tehnologija mesa
Osnivač i izdavač: Institut za hihijenu i tehnologiju mesa, Beograd

UDK: 637.5.065:579.8 ; 665.52
ID: 201658892

Preview paper
Pregledni rad

Use of essential oils in order to prevent foodborne illnesses caused by pathogens in meat

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A b s t r a c t: Although food industry has improved production techniques and slaughter hygiene, pathogens found in meat, such as Salmonella spp., Campylobacter spp. and E. coli still cause a number of foodborne illness outbreaks yearly all over the world. The overuse of antibiotics and disinfectants in both veterinary and human medicine practice has led to phenomenon of multi-drug-resistance of bacteria, which highlights the research needs on new antimicrobial agents. One of the alternatives is use of essential oils, which are aromatic oily liquids obtained from plant material by different methods. It has been proved that essential oils exhibit variable antibacterial activity, depending on the type of bacteria as well as on the chemical composition of essential oil being used. Essential oils (Eos) have antioxidant role, and inhibitory role not only to pathogens, but also to the spoilage microorganisms, which subsequently affects quality and extends meat shelf-life in order to produce safer and healthier product.

***Key words:** plant essential oils, antibacterial properties, food borne pathogens, meat.*

Introduction

In the recent years, food safety issues have become one of the main public health concerns. In 2005, WHO reported 1.8 million of death caused by diarrheal diseases, mostly associated with contaminated food and drinking water (*Newell et al.*, 2010; *Sofos*, 2008). Before 1970's *Salmonella* spp., *Shigella* spp., *Clostridium botulinum, Staphylococcus aureus, Bacillus cereus* were recognized as the major causes of gastrointestinal disease, and during the 1980's and 1990's *Campylobacter* spp., *Yersinia enterocolitica, Listeria monocytogenes, Escherichia coli O157:H7, Vibrio cholerae non O1, Vibrio vulnificus, Norovirus, Cryptosporidium parvum, Cyclospora cayetanensis, Enterobacter sakazakii* and prions were added on the list of food pathogens, but it is alarming that in about 50% of cases causative agents still remain unknown (*Sofos*, 2008; *Newell et al*., 2010; *Linscott*, 2011). *Salmonella* spp., *Campylobacter* spp., enterohaemorrhagic *E. coli,* including serotype O157:H7, present microbial pathogens of current concern in food, especially in meat, which presents valuable source of proteins, fat, Fe ion and B_{12} vitamin, and has the main role in human diet, while *Listeria monocytogenes* can be found in ready-to-eat meat and poultry products (*Bacon and Sofos*, 2003; *Sofos*, 2008; *Baltić et al*., 2010; *Linscott*, 2011; *Velebit et al*., 2012, *de Castro Cardoso and dos Reis Baltazar Vicente*, 2013). According to farm-to-fork approach in food production, monitoring of foodborne illnesses and pathogens as well as structured approaches to food safety, such as HACCP principles, have been implemented in the food chain (*Newell et al*., 2010). Despite efforts and improvements in slaughter hygiene and food production techniques in food industry, foodborne pathogens found in meat still cause a number of foodborne illness outbreaks yearly all over the world (*Burt*, 2004; *Sofos,* 2008; *Newell et al.,* 2010; *Linscott*, 2011). The overuse of antibiotics in order to reduce pathogens in human medicine, as well as in veterinary practice, has led to phenomenon of multi-drug-resistant bacteria (*Doyle and Erickson*, 2006; *Sofos* 2008; *Tohidpour et al*., 2010). Intestinal infectious diseases and bacterial resistance are not the only problem associated with meat safety. Salt is most common used additive which has been used since

Acknowledgments: This paper was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Project TR 31034.

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ancient times for the preservation of meat products and plays great role in sensory and textural properties of meat and meat products. In spite of advantages, use of salt has shortcomings because it is linked to hypertension and consequently increased risk of cardiovascular disease, which is why reducing salt intake presents a new trend, but also a challenge for meat industry (*Desmond*, 2006; *Sofos* 2008; *Weiss et al*., 2010). Reducing NaCl levels below those typically used, without any other preservative measure, reduces product shelf life and allows spoilage flora to grow and render product unsafe for consumption (*Desmond*, 2006; *Weiss et al*., 2010). All of these have led to need for new methods of eliminating or reducing foodborne pathogens and spoilage microorganisms, possibly in combination with the existing and already used methods (*Burt*, 2004). One such possibility is the use of essential oils (EOs), which present a better choice than some synthetic chemical additives, especially for ”organic” and ”natural” food production, which has become popular mostly in the Western society, and is widely accepted by consumers (*Burt*, 2004; *Sofos* 2008; *Gutierrez*, 2009; *Velebit et al*., 2012). Essential oils are aromatic oily liquids obtained from plant material by different methods (*Burt*, 2004). EOs are also known as volatile or ethereal oils, and they have been used since ancient times for their perfume, flavor and preservative properties (*Bauer et al*., 2001; *Burt*, 2004). In adition to antibacterial properties, EOs also possesses antiviral, antimycotic, antitoxigenic, antiparasitic and insecticidal properties. Although some of these properties have been recognized long ago, in recent years scientific interest for essential oils and their application in food is renewed (*Burt*, 2004).

