 kg on average since the 1960s (FAO). Their nutritional value is based on essential fatty acids which in seafood are recognized as polysaturated fatty acids like omega 6 and omega 3.

They rot fast causing bad smell and flavour, changes in texture, and changes in colour which are caused by oxidative degradation like photo-, thermal- or enzymatic oxidation of lipids and auto-oxidation; this latter, defined as the spontaneous reaction of atmospheric oxygen with lipids, is the most common process causing oxidative deterioration (Shahidi & Zhong, 2005). This is why their preservation is of great concern in a sense of extending their shelf life, and it has always been going back in history with salting, smoking, marinating, drying, etc. They begin their microbial spoilage process right after being caught because they contain a lot of amino acids and water content, high after life, pH, and trimethylamine oxide which is suitable for growth of bacteria such as *Preudomonas* and *Shewanella* (Chaillou et al., 2015; Giuffrida et al., 2013; Gram & Dalgaard, 2002; Gram & Huss, 2000).

As Hassoun & Emir Çoban (2017) discussed, due to essential oils, effective antimicrobial and antioxidant activities they could be useful in this area of seafood preservation as consumers are more intrigued and willing to replace synthetic preservation additives which control water and enzymatic activity. Preservation in seafood in general is based on several techniques which are based on several parameters like temperature control, oxygen availability, microbial loads, etc.

Application methods with the goal of preserving seafood are by direct treatment, meaning the essential oils are added to products during manufacturing and processing, in incorporation with packaging like edible films and coating or introducing them as feed and based on research from many different authors (Hassoun & Çoban, 2017) conclude that direct application of essential oils on seafood proved to be less beneficial than *in vitro* because higher concentration is needed to achieve the same results, also even small concentration of directly applied essential oils onto the seafood results in negative sensory parameters. Better application methods were suggested like edible coating films saturated with essential oils or sedating with essential oils right after harvest. Some studies showed that it is possible to incorporate essential oils into fish feed which goes directly into their flesh, making them inhibit the growth of microorganisms and lipid oxidation (Alvarez et al., 2012). Such experiment was performed on gilthead seabream, which was fed with high doses of thymol groups and resulted in lower counts of microorganisms such as *Enterobacteriaceae* and coliforms (Hernandez et al., 2015).

Another such example which was researched is preservation with essential oil extracted from oregano leaves (*Origanum vulgare*) which is an essential part of Mediterranean diet easily found along the coast, which could bring an effective and easily accessible food preservative on already freshly caught fish while cutting on the storage and transport expenses. Carvacrol and thymol as active found in oregano leaves showed to be effective antimicrobial compounds, the same principle of use and experimentation showed the practicality of thyme (Thymus vulgaris), basil (*Ocimum basilicum*), clove (*Eugenia caryophyllata*), sage (*salvia officinalis L*.), *Zataria multiflora Boiss*, turmeric (*Curcuma longa*) and lemongrass (*Cymbopogon citratus*) and lemon (*Citrus limon*) and their essential oils on preservation of seafood. (Rodriguez-Garcia et al., 2016; Hyldgaard et al., 2012; Kostaki et al. 2009; Suppakul et al., 2003; Emir Çoban & Patir, 2013; Çoban et al., 2015; *Emir Çoban & Tuna Kelestemur, 2016;* Masniyom et al., 2012; Alfonzo et al., 2017; Goulas & Kontominas, 2007; Vatavali et al., 2013 as cited in Hassoun and Çoban, 2017)

Table 1 Summary of examples found in studies researching antimicrobial activity of essential oils in seafood products

Product	EO /components	Target of interest	Application method /incubation /storage time /temperature	Evaluation /inhibition /sensory analysis /toxicity	Reference
White Hard Clam (Meretrix lyrata)	Turmeric (Curcuma longa), Galanga (Alpinia galanga) Powder and Lemongrass (Cymbopogon citratus)	Shelf-life, firmness (N), total plate count (cfu/g), sensory score	White hard clam meat was then treated with turmeric, galanga powder and lemongrass esssential oil in different concentration to demonstrate their effects on physico-chemical, microbial and sensory characteristics during chilled storage. White hard clam meat was dipped in treatment solution for 15 minutes at 28°C. Then it was dripped in 5 minutes, packed in polyethylene bags, stored in cool store at 4°C for 12 days. turmeric: galanga powder:	turmeric: galanga powder: lemongrass essential oil (0.5%:0.5%:0.75%) could effectively maintain physico-chemical, microbial and sensory characteristics of white hard clam muscle	Phuoc Nguyen, 2020

Product	EO /components	Target of interest	Application method /incubation /storage time /temperature	Evaluation /inhibition /sensory analysis /toxicity	Reference
			lemongrass essential oil (0.25%:0.75%:0.5%; 0.5%:0.5%:0.5%; 0.75%:0.25%:0.5%; 0.5%:0.5%, 0.75%; 0.5%:0.5%:1.0%)		
Rainbow Trout (Oncorhynchus mykiss) Fillets	Laurel Essential Oil (1,8-cineole (62.36%), a-terpinyl acetate (10.54%) and sabinene (8.44%) and Vacuum Packing	total viable counts, total psychrotrophic bacteria, Pseudomonas, lactic acid bacteria, Enterobacteriaceae, coliforms), chemical (TVB-N, pH) and sensory quality	Application by spraying 1%, 2% laurel treated vacuumed samples	No significant difference in total viable count of control group and 1% laurel oil, 2% affected significantly on the total viable counts and microorganism count and extended shelf life for 4 days/ laurel essential oil creates pleasant smell	AKSOY & SEZER, 2019

Product	EO /components	Target of interest	Application method /incubation /storage time /temperature	Evaluation /inhibition /sensory analysis /toxicity	Reference
Rainbow Trout (Oncorhynchus mykiss) Fillet	Hydro-ethanol extract of *Salvia officinalis* L. (control, 2%, 4% and 6% extracts), polyethylene bags	Shelf life, composition (protein, ash, fat, total, volatile basic nitrogen, thiobarbituric acid, free fatty acids) total bacterial (Psychrophilic bacteria, Enterobacteriaceae, H2S producing bacteria),Pseudomonas, sensory evaluation(color, odor, texture and total acceptability)	The fish fillets were cleaned of blood and viscose layers before being treated and the extract of *Salvia officinalis* L. was prepared by firstly drying the plant, turning it to powder and treating with ethanol and a solvent. Fillets were selected and prepared in 4 treatments (control, 2%, 4% and 6% extracts). Each fillet was immersed in the extract for 10s and then transferred into polyethylene bags. They were monitored for 25 days at a refrigerated temperature (4 ± 1 °C) for microbial, chemical and sensory tests.	The extract of *S. officinalis* L. caused a lowering in the production of TVB-N and FFA, however, the shelf life of fish fillets was generally prolonged due to the extract. The use of hydro-ethanol extract of *S. officinalis* L. decreased the microbial growth (the 6% group showed the lowest microbial growth other groups). Regarding the sensory	Mehdizadeh et al., 2019

Product	EO /components	Target of interest	Application method /incubation /storage time /temperature	Evaluation /inhibition /sensory analysis /toxicity	Reference
				evaluation, decrease in odor was noticed and the control group received a lower rating for general acceptability.	
Hybrid Striped Bass, *Morone saxatilis* x *M. chrysops*	edible zeinbased coatings, nisin and lemongrass essential oil (LG) (8%), polyvinyl chlorine (PVC) vacuum-packaging	Assessment of antibacterial action against *L. monocytogenes* and spoilage organisms in edible zeinbased coatings with absorbed nisin and lemongrass essential oil	Lemon grass essential oil was produced from 20 plant materials and concentrations of 30,000; 60,000; 90,000; and 120,000 IU)/15 mL of coating solution were used on microbiological media which contained the problematic bacteria *L. monocytogenes*. The coating solution was applied on the fish fillets by using a spraying method	Corn zein-based edible coatings are suitable for the reception of nisin and LG. Moreover, samples treated with nisin were the most effective against *L. monocytogenes* in PVC and vacuum-packaged storage	Hager et al., 2018

Product	EO /components	Target of interest	Application method /incubation /storage time /temperature	Evaluation /inhibition /sensory analysis /toxicity	Reference
			and the fillets were then wrapped with PVC (polyvinyl chlorine) or vacuum-package in barrier bags with a 95% vacuum. All treated samples were stored at 4 ± 1°C, PVC for 14 days and vacuum-packaging for 42 days.	(reduction of 3.5log and 3.7log). Samples treated with Lemon grass oil reduced *L. monocytoge-nes* counts by 2.5log in PVC and 1.7log in vacuum-packaged samples.	
Atlantic salmon (Salmo salar)	Coriander(1%), , garlic (1%), rosemary (0.3%), and orange peel (1%) oils	the effects of coriander garlic, rosemary and orange peel oils on the survival of *Salmonella Enteritidis* and *Listeria*	Before being treated with EO salmon samples were tested for the presence of *Salmonella Enteritidis* and *L. monocytogenes*. Coriander (0.125mL), garlic (0.125mL), orange peel (0.125mL), and rosemary oils (0.037mL) were applied to each	*Salmonella Enteritidis* population was reduced to 4.3960.15 log cfu/g in first group (Control), 3.7460.04 log cfu/g in second group (Coriander oil), 3.6560.07 log cfu/g in	Tosun et al., 2017

Product	EO /components	Target of interest	Application method /incubation /storage time /temperature	Evaluation /inhibition /sensory analysis /toxicity	Reference
		monocytogenes on fresh salmon	fish after which the samples were placed in sterile plastic bags and stored at 2 ± 1 °C for 96 hours. The samples were analyzed every 24 hours.	third group (Garlic oil), 3.9160.14 log cfu/g in fourth group (Rosemary oil) and 3.7460.11 log cfu/g in fifth group (Orange oil). In the untreated samples the population of bacteria increased during the storage period.	
Crabsticks (Golden Champ)	Lemongrass EO (0.5%), pure water-based extract (WBE), 30% ethanol, sterile water, vacuum-	Assess the antimicrobial property of lemon grass WBE and LG commercial essential oil at different	The crabsticks were treated with a 500-µl aliquot of dilution of the culture (7 log cfu/ml) to a final population density of approximately 4 log cfu/g. 500 µl of either pure WBE, 0.5%	Lemon grass treatments reduced the numbers of *Listeria monocytogenes* on crabsticks and there was no negative effect	Ramroop & Neetoo, 2018

Product	EO /components	Target of interest	Application method /incubation /storage time /temperature	Evaluation /inhibition /sensory analysis /toxicity	Reference
	package (MULTIVAC)	concentrations against *Listeria innocua* (a surrogate organism for *L. monocytogenes*) *in vitro* on crabsticks, sensory evaluation (color, aroma, texture, general appearance) of treated and untreated crabsticks	essential oil of lemon grass, sterile water (solvent control for WBE) or 30% ethanol (solvent control for EO) were also applied to the surface. Only two crabsticks were treated with *L. innocua*. Finally, the inoculated crabsticks were vacuum-packaged (MULTIVAC) and kept at 4 °C for 15 days of storage. Every 5 days a microbial assessment was completed.	on the sensory attributes. Lemon grass essential oil performed more effectively than the water-based extract.	
Silver Carp (Hypophthalmichthys	Cinnamon, thyme, and rosemary essential oils (EOs), shallot and turmeric	Assess the antimicrobial activity of thyme, cinnamon, and rosemary	Minced fish samples were treated with 1 x 10^4 cfu/g of *L. monocytogenes*. Following, they were then treated with EO at concentrations of 0.4, 0.8, and 1.2	The taste and general acceptability of minced fish treated with thyme EO at 0.8 and 1.2% was	Abdollahzadeh et al., 2014

Product	EO /components	Target of interest	Application method /incubation /storage time /temperature	Evaluation /inhibition /sensory analysis /toxicity	Reference
Molitrix)	Extracts, plastic bags	EOs and the water extracts of shallot and turmeric against L. *monocytogenes*, sensory evaluation	mL of EO/100 g minced fish (% volume/weight) levels, nisin at 500 or 1000 IU/g level. The samples were then packed in plastic bags and kept at refrigerator temperature (4℃ ± 1℃) for 12 days.	unacceptable, and the odor of minced fish treated with EO at 0.4, 0.8, and 1.2% was equal to that of the control sample, or higher. Thyme EO presented the largest antimicrobial activity against *L. monocytogenes* and rosemary EO was the least effective.	

Considering the sensory properties of treated fish, in high concentrations, essential oils can cause allergic reactions, bad aftertaste, strong smell which making it repulsive. Due to that, scientists always try to describe the antimicrobial effectiveness with the lowest amounts (concentrations) needed to inhibit the growth of bacteria and other microorganisms (Burt, 2004; Hyldgaard et al., 2012; Mann & Markham, 1998). One of the ways to reduce bad sensory properties of the seafood is by example using coatings enriched with cinnamon essential oil which proved to be effective in rainbow trout in one of the researches done (Andevari & Rezaei, 2011), not only did it reduce the smell and flavour but it helped inhibit growth of bacteria (Alfonzo et al., 2017).

During manufacturing process, essential oils showed to have synergism with modified atmosphere packaging and vacuum packaging, in studies with Mediterranean and trout fillets and oregano essential oil and common carp using cinnamon that showed to be an effective way of preservation technique as just one of many examples (Atrea et al., 2009; Frangos et al., 2010; Zhang et al., 2017).

In conclusion, the preservation of seafood indicated that the use of essential oil as antimicrobial effective additives showed great variations, which on the other hand can be explained by many factors which change the effectiveness of essential oils, both geography, seasonality etc. Moreover, if not applied *in vitro*, as seafood preservatives they are required in higher concentrations which again lead to often bad organoleptic effects but nevertheless they have a high potential is further studies are taken to substitute synthetic preservatives (Hassoun & Emir Çoban, 2017).

3.3 Application of essential oils in meat preservation

Jayasena & JO (2013) describe that in recent times the consumer demand for heavily processed meat have fallen, instead consumer demand less processed, clean-label, and naturally processed meat and meat products. The goal here is to find and replace synthetic additives which would not cause food borne allergies and contain some carcinogenic and toxic components which were reported over the years with additives more attractive to consumers with more organic and natural ingredients, like essential oils just as one of the natural alternatives because they showed better sensory qualities, better storage stability as well as less storage than other raw materials for preservation like crude spices etc. (Mariutti et al., 2011; Tipsrisukond et al.,1998. Many researchers reported some of the following essential oils as effective in preservation of meat and meat products; basil, marjoram, coriander, basilica, balm, oregano, thyme, clove etc. (Barbosa et al., 2009; Chouliara & Kontominas, 2006; Dzudie et al., 2004; Fratianni et al., 2010; Govaris et al., 2010, Menon & Garg, 2001; Skandamis and Nychas, 2001; Skandamis et al., 2002; Solomakos et al., 2008; Tsigarida et al., 2000 as cited in Jayasena & Jo, 2013).

Meat and meat products are rich in essential nutrients but are prone to spoilage due to some factors like pH and water content which fall into microbiological ranges prone to growth of microorganisms, and bacteria which cause spoilage if factors of hygiene, temperature and meat acidity are not taken care of under certain standardized regulations (Dave & Ghaly, 2011). Some of the common preservation techniques and regulations are treatments like freezing, chilling, drying, heating, ultrasound, ionizing radiation, high pressure, packaging, modified atmosphere, washing, adding sulphites and nitrites and many others (Table 2 from Jayasena & Jo, 2013 but modified from Mor-Mur & Yuste, 2009).

As meat enters the production chain, in each stage of processing, the spoilage levels up, meaning spoilage levels up during preparation, storage, distribution, selling, etc. The main roles in microbial spoilage include lipid oxidation and autolytic enzymatic spoilage which later in the production along with residual toxic additives lead to environmental and economic losses and impacts such as pollution outside the processing plant (Dave & Ghaly, 2011).

Spoilage in meat is caused by many common bacteria, yeasts, and moulds, some which are Pseudomonas, *Brochotrix thermosphacta*, *Acinetobacter*, *Lactobacillus* spp., *Enterobacter*, *Candida*, *Rhizopus*, *Fusarium,* etc. Such microorganisms cause decomposition and degradation of lipids and proteins found in the meat which leads to bas sensory characteristics like bad smell, bad flavour, texture and colour changes, and changes in pH and can cause gastrointestinal illnesses if consumed in great concentrations (Borch et al., 1996; Dave & Ghaly, 2011, Fratianni et al., 2010; Karabagias et al., 2011; Lucera et al., 2012; Mor-Mur and Yuste, 2010; Rydlo et al., 2006 as cited in Jayasena & Jo, 2013).

Table 2 Summary of examples found in studies researching antimicrobial activity of essential oils in meat products

Product	EO /components	Target of interest	Application method /Incubation /storage /temperature	Evaluation /inhibition /Sensory analysis /toxicity	Reference
beef	Pomegranate peel extract and Thymus kotschyanus essential oil.	odour, colour, pH, thiobarbituric value for lipid oxidation levels and *Pseudomonas* spp. total viable counts, lactic acid and *Listeria monocytogenes*	Pomegranate peel extract and *Thymus kotschyanus* essential oil incorporated in chitosan-starch composite films. 21 days at 4 °C.	EO incorporated films showed anti-listeria effects. The positive effects of EO incorporated films increased with concentrations of PPE or and TEO additives. Sensory parametres proved to be acceptable. CH-S-PPE 1%-TEO 2% had the best results.	Mehdizadeh et al., 2020
Minced beef meat	Antibacterial activity of ovendried, freeze-	Mesophilic aerobic microorganisms,	6 days in refrigerated storage (4-8 °C)	Garlic derived as freeze-dried fresh garlic and the spray-dried microencapsulated essential oil at a	Najjaa et al., 2020

Product	EO /components	Target of interest	Application method /Incubation /storage /temperature	Evaluation /inhibition /Sensory analysis /toxicity	Reference
	dried and spray-dried microencapsulated EO of Allium sativum	coagulase-positive staphylococci, Escherichia coli, Salmonella sp., and the sulfite-reducing anaerobes		concentration of 20% proved to be effective in preservation.	
pork tenderloins meat batters	Plasma enhanced-nutmeg essential oil solid liposome treatment on the gelling and storage	effect on the oxidation of proteins and lipid, thermal gelatin and water holding capacity related to storage and gelling	pork tenderloins (100 g) were chopped with 50 mL of 1% NaCl solution and 1% (w:w) NEO-SLP or P-NEO-SLP, gels of the meat batters were prepared by heating the pastes at 75 °C for 20 min after that the gels were cooled	results showed NEO-SLP and P-NEO-SLP addition increased water holding capacity and gel strength integrity which is in direct relation to a good preservation effects	Zhu et al., 2020

Product	EO /components	Target of interest	Application method /Incubation /storage /temperature	Evaluation /inhibition /Sensory analysis /toxicity	Reference
			immediately and stored at 4 °C before using, microbiological count during 4 days storage was evaluated by plate counting method		
Chicken breast fillet	Summer savory (*Satureja hortnesis*) essential oil (1, 2 and 3%), cold plasma, chitosan	Assessment of possible prolongation of shelf life for chicken breast fillets by using chitosan and low density polyethylene bilayer film	Polyethylene bilayer films were cut and cleaned with acetone and then treated for 10s with cold plasma. Then, chisotan powder was dissolved and then applied to film layers. Essential oil of summery savory was applied to the prepared films, in the concentration	The samples protected by film bilayers containing summer savory essential oil had a positive effect on prolonging the shelf-life of chicken breast fillets. Moreover, the research confirmed that cold plasma can be used as a new way of packaging meat. Sensory parameters, depend on the treatment, some are undesirable.	Moradi et al., 2020

Product	EO /components	Target of interest	Application method /Incubation /storage /temperature	Evaluation /inhibition /Sensory analysis /toxicity	Reference
		with method cold plasma incorporated with summer savory essential oil (SEO)	of 1,2 and 3%. Chicken fillets were put in polyethylene dishes and sealed with films. The samples were divided in the following categories: non plasma-treated polyethylene (PE), plasma-treatment chitosan with PE without essential oil, and plasma-treatment chitosan with PE containing summer savory essential oil 3The samples was refrigerated (at 4 ± 1 °C) for 13 days.		

Product	EO /components	Target of interest	Application method /Incubation /storage /temperature	Evaluation /inhibition /Sensory analysis /toxicity	Reference
Sucuk (dry fermented sausages – beef, fat, sugar, salt, spices, nitrite)	chitosan (C), chitosan enriched with thyme (CT) or rosemary (CR) essential oils, potassium sorbate (PS)	Assessment of the effect of chitosan or chitosan infused with rosemary, thyme essential oils on the growth of fungus in fermented sausages.	The beef was minced, seasoned and then put in collagen casing by using a vacuum stuffer. The drying process was as followed: 4 days at 20–22 °C and 85–92% RH under an air flow rate of 0.8–1 m/s, 8 days at 18–20 °C and 75–85% RH under an air flow rate of 0.5 m/s. The sausages were then coated with distilled water, 1% acetic acid, 20% potassium sorbate, 1% chitosan, 1% chitosan–thyme and 1% chitosan–rosemary.	Chitosan and the combination of chitosan with thyme or rosemary EO gives sufficient protection to dried sausages in protection from superficial fungal growth and oxidative quality. PS-, C-, CT- and CR-treated sausages had the odor acceptability decrease during storage, however, CT and CR sausages helped the preservation of the characteristic acidic and spicy odor. The taste quality decreased for all samples in the last month of storage. Not toxic – possible alternative in food storage	Demirok Soncu et al., 2020

Product	EO /components	Target of interest	Application method /Incubation /storage /temperature	Evaluation /inhibition /Sensory analysis /toxicity	Reference
			After being coated in solutions the sausages were dried and then put in polyethylene bags. They were stored at 4 ± 1 °C under 65 ± 2% RH for 3 months.		

Food-borne diseases in meat are caused by spores which need to be killed at much higher temperatures than normal vegetative cells of the following common microorganisms like *Salmonella* spp., *Campylobacter jejuni*, *E. coli*, *Listeria monocytogenes*, *Clostridium* spp. and *Aeromonas hydrophila* (Cousin, 1989).

Essential oils as microbiological active agents in preservation of meat can be also added in synergy with some other preservation technologies like additives controlling temperature and acidity, modified hydrostatic pressure, other natural preservatives like nisin and low dose radiation the same way as application of essential oils in preservation of seafood or other food items which require more advanced packaging (Al-Reza et al., 2010; Skandamis & Nychas, 2001; Devlieghere et al., 2004; Zhou et al., 2010; Chouliara et al. 2005, as cited in Jayasena & Jo, 2013).

Some of the limitations of essential oils used as preservatives in meat production are the possibility of lower effectiveness of their microbiological action if mixed with some of the other meat ingredients not using *in vitro* application also the effectiveness may vary based on the time of harvest, concentration levels, part of the plant used and geography of essential oils and contents of the meat such as carbohydrates, proteins, fat etc. (Burt, 2004; Busattaa et al., 2008). However, the future of essential oils in meat products is bright; there are many oils which proved to be effective with their active compounds with which they degrade and destroy the unwanted bacteria, yeasts, and moulds. Further research is needed and improvements in organoleptic impacts of certain oil but overall a very interesting and effective future alternative method of meat and meat products preservation technique (Jayasena & Jo, 2013).

3.4 Application of essential oils as fruits and vegetables preservatives

Solgi & Ghorbanour (2014) mention that the role of essential oils in horticultural sciences is mostly based on sterilization of explants by an *in vitro* culture, extending the life of flowers in vases and cut flowers which is not much explored yet and lacks in data available and extending the shelf life of vegetables and fruits which is the most important aspect because their postharvest time is short as they are eaten freshly. As the international distribution and technology got more advanced, the refrigeration for the cold distribution appeared but just that is not enough to assure fruit and vegetables stay safe and high in quality, which is a main priority to the consumers.

Postharvest causes fruit worsening in a form of changes in texture and colour, weight loss due to dehydration, lower acidity levels, and microbial spoilage. Some fruits like banana, peach, plum, tomato, avocado, etc. experience increased respiration and ethylene biosynthesis rates while other fruits which are non- climacteric like grapes, peppers, citruses etc. experience do not intake ethylene for ripening. Fungicides are usually used on the postharvest fruits in a way of large volume dip solutions to reduce pathogens but to find a natural antimicrobial agents would be of great value because it would reduce the 'toxic pack house effluent'. In organic farming, for example, essential oils and plant derived extracts are available as pre-harvest fungicides, but postharvest natural fungicides studies are still missing. Not only do synthetic fungicides harm the human health but also the environment by accumulation in the soil and water and overall resistance to pathogens is increasing (Tzortzakis, 2007; Regnier et al., 2008). Essential oils would be a great alternative to synthetic fungicides, researchers believe because they also can control the decay, gas exchange through coating, so moisture remains, and mostly it is nonspecific meaning works on a large spectrum of microorganisms as they do in their original plants to fight fungi and other harmful pathogens (Plooya et al., 2009). To reduce the residuals of fungicides and their often application to the fruit orchards, effective postharvest mycobiocides are considered (Solgi et al., 2010; Regnier et al., 2010). Meaning, horticulturists rather turn to modified atmosphere packaging, so postharvest packaging control, in a way that antimicrobial agents from essential oils would slowly be released from the packaging onto the fruits (Maqbool et al. 2011). Some of such essential oils which proved to be efficient in combination with modified atmospheric packaging are eugenol, tymol, and menthol for prolonging shelf life of fruits and vegetables based on research taken (Serrano et al., 2008). The only worry is the organoleptic impact which needs more research.

Table 3 Summary of examples found in studies researching antimicrobial activity of essential oils in fruits and vegetables

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
Nectarines (*Prunus persica L. Bath*)	*Cinnamon zeylanicum* (CEO) – *cinnamaldehyde* (80.82%), *Zataria multiflora* (ZEO) – *Thymol* (32.68%) and *Carvacrol* (30.57%) for ZEO, and *Satureja khuzestanica*	*Botrytis cinerea* and *Rhizopus stolonifer*	*In vitro* / *in vivo*	75, 150, 300, 600, and 1200 μL/L (v/v) of each Eos. Fungi inoculation – 5mm incubation at 25 ± 2 °C under 12 h light and 12 h dark period.	In *in vitro* ZEO and CEO showed positive results against fungi by mycelia inhibition but none of the EO complete inhibition, *in vivo* the same concentrations showed less antifungal activity.	NA	Further investigation needed.	Tahmasebi et al., 2019

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
	(SEO) - *carvacrol* (38.43%)							
Papaya Maradol (*Carica papaya L*)	chitosan-*Ruta graveolens L*	*Colletotrichum gloesporioides*	*In vitro* / *in situ*	chitosan (CHI) (2%) emulsion added with *Ruta graveolens L.* essential oil (REO) (0.5%, 1.0% and 1.5%) to control *C. gloesporioides* growth in both "*in situ*" and "*in vitro*"	*In vitro* studies showed a decrease on fungal growth (mycelia diameter) with the increase of REO concentration, Studies "*in situ"* on papaya fruit during 12 days at 20 °C, showed a	No effect on the organoleptic characteristics.	NA	Peralta-Ruiz et al., 2020

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
					reduction of the *C. gloesporioides* and by 100% with treatments of CHI-REO 1.0 and 1.5%, in addition emulsion was efficient to reduce fruit microbiota.			
Fresh cut cucumber	saffron petal extract (SPE; 1%–4%) incorporated into Konjac	Extended shelf life, (*Escherichia coli, Shigella sonnei,*	*in vitro* and *in vivo*	Sliced cucumbers coating in a solution of SPE and KGM / samples kept at	KGM films incorporated SPE reduced mesophilic bacteria and	NA	Non toxic – safe edible film.	Hashemi & Jafarpour, 2020

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
	glucomannan (KGM) edible films	*Salmonella Typhi*, *Staphylococcus aureus*, *and Bacillus cereus*)		4°C for 5 days at a relative humidity of 80%–90%	fungi populations, microbial load significantly decreased as the concentrations of SPE increased/ KGM + 4% of SPE is the most effective treatment in decreasing the microbial content.			
fresh pistachio	alginate coating	Total phenolic compounds,	Immersion in coating	Immersion in coating solution,	Edible coating with thyme's	NA	NA	M. Hashemi et al., 2020

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
(*Pistacia vera* L.)	enriched with Shirazi thyme *Zataria multiflora Boiss*) essential oil.	antioxidant capacity, peroxide value, free fatty acid content, aerobic mesophilic bacteria, mold and yeast count.	solution.	dried then immersed in calcium chloride (2% w/v) for 2 min to allow gel formation and coated pistachio was stored at 3 ± 1 °C and 80 ± 5% RH for 39 days.	EO contributed to higher values of phenolic content and antioxidant activity compared to control, reduced mold and yeast growth.			
laboratory medium and tomato juice	Synergism of oregano and thyme thymol essential oils.	*Leuconostoc citreum*	Agar well diffusion assay	25 °C for 48h	Both EOs (0.156 µL/mL in total) decreased the populations of *L. citreum* were	Should be investigat ed.	NA	Lee et al., 2020

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
					significantly lower (5.7–6.5 log CFU/mL) (*P* ≤ 0.05).			
Tomato (*Solanum lycopersicum*)	*Tetraclinis* articulate (α-pinene, β-caryophyllene, caryophyllene oxide), vegetative, flowering and fructification stages	*Botrytis cinerea*	*In vitro/in vivo*	The EOs were emulsified with agar at concentrations (10, 12.5, 18.75, 25, 50, 100 and 200 μg mL−1) and then fungi culture disks of 5 mm diameter were plated on the prepared medium and incubated at	*In vitro* - antifungal activity of *T. articulata* EO varied with the phenological stage, EO extracted at flowering stage showed the best results *In vivo* - T. articulata EO	NA	Non - toxic	Rguez et al., 2020

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
				25 °C for 3 days.	inhibited B. cinerea infection in a tomato fruit at concentration of 100 µg mL−1 (low).			
Jack fruit (*Artocarpus heterophyllus Lam*)	*Vetiveria zizanioides* and *Ocimum basilicum*	*Staphylococcus aureus, Escherichia coli, Bacillus cereus, Enterobacter aerogenes, Klebsiella pneumonia, Pseudomonas aeruginosa*	Broth dilution method/*in vitro*	EOs concentrations (1X MIC, 2X MIC and 4X MIC), the Inoculum mixed with all three concentrations in culture medium, incubated at 37 °C. 4 h in 24h	*V. zizanioides* and *O. basilicum* showed activity against *Staphylococcus aureus, Proteus mirabilis, Streptococcus mutans, Rhizopus*	Suggests enchanci ng biologica l activities at lower concentra tions to avoid bad sensory	NA	Atif et al., 2020

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
		and Streptococcus mutans, Penicillium notatum, Aspergillus niger, Aspergillus flavus and Rhizopus microsporus		intervals for bacteria whereas, 0, 12, 24, 36, 48, 60, 72, 84 and 96 h intervals for fungi culture.	*microsporus* , *S. aureus, P. mirabilis, Enterobacter aerogenes, Penicillium notatum. Ocimum* EO reduced the spreading of *P. notatum* on the surface which causes rotting.	propertie s.		
Shredded carrots	*Origanum majorana* and ascorbic acid	Finding an alternative to chlorine preservative,	Hydrosol treatment - marjoram hydrosol	4 °C for 9 days	Hydrosol application of marjoram increased	Ascorbic acid maintaine d original	Non toxic	Xylia et al., 2019

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
		extend shelf-life	(Hyd) (1:15 v/v), ascorbic acid (AA) (1%) and their respective combinations		respiration after 6 and 9 d of storage at 4 °C and 90% RH. Ascorbic acid increased total phenolic content, antioxidant activity, combination of Ascorbic acid with marjoram EO increased carotenoid amount. Decay incidents	color and aroma, Majroram EO acceptable in aroma but darkens the colour, both ascorbic acid and marjoram prove to preserve		

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
					decreased either alone by marjoram or ascorbic acid or with their action together.	carrots		

3.5 Application of essential oils in bakery products

Bakery products are very susceptible to spoilage and short shelf life, so to investigate the use of essential oils and their microbiological activity is very interesting as they could be safe and edible new alternatives to usually synthetic additives. There are some legal aspects which need to be considered if using essential oils as preservatives in bakery products, but thankfully they are listed on the GRAS (Generally Recognized as Safe) list which is under FDA (Food and Drug Administration) approval and supervision, that they can be used as a part of bakery products in the United States (FDA, 2017). Essential oils can be added either as a part of ingredients list or as a part of packaging materials. Some previous research showed that thymol and carvacrol could be effective in preservation of breads and cakes just to set an example from studies taken (Debonne et al., 2018; Mani Lopez et al., 2018; Nanasombat et al., 2010; Faccin et al., 2015; Goncalves et al., 2017; Lopes et al., 2013; Passarinho et al., 2014 as cited in Gavahian, 2018).

Essential oils as a part of packaging materials for bakery goods, with a goal of extending the shelf life even more than packaging does by itself, showed to be beneficial because added active components found in essential oils could provide better protection. Modified atmosphere, replacing oxygen, which allows faster spoilage due to aerobic conditions which benefits in the growth of microorganisms overall, with a mixture of different gases or active agents is more beneficial (Ghidelli & Perez-Gago, 2017). Otoni et al. (2014) showed that once incorporated inside the packaging essential oils of oregano and clove buds in combination with modified polypropylene packaging showed antifungal properties, the same effect (Balaguer et al., 2013) showed that cinnamaldehyde found in cinnamon essential oil which inhibited the growth of *A. niger* and *P. expansum* but the incorporation in the packaging was in a form of a protective gliadin films.

The problem with using essential oils in packaging is that it often affects the sensory properties in a sense that aroma and smell stay after even after removal from the packaging, which proved to be unacceptable in consumers’ eyes. In research from Passarinho et al. (2014) where oregano oil proved to show antifungal properties, the taste was repulsive and overflowed the antifungal benefits.

Table 4 Summary of examples found in studies researching antimicrobial activity of essential oils in bakery products

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
Bread	citral and eugenol	*Aspergillus niger*	antimicrobial sachet containing citral and eugenol	The packaged bread was stored in incubators at 25 °C for control group and 35 °C for bread with antimicrobial satchet	Eugenol and citral appear to be a potent bacteriostatic combination, their direct mechanism provided damage to the cell membranes of *A. niger*. Eugenol is mainly responsible for the permeability of damaged cell membrane, and	Antimicrobial sachets containing citral and eugenol prolong shelf life of bread without producing unpleasant odour.	Non toxic	Ju et al., 2020

Product	EO /components	Target of interest	Application method	Incubation /storage /temperature	Evaluation /inhibition	Sensory analysis	Toxicity	Reference
					citral causes membrane lipid peroxidation.			
Bread	*Origanum vulgare* Linneus and *Thymus vulgaris*	To extend shelf-life of bread, antioxidant activity, encapsulation efficiency, polydispersion index, and zeta potential.	*in situ* of zein nanocapsules containing EO.	90 days, 4 °C and 20 °C storage	It extended the shelf life by a period of 21 days with no microorganism appearing. Also, nanocapsules have high termal capacity meaning they protect EO during baking.	NA	NA	Gonçalves da Rosa et al., 2020

Table 5 Modified table: "Summary of conducted studies on bakery products preservation by essential oils" by (Gavahian et al., 2018)

Bakery product /culture media	Incorporated essential oil	Investigated microorganisms	Key findings	Reference
wheat flour agar medium	Clove (*Syzygium spp*), Basil (*Ocimum spp*), Neem (*Azadirachta sp.*), Ajwain (*Trachyspermum sp*.), cinnamon	*Penicillium oxalicum*, *Aspergillus flavus*	Clove essential showed to be the only effective preservative.	Mishra et al., 2014
Cake	Clove	Coliforms, molds, yeasts	Clove EO retarded the oxidation of the cake. By increase of EO concentration from 400 to 800 ppm it improved the antimicrobial activity but affected the sensory characteristics of the cake negatively.	Ibrahium et al., 2013
Cake	thyme (*Thymus vulgaris*)	*Candida albicans*, *Enterococcus faecium*,	Both free and encapsulated thyme EO showed antioxidant and antimicrobial activities during	Goncalves et al., 2017

Bakery product /culture media	Incorporated essential oil	Investigated microorganisms	Key findings	Reference
		Enterococcus hirae, Escherichia coli, Salmonella choleraesuis, Staphylococcus aureus , Salmonella typhimurium, Pseudomonas aeruginosa, and Aspergillus niger	one month in the storage. Free essential oil MIC value was higher than of the encapsulated essential oil. The shelf life of the product was prolonged.	
Cake	Chamomile (*Matricaria chamomilla* L.)	yeast and mold	Chamomile EO inhibited the mould growth on the cake at higher concentrations, but not as well as sodium sorbate. Chamomile EO improved the oxidative stability of the cake. Sensory scores were lower as concentration of the EO was higher.	

Bakery product /culture media	Incorporated essential oil	Investigated microorganisms	Key findings	Reference
Bread	Aqueous Yerba Mate (*Ilex paraguariensis*) Extract	Fungi	Bread mixed with Yerba Mate extract improved the shelf life due to the phenolic compounds.	Faccin et al, 2015
Wheat Bread	Aqueous garlic (*Allium sativum*) extract			
bread model system	Mexican oregano (*Lippia berlandieri Schauer*)	*Aspergillus spp*	EO increase in concentration prolonged the shelf-life by inhibition of fungi. Variation in the ration of thymol and carvacrol showed no difference in the fungi growth kinetics.	Avila Sosa Sanchez et al., 2015
Bread	Lemongrass (*Cymbopogon citratus*)	*Penicillium expansum*	750 ppm of lemongrass EO inhibited *P. expansum* for 3 weeks at 20°C and inhibition increased with increased EO concentration. Lemongrass EO did not affect sensory parametres.	Mani López et al., 2018
Par-baked wheat and sourdough	thyme (*Thymus zygis*)	*Aspergillus niger*, *Penicillium paneum*	Thyme did not improve the shelf-life of par-baked bread in this experiment. Thyme essential oil negatively affected sensory parametres in	Debonne et al., 2018

Bakery product /culture media	Incorporated essential oil	Investigated microorganisms	Key findings	Reference
breads			sense of bad smell and flavour.	

As previously mentioned, a great problem is in the leftover smell and aroma, so further scientific investigations needs to be undertaken to find essential oils with both antimicrobial and antifungal properties as well as acceptable levels of smell and aroma. Consumers would also be attracted into buying bakery product with additional natural additives which also may attract them with their health benefits (Gavahian et al., 2018).

3. 6 Toxicity and Safety

Many essential oil major active compounds are used in the food industry in European Union as a part of the food system in which they are mostly registered as food flavouring and additives with a low risk to the public health, added and approved by the European Commission. Following that positive attitude, the United States also followed the policy trend on essential oil use as the United States food and Drug Administration approved many of the same active essential oil compounds. As studies are getting more detailed and are more targeted towards research in the field of toxicity of certain essential oil compounds, many of them have been removed or added along the years. The process of an essential oil active compound being added as a new food preservative while being considered as a new additive, there is a great amount of administration and safety guidelines to be taken beforehand, meaning metabolic and toxicological studies (Burt, 2004). As previously mentioned, essential oils are still seeking for a standardized test, especially as food additives as they are not considered a food or an typical additive, there also needs to be taken in consideration their natural origin and use throughout history meaning they could fall in an intermediate significance in testing among natural additives.

Essential oils played an essential role in history as part of everyday life, an example of such oil as cinnamon oil which was used in many ways in ancient Egypt, from embalming the bodies to being used as a breath freshener or as an ointment used for wounds cause by different animals. As humans saw essential oils as miracle remedy given by nature, their toxicity was not questioned much, rather it was almost unexplored. Nowadays, due to many governmental measures that need to be taken beforehand, essential oils need to be of standards which are safe and risk free regardless of their intended use. For the purpose of essential oils to be added as food additives, traditional ways of toxicological tests are taken, meaning performing tests on animals, but to test every active compound of essential oils is not beneficial financially and that is why this kind of testing is falling in popularity. Such tests are intended to show the safe levels of the amounts that would be taken on a daily base by humans, and are only taken when all the other laboratory tests are performed or are showing that it is safe to test on animals. In recent times, the toxicological tests are starting to be based on observing the chemical structures and composition of natural products which is crucial in understanding the toxicity of essential oils, more to their active compounds, in regards to scientific advances since many of the constituents have already been studies and discovered. Evaluation of toxicity nowadays is performed through a series of analytical laboratory methodology tests which are faster, more economically efficient, and more precise (Buchbauer & Baser, 2009).

The most recent new REGULATION (EC) No 1334/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC describes how essential oils and other raw and natural complex materials, would be used as sources of food flavouring and natural additives which would help trade and commercial use of such materials in a way that the materials would be more clearly labelled. The Regulation addresses "biologically active substances" which are put in a number of annexes and describes exactly the maximum levels (mg/kg) that can be used or substances which are banned in food products and beverages. The main aim of this regulation is to improve the trade of such materials as essential oils and extracts internationally with the European Union, and another thing, to define natural products as such to be more representable and clearly labelled to consumers as they are more attracted to natural over synthetic additives (Demyttenaere, 2011).

3.7 Real-life examples of essential oils as food preservatives

Divoženka is "Lemonade that can be made once a year" which was made by Anna Grosmanova in the University of Life Sciences in Prague as a part of her diploma thesis all of the ingredients are local and sourced from Czech Republic. Her diploma thesis was examining the ways in which lemonades could be preserved and contain low amounts of sugar. She found a way to do this using lemon grass, which also served as a nice flavouring additive in combination with elderberry flowers, and that is how the first flavour of Divoženka lemonade was made called "Diplomka". There are two more flavours, "Indianske leto" with melon flavour added to the existing first flavour and “Polibeni” with red currant, raspberry, and basil (Grosmanova, 2016). In “Polibeni” flavour, the presumption is that basil serves as a preservative (Lachowicz et al., 1998).

Gama CYCROM and DMC BASE NATURAL are products made by Spanish company DOMCA, which makes innovative food solutions. Gama CYCROM is a natural preservative based on synergistic combination of different aroma and spice extracts. Once incorporated in food it acts as a protection against *Listeria monocytogenes*, *aureus*, *E.coli*, and *Clostridium* and as an overall contributor to microbiological stability (“Gama CYCROM,” 2020). DMC BASE NATURAL is made of a mix of organic acids, aromatics like tea, orange, and rosemary which add to the preservative value of this product. It is used in preservation of fresh meat products and it acts as a protectant against *Salmonella*, *E. coli*, *Listeria*, *Staphylococcus*, and *Campylobacter* (“DMC BASE NATURAL,” 2020).

Another company which produces natural preservatives is Bavaria Corp. Their line of products called bioTecta™ uses all natural products with antimicrobial activity from the Allium family with the purpose of initial elimination of pathogenic bacteria and extension of a shelf life in cooked or fresh meat, seafood, poultry, deli entrees, sauces, etc. without intervening in with the tangible characteristics of food. Its benefits are that it holds a declaration that is consumer friendly and eradicates listeria monocytogenes bacteria (“BioTecta™,” 2018). Another one of their lines of natural food products is called PROTECTA™ with the same purpose as bioTecta™ line but it can additionally also be mixed with spices, injections and marinades. PROTECTA™ benefits, alongside the same benefits bioTECTA™ has, include eradication of Salmonella, E.coli, Campylobacter, Listeria, moulds and yeasts and it is USDA approved with FSIS Directive 7120.1 (“PROTECTA™ – Natural food safety products,” 2018).

NATUREX is a company which is in a bigger international group specializing in making natural ingredients with a goal to produce new solutions for the food, health and beauty sector. Their XtraBlend RN product is a new solution to extendin the shelf life of dressings, mayonnaise and other lipid emulsions by acting as a replacement for popular artificial antioxidant EDTA ethylenediaminetetraacetic acid. The botanical blend in XtraBlend RN has free radical eradication properties and it chelates lipid emulsions well while leaving original taste and flavour untouched (“DISCOVER XTRABLEND RN: A NATURAL ALTERNATIVE TO EDTA,” 2018). XtraBlend RN is a blend of spinach and rosemary extracts which were picked from over 500 botanicals tested, and it comes in a powder form as a final product which is added during water phase, the same way as EDTA (foodnavigator.com, 2018).

3.8 Sensory parameters (organoleptic impact) and consumer acceptability

The sensory parameters are affected by the flavour and fragrance exerted by essential oils which first act on taste and smell triggers in our mouth and nose if used as food additives. Humans through evolution were drawn to essential oils due to their very specific and strong aroma.

By means of processing the essential oils for flavouring purposes, as their chemical composition varies on different environmental factors their flavour does too. The crude essential oil's flavour comes from factors of location, part of plant used, isolation and seasonality. Once in the processing plant crude oil goes through a number of purifying and distillation processes to remove natural impurities such as wax. After the processed oil goes through gas or liquid chromatography, to determine their function as a flavouring additive, from there the main goal is to achieve a certain desired flavour which may lead to further distillation until the final product is made (Buchbauer & Baser, 2009).

Another way to decrease the organoleptic effects of essential oils is the use of emulsions that can help increase the organoleptic quality and antioxidant stability of the product (Acevedo-Fani et al., 2016; Alfonzo et al., 2017; Calo et al., 2015; Ozogul et al., 2017; Perricone et al., 2015). The combination of such emulsions with essential oils helps create a barrier between the oil and the food matrix which then helps raise the antimicrobial activity (Sugumar et al., 2016). Moreover, essential oils have been increasingly used as a way to prolong the shelf life of food products and ensure sensory quality (Atarés & Chiralt, 2016; Kapetanakou & Skandamis, 2016; Maisanaba et al., 2017; Ribeiro-Santos et al., 2017). This practice is known as active food packaging and signifies the incorporation of essential oils into the packaging which are then monitored and released in order to prolong the organoleptic properties and microbiological integrity (Atarés & Chiralt, 2016; Ribeiro-Santos et al., 2017).

Within the group of essential oils there is a noticeable difference in their respective qualities, both physical and chemical, such as odour, optical and rotation properties, however, they are all immiscible with water and soluble in ether, alcohol and organic solvents. What is more important is that all essential oils have over 200 components that affect their odour and flavour, which correspondingly has a direct influence on their use in food preservation. Essential oils which are extracted from stems, leaves or flowering tops can be used as flavouring in beverages and liquors, while essential oils from herbs and species can be used as additives or functional ingredients in food as a way to change or enhance the flavour or aroma (Kalantari et al., 2012). A plethora of research projects examine the effect of specific oils on food, one of which pointed to the effects of essential oils from seeds of *Piper guineense,* flower buds of *Eugenia aromatic* and kernels of *Monodora Myristica* on store grains. It showed that grains treated by the mentioned oils were preferred during examination than those that were not treated (Okunola et al., 2007). Essential oils extracted from lavender are often used as natural food flavours and are specifically prominent in the production of baked goods, desert-like foods such as candy, gelatine or pudding, and also non-alcoholic beverages (Lis-Balchin, 2004). Oregano essential oil and caprylic acid are mostly utilized in meat and during research it was shown that oregano essential oil ensured a stable pH during storage, and even though the odour of the cooked meat was enhanced while being treated the EO was still satisfactory. However, when used with minced meet it performed even better and helped the colour, flavour and the odour (Hulankova et al., 2013). Interestingly, it is confirmed that fresh basil used as a food additive performed worse than dried basil which improved the organoleptic quality of olive oil (Di Giovacchino et al., 1996). As it was already mentioned, the components of essential oils can have great influence on the qualitiy of the food and some components can even give specific and distinctive flavours to foods. One of them is Carvacrol which changes the aroma of fish and delays spoilage in kiwi and melon (Roller and Seedhar, 2002). Carvacrol, together with carvone, citral, eugenol, thymol, etc., is approved by the European Commission as food flavouring with no health risks. Lemon essential oil can be used in preservation of apple juice or pasteurized milk and its sensory impact on food is decreased by its rich and dominant flavour (Espina et al., 2012). Mentha oil and its vapours are used in fruit juices, and it was found that its addition doesn’t change the odour, colour and it ensures longer storage period (Tyagi et al., 2013). Furthermore, oregano and rosemary essential oils are found to better the oxidative and fermentative qualities of cheese, which then have overall better stability and lower acidity while stored, and protect from lipid oxidation and fermentation (Olmedo et al., 2013). Essential oils extracted from thyme, clove and summer savoury can be used with ketchup and tomato paste as they have a good antifungal activity (Olmedo et al., 2013). During the application of thyme oil on tomato paste it is possible to note changes in the organoleptic properties of the paste, also seen in other food products, however this can be approached/solved in three different ways; accepting the essential oil as a part of the new flavour, using essential oil in products that have a distinctive and strong taste and in that manner minimizing the influence of the EO, or finally, using only certain components of the essential oil, those which would have a minimal effect on the organoleptic properties of food (Omidbeygi et al., 2007). Another possible solution is the use of essential oil vapours as they do not present a direct contact between the food and the essential oil and thus have lesser effect on its properties, mainly flavour or odour. Nonetheless, the use of undiluted essential oil can sometimes lead to extensive changes in flavouring or appearance. One example is the use of undiluted thyme, oregano, and rosemary essential oil in lettuce, cabbage and peppers, as the produce gains an undesirable odour and appearance (Scollard et al., 2013; Uyttendaele et al., 2004). Given this information, it is suggested to dilute the essential oils before use or to combine them with other essential oils or plant material (Scollard et al., 2013). The article points out that the choice of the essential oils and its concentration is highly important as this can greatly affect the properties of the food to which it is applied, for example, strong aroma can cause change in organoleptic properties (Krisch et al., 2011). Finally, superficial fluid extraction is offered as an environmentally friendly way of extracting essential oils from vegetable substrates and extracts obtained in such way can keep the organoleptic characteristics of starting materials (Mariod, 2015).

3.9 Future of essential oils

In the future, competition against different foodborne diseases and allergies is expected. People experience and develop more of those problems as the world develops higher consumer demand every day. Essential oil active antimicrobial compounds are leading to be safer than synthetically made food additives as research on this topic becomes of larger spectrum, also synthetic additives are nowadays falling behind on the trend of so-called green consumerism increasingly. For now, it is important to carry on with research about safety and toxicity of essential oils if they were to be used in higher concentrations in the future among food systems familiar in everyday use and consumption. Another important issue that needs to be taken into consideration is the organoleptic impact; there should be no flavouring or smell disturbances or changes if they are not considered intentional among food products with the application of essential oils as food preservatives (Burt, 2004).

4 Conclusion

- Through exploration of studies the aim of this bachelor thesis is fulfilled due to experiments that showed that the potential use of essential oils is indeed effective in food preservation
- Antimicrobial, antioxidant and antifungal properties of essential oils are explained through their active compounds and their mode of actions, especially on bacterial cells
- In the chapters discussing essential oils application for seafood, meat products, fruits and vegetables, and bakery products preservation it has been concluded that essential oils' antimicrobial action varies greatly depending on concentrations, mode of application, and organoleptic impact, therefore consumers acceptability
- In my opinion, this is a very interesting concept of using essential oils as preservatives, especially in the aspect of nontoxic residuals which are usually left in the water, soil and air and affect the whole life cycle of living beings here on Earth

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