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Review scientific paper

THE EFFECT OF ESSENTIAL OILS ON PATHOGENIC BACTERIA IN MEAT

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Summary

Meat is a nutritionally valuable food which is susceptible to microbiological contamination and spoilage. Major foodborne pathogenic bacteria, such as *Salmonella spp*., *Listeria monocytogenes* and *Yersinia enterocolitica*, are often associated with meat. These causative factors are at the top of the list of reported zoonoses in humans. In order to increase the microbiological quality of meat, alternative substances have been used in recent decades, including essential oils, compounds obtained by extraction from plants, which are a “safe” alternative to chemical and synthetic additives, in order to achieve antimicrobial and antioxidant effects. Many studies show the antibacterial effect of essential oils. The effect of essential oils on bacteria includes the inhibition of growth (bacteriostatic effect) or destruction of bacteria (bactericidal effect). This paper presents commercially available essential oils recognized as safe for use in food industry, their antibacterial effects against food-borne bacteria *in vitro*, the mechanisms of action of these essential oils on Gram-positive and Gram-negative bacteria, as well as their application on a meat model.

Key words: antibacterial activity, plant extracts, mechanisms of action, microbiological quality.

INTRODUCTION

Despite the progress in the field of food safety and the production process hygiene, through the application of good production and good hygiene practices as well as food safety systems, the modern meat industry still faces the problem of controlling food-borne pathogenic bacteria (Saad et al., 2019). The causative factors of the leading foodborne diseases, such as *Salmonella spp*., *Listeria monocytogenes* and *Yersinia enterocolitica*, are, according to the reports of the European Food Safety Agency and the European Center for Disease Prevention and Control, of great health and socioeconomic importance at the global level (EFSA and ECDC, 2022). The World Health Organization reported over 600 million cases of food-borne diseases annually globally, of which 420 000 are deadly. Of these, 40% occur in children under the age of five (WHO, 2015).

Food-borne pathogenic bacteria, in addition to being responsible for a hospitalizing people worldwide, also affect the reduction of the shelf life of meat and meat products. Extending the shelf life, among others, is achieved through the use of additives. Despite the proven effects of reducing microorganisms, the frequent use of additives leads to the accumulation of harmful residues in the food chain, and thus to harmful effects on human health. Consumer concern about the negative effects of chemical additives and salt on health, in addition to resistance to antimicrobial drugs, imposes the need to find new antimicrobial substances (Burt, 2004). In recent years, essential oils have been used as new, alternative compounds to reduce the number of pathogenic, food-borne microorganisms, as well as bacteria that cause food spoilage. Essential oils are complex mixtures, often composed of hundreds of individual plant components. The chemical composition of essential oils affects their antimicrobial potential and varies depending on the geographical origin of the plant, soil composition, stage of development and plant parts.

The antibacterial effect of essential oils is based on several different mechanisms of action on bacterial cells (Rao et al., 2019). Their potential usage in meat and meat products, with the aim of creating a safer and longer-lasting product, as well as their role in reducing the incidence of new diseases caused by food-borne pathogenic bacteria, such as *Salmonella spp*., *Listeria monocytogenes*, *Escherichia coli* and *Yersinia enterocolitica*, has been the subject of studies in recent decades (Kocić-Tanackov et al., 2017; Vidaković Knežević et al., 2021; Šojić et al., 2023).

ESSENTIAL OILS

Etheric oils, also known as essential oils, are concentrated oily liquids that contain volatile aromatic compounds with a characteristic smell (Burt, 2004). According to the definition of the ISO (International Organization for Standardization), essential oils are products from natural raw materials of plant origin, obtained by steam distillation, by mechanical processes from the epicarp of citrus fruits or by dry distillation after separation of the aqueous phase by a physical processes (ISO, 2021). These oils are synthesized during secondary metabolism in all parts of plants (Akthar et al., 2014), including flowers, buds, leaves, bark, seeds, fruits, and roots, and stored within secretory cells, cavities, ducts, and epidermal cells (Burt, 2004).

The most common method of obtaining essential oils is steam distillation (Rao et al., 2019). The yield of essential oils depends on a number of factors. Younger plants give a slightly higher yield, while older plants produce thicker and darker essential oils. The same applies to cultivated plants, compared to wild, i.e. self-growing. The method of obtaining essential oils also affects their yield. To achieve the maximum yield, the length of time of distillation is important, as is the temperature of extraction (da Silva et al., 2021).

Essential oils are soluble in lipids and organic solvents (Akthar et al., 2014), while they are poorly soluble in water (Rios, 2016). At room temperature, they are mostly liquid. The chemical composition and quality of essential oils of the same plant species depend on the genotype, the area where the plant grow, climatic conditions, the vegetative phase and plant parts used to obtain essential oils, storage conditions, and the method of obtaining essential oils (Rios, 2016). The term chemotype is used to describe essential oils of the same plant species that vary in chemical composition. Usually, essential oils are mixtures of a different components (Rao et al., 2019), some of which are found in traces, while others are present in over 85% of the total composition (Table 1) (Burt, 2004; Vidaković Knežević et al., 2023). These components are classified into groups, such as terpenes, terpenoids, low molecular weight aromatic and aliphatic compounds that determine the biological properties of essential oils (Bakkali et al., 2008; Rao et al., 2019).

Table 1 Main components of selected essential oils and their presence (Vidaković Knežević et al., 2023)

Number	Essential oil	Latin name	Component name	Presence of component (%)
1.	Basil	*Ocimum basilicum*	Estragol	69.52
2.	Black pepper	*Piper nigrum*	β-Pinene	19.31
3.	Thai ginger	*Zingiber cassumunar*	Sabinene	39.17
4.	Cinnamon	*Cinnamomum zeylanicum nees*	Cinnamaldehyde	74.93
5.	Clove	*Syzygium aromaticum L.*	Eugenol	85.14
6.	Everlasting flower	*Helichrysum italicum*	Caryophyllene	21.48
7.	Fennel	*Foeniculum vulgare*	Anethole	88.42
8.	Angelica	*Angelica archangelica*	β-Phelandrene	41.57
9.	Hyssop	*Hyssopus officinalis*	Cis-Pinocamphone	27.42
10.	Lavender	*Lavandula angustifolia*	Linalyl acetate	25.33
11.	Lemon	*Citrus limonum*	Limonene	79.72
12.	Myrtle	*Myrtus communis*	α-Pinene	35.47
13.	Oregano	*Origanum vulgare*	Carvacrol	81.00
14.	Rosemary	*Rosmarinus officinalis*	α-Pinene	28.23
15.	Sage	*Salvia officinalis*	Linalyl acetate	56.41
16.	Thyme	*Thymus vulgaris*	p-Cymen	40.91
17.	Heather	*Satureja montana*	Carvacrol	50.45
18.	Yarrow	*Achillea millefolium*	Sabinene	22.70

Nowadays, over 3 000 essential oils are known and about 300 are commercially important (Bakkali et al., 2008). The leading countries in the production of essential oils are China and India, followed by Indonesia, Sri Lanka and Vietnam. In Europe, France and Germany produce the largest quantities of essential oils, followed by Spain, Greece and Great Britain. The largest quantities of essential oils are used in the food and beverage industry (35%), cosmetics and aromatherapy (29%), household products (16%) and the pharmaceutical industry (15%) (Barbieri and

Borsotto, 2018). The wide use of essential oils in various domains indicated that quality criteria should be set. In addition to the ISO standard (ISO, 2021), many associations and companies, such as the Association Française for Normalization, Generally Recognized As Safe (GRAS), Fragrance Materials Association, the International Fragrance Association, Bundesinstitut für Risikobewertung, Research Institute for Fragrance Materials and the Scientific Committee on Consumer Safety, have set composition and quality criteria (Rios, 2016).

The prerequisite for the use of essential oils in the food industry, according to GRAS, is the chemical identification and purity of the substance, as well as the determination of the presence of secondary components. Therefore, reliable methods for the analysis of essential oils have been developed. The application of gas chromatography (GC) and gas chromatography with mass spectrometry (GC-MS) techniques are most common in the analysis of essential oils today. These methods make it possible to determine the chemical composition of essential oils and the concentration of their compounds. Different batches of commercial essential oils can vary in chemical composition. Therefore, according to GRAS, it is necessary to define the maximum allowed concentration of certain compounds (Smith et al., 2005). In the food industry, the use of a large number of essential oils is permitted, including the essential oils of angelica *(Angelica archangelica*), anise (*Pimpinella anisum L.*), basil (*Ocimum basilicum L.*), cinnamon (*Cinnamomum zeylanicum Nees*), coriander (*Coriandrum sativum L.*), fennel (*Foeniculum vulgare Mill.*), ginger (*Zingiber officinale Rosc.*), lavender (*Lavandula officinalis Chaix.),* lemon (*Citrus limon*), black pepper (*Piper nigrum L.*), rosemary (*Rosmarinus officinalis L.*) , oregano (*Origanum vulgare*), sage (*Salvia officinalis L.*) and thyme (*Thymus vulgaris*) (FDA, 2021).

Antibacterial effects of essential oils

The effect of essential oils on bacteria is reflected in the inhibition of their growth (bacteriostatic action) or the destruction of the bacterial cell (bactericidal action) (Swamy et al., 2016). Antibacterial activity, based on the mentioned effects, is measured by minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) (Burt, 2004).

There is no standard method for evaluating the antibacterial activity of essential oils. For this purpose, the NCCLS (National Committee for Clinical Laboratory Standards), CLSI (Clinical and Laboratory Standards Institute) and EUCAST (the European Committee on Antimicrobial Susceptibility Testing) guidelines were primarily developed for the assessment of antibiotic activity (Rao et al., 2019).

Different types of *in vitro* tests are applied in order to determine the antibacterial activity of essential oils, and their selection depends on several characteristics, of which the complexity of the technique and the cost are the most relevant. Rapid determination of the antibacterial activity of essential oils is usually performed by the agar diffusion or disk diffusion method, when essential oils are added to wells made in agar or to filter paper discs placed on the surface of the agar containing the inoculated target bacterial isolate. After incubation, a zone of microorganism growth inhibition appears, which represents antibacterial activity (Swamy et al., 2016). The intensity of antibacterial activity is most often determined by diluting essential oils in broth using the microdilution method. This pattern of testing the antibacterial activity of essential oils is more sensitive, and the determination of MIC is based on measuring optical density, counting surviving bacterial colonies or using resazurin as an indicator (Burt, 2004).

In addition to non-standardization of methods, difficulties in determining the antibacterial activity of essential oils are their insolubility in water and volatility. Of particular importance is the hydrophobicity and high viscosity of essential oils, which cause irregular distribution through the medium when applying the diffusion method, as well as unequal solubility in dilution methods. This can be avoided by using solvents such as ethanol, methanol, Tween-20, Tween-80, acetone, dimethyl sulfoxide (DMSO), n-hexane, and propylene glycerol. The results of testing the sensitivity of microorganisms to essential oils depend on the applied method, type and amount of nutrient medium, inoculum concentration, temperature and incubation time, as well as the origin of the essential oils used (Burt, 2004).

Mechanisms of action of essential oils on bacteria

Despite numerous tests on the antibacterial effect of essential oils, their mechanisms have not yet been fully explained. In general, these mechanisms can be attributed to physical, chemical or biochemical changes in bacteria exposed to essential oils. Different components may act by different mechanisms and may target different types of bacteria. Gram-negative bacteria have been shown to be less sensitive to essential oils than Gram-positive bacteria (Burt, 2004), which is a result of differences in their structure. The structure of the cell wall of Gram-negative bacteria is more complex. A thin layer of peptidoglycan is surrounded by the outer membrane. These two structures are tightly linked by Brown's lipoproteins. The outer membrane of Gram-negative bacteria is made of a lipid bilayer connected by polysaccharides to the inner membrane. The outer membrane creates a barrier and protects the cell from essential oils (Burt, 2004; Böhme et al., 2013). However, small hydrophilic solutes can pass through the outer membrane via porin proteins that serve as hydrophilic transmembrane channels.

On the other hand, the cell wall structure of Gram-positive bacteria allows easy penetration of hydrophobic molecules. Such molecules, like essential oils, act on the cell wall and the cytoplasm (Nazzaro et al., 2013). Common factor, regardless of their composition, is that essential oils, as typical lipophiles, pass through the cell wall and cytoplasmic membrane, disrupting the structure of polysaccharides, fatty acids and phospholipids. Damage to the cytoplasmic membrane causes its permeability and leakage of cell contents, ion loss, proton pump collapse, adenosine triphosphate (ATP) depletion, and cell lysis (Burt, 2004; Böhme et al., 2013; Rao et al., 2019).

The main components of some essential oils, carvacrol and p-cymene, induce the synthesis of heat stress proteins involved in various processes of polypeptide synthesis and release. In *Escherichia coli* O157:H7 cells treated with carvacrol, there is a significant increase in the amount of heat stress protein (GroEL) and inhibition of flagellin synthesis, resulting in cell immobility. Salmonella cells treated with sublethal concentrations of thymol synthesize a set of chaperone proteins (DnaK, GroEL, HTpG), outer membrane proteins (OmpX, OmpA) and proteins that are directly or indirectly involved in citrate metabolism and ATP synthesis. These different actions categorize thymol as a major stressor for Salmonella cells (Di Pasqua et al., 2010). Studies also show a decrease in the pH value of bacterial cells exposed to the essential oils. By treating the cells of *Salmonella* Typhi, the MIC of the essential oil of licorice reduces the pH value from 6.59 to 5.44, and in *Escherichia coli* O157:H7 from 6.23 to 5.20 (Turgis et al., 2009). Considering the large number of different groups of chemical compounds within the essential oil, it is most likely that their antibacterial effect cannot be attributed to a single mechanism, but that there are several targets in the cell. Also, despite the large number of chemical components within the essential oil, the antibacterial effects are usually attributed to the most abundant component, or to the synergistic action of several components (Burt, 2004).

Application of essential oils on the meat model

Meat is a nutritionally valuable food in the human diet. However, pathogenic bacteria transmitted through food can be, due to their presence in meat, a hazard for consumers if the meat is not sufficiently thermally processed, that is, if its cross-contamination occurs. Meat can be a source of human infections with bacteria such as *Salmonella spp*., *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter spp*.

and *Yersinia enterocolitica* (Vidaković et al., 2020; Velhner et al., 2020; Ljubojević Pelić et al., 2021).

By applying essential oils of oregano and thyme in minced meat with less fat (≤ 7%) to the population of *Listeria monocytogenes*, a significant reduction was achieved during storage at 4°C for 4 days. The first reduction in the number of *Listeria monocytogenes* was observed on the second day in samples treated with essential oil of oregano in the amount of 0.36 µL/g, and essential oil of thyme in the amounts of 0.36 µL/g and 0.72 µL/g. Oregano and thyme essential oils in an amount of 0.36 µL/g reduced the number of *Listeria monocytogenes* almost identically, i.e. by 0.26 $\log_{10}$ CFU/g and 0.27 $\log_{10}$ CFU/g, while thyme essential oil in an amount of 0.72 µL/g reduced the number of *Listeria monocytogenes* by 0.42 $\log_{10}$ CFU/g. At the end of storage, i.e. on the 4th day, the reduction in the number of *Listeria monocytogenes* ranged between 0.25 $\log_{10}$ CFU/g and 0.51 $\log_{10}$ CFU/g when applying essential oil of oregano, i.e. between 0.22 $\log_{10}$ CFU/g and 0.29 $\log_{10}$ CFU/g when applying thyme essential oil. The applied concentrations of essential oils corresponded to the MIC and 2MIC values obtained using the broth microdilution method (Vidaković Knežević et al., 2023).

Parsley essential oil applied to a prosciutto model showed its antimicrobial activity at a concentration of 6%, while in an *in vitro* test the MIC was 0.018% (Gill et al., 2002). On the other hand, a high concentration of essential oils on/in meat and meat products can affect their organoleptic properties. Essential oils have an intense aroma even at very low concentrations (Chivandi et al., 2016). Reducing the organoleptic effect of added essential oils in the matrix of meat or meat products can be achieved by encapsulating essential oils in a nanoemulsion. This increases the stability of volatile components, protecting them from interaction with the meat matrix while increasing antimicrobial activity through increasing passive cellular uptake (Donsì et al., 2011; Pan et al., 2014). Reducing the concentration of essential oils, without impairing its antimicrobial activity, in food models, can be achieved by combining two or more essential oils or their compounds. Thus, synergistic action is achieved (Gutierrez et al., 2008). The synergistic effect of essential oils of mastic tree (*Pistacia lenticus*) and heather (*Satureja montana*) was observed in the model of minced beef against *Listeria monocytogenes* (Djenane et al., 2011), as well as essential oils of basil and rosemary in chicken meat against *Salmonella* Enteritidis (Stojanović -Radić et al., 2018).

The application of essential oils, as preservatives in meat and meat products, requires a good knowledge of their properties, mode of action, antimicrobial potential and interaction with matrix components. Fat content, protein content, water activity, pH and enzymes can reduce the potential effect of essential oils in the matrix of meat and meat products. High concentrations of fats, proteins and carbohydrates can protect bacteria by creating a protective coat and absorbing essential oils (Burt, 2004; Böhme et al., 2013).

CONCLUSION

The application of essential oils against pathogenic bacteria in meat has proven to be successful in laboratory conditions. Essential oils act by different mechanisms against Gram-positive and Gram-negative bacteria in very low concentrations. However, the application of essential oils in meat models, i.e. food, requires additional research. Food components often protect bacterial cells and allow them to recover faster, which is why larger amounts of essential oils are needed in meat models. On the other hand, as aromatic mixtures, essential oils impair the organoleptic properties of meat, i.e. food. Today, studies are focused on finding different strategies in order to enable the use of essential oils in food production, as an alternative to different additives and chemical substances.

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