Composition of EOs

Essential oils are the low molecular weight liquids, limpid, rarely coloured, volatile mixtures, which are lipid soluble and soluble in organic solvents (*Burt*, 2004; *Bakkali et al*., 2008; *Marković et al.,* 2008; *Tajkarimi et al*., 2010; *Lv et al.,* 2011; *Bajpai et al*., 2012). Essential oils play role in plant defense and some are always present, while others are produced as a response to microbial invasion or physical injury (*Hyldgaard et al*., 2012). They are synthesized by different plant organs, such as flowers, leaves, seeds, fruits, roots, buds, stems, twigs, wood or bark, and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes from which they are obtained (*Burt*, 2004; *Bakkali et al*., 2008; *Tajkarimi et al*., 2010; *Lv et al.,* 2011; *Bajpai et al*., 2012). Several methods including steam and hydro distillation, solvent extraction, and expression are used for extracting essential oils (*Burt*, 2004; *Bakkali et al*., 2008; *Tajkarimi et al*., 2010). The most common and the simplest method for producing EOs for commercial purposes is steam distillation. More expensive method is extraction by means of liquid carbon dioxide under low temperature and high pressure produces. EOs produced in this way have more natural organoleptic characteristics, and exhibit greater antimicrobial activity (*Burt*, 2004).

Essential oils may have different properties depending on climate, soil composition, age and vegetative cycle stage, which is why they have to be extracted under the same conditions, from the same organ of the plant which has been growing on the same soil, under the same climate and has been picked in the same season (*Burt*, 2004; *Bakkali et al.,* 2008). Antimicrobial activity also varies, and it is strongest in EOs produced from herbs harvested during or immediately after flowering. Because EOs are volatile, in order to maintain their characteristics after extraction, they need to be stored in airtight containers away from light (*Burt*, 2004).

Essential oils are complex mixtures, and contain between twenty and sixty, and some of them more than sixty individual components, which may be determined by gas chromatography/mass spectrometry. The concentration of components is quite different, and major components can constitute up to 85% of the EO, while other components can be found only in traces. These major components determine the biological properties of the essential oils. However, studies conducted on sage, thyme and oregano have shown that minor components have a critical part to play in antibacterial activity, probably by producing a synergistic effect between major components of EOs. Also, it is proved that oil as a whole possess better antibacterial activity than only a combination of major volatiles of the oil (*Burt*, 2004; *Bakkali et al*., 2008).

The components of essential oils usually are divided into two groups, the main group which is composed of terpenes and terpenoids, and the other composed of aromatic and aliphatic constituents.

Terpenes are made from combinations of several isoprene (5-carbon-base, C5) units. The monoterpenes (C10) and sesquiterpenes (C15) present main classes of terpenes. Monoterpenes constitute 90% of the essential oils and work as carbure, alcohol, aldehyde, ketone, ester, ether, peroxide and phenols. The sesquiterpene compounds contain three isoprene units and the functional properties are very close to monoterpene compounds. Hemiterpenes, diterpenes, triterpenes and tetraterpenes terpenoid (terpene which contain oxygen) also exist, but in lower concentrations then monoterpenes and sesquiterpenes.

The aromatic compounds are the derivatives of phenylpropane and they consist of aldehydes, alcohols, phenols, methoxy and methylenedioxy in nature. A few nitrogen and sulfur compounds present in EOs are also characterized as plant essential constituents (*Bakkali et al.,* 2008; *Bajpai et al.,* 2012).

Despite a widespread opinion that the phenolic components are responsible for the antibacterial properties of EOs, recent studies showed that nonphenolic compounds of oils extracted from oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, purple and bronze muscadine seeds and sage also exhibit antibacterial activity against Gram-positive, as well as against Gram-negative bacteria (*Tajkarimi et al.,* 2010).

Antibacterial and antioxidant properties and mechanism of action of EOs

Before they are added to the meat, antibacterial activity should be tested *in vitro* conditions. The minimum inhibitory concentration (MIC), which is defined by most authors as a measure of the antibacterial performance of EOs, should be determined at first (*Burt* 2004; *Lv et al.*, 2011). Minimum inhibitory concentration of EOs can be detected by diffusion, dilution or bioautographic methods, of which, diffusion method is mostly used in experiments for screening for antibacterial activity, while agar or broth dilution methods are used to determinate strength of antibacterial properties. Scanning electron microscopy is method of choice for observation of physical effects of antibacterial activity (*Burt*, 2004). Although tests for determinations of MIC are not standardized, the NCCLS (*National Committee on Clinical Laboratory Standards, actually CLSI – Clinical and Laboratory Standards)* method for antibacterial susceptibility testing, which is mainly used for the testing of antibiotics, has been modified for testing EOs (*NCCLS*, 2000; *Burt*, 2004). Even so, comparison of published data is complicated because outcome of a test is affected by different factors, such as the method used to extract the EOs from plant material, the volume of inoculum, growth phase, culture medium used, pH of the media incubation time, temperature of incubation and many others, which is why it is preferable for researches to determine MIC by themselves before conducting the experiment (*Burt*, 2004).

Also, it is important to be familiar with the mode of action of EOs, in order to choose the proper one, depending on what the active component of EO is and which bacteria are tested to be inhibited. Since essential oils consist of large number of components, their antibacterial activity is not based on one specific mechanism, but there are several targets in the cell. Interaction and damage of bacterial cell membrane is considered to be the main mode of antibacterial action of EOs. Hydrophobic nature of EOs makes them to interact well with lipid membrane of bacterial cell membrane and mitochondria and cause permeabilization of the membranes. Changes in membrane permeability occur as a result of loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool, which eventually lead to leaking of intracellular constituents, coagulation of cell contents, lysis and cell death. The chemical structure of the individual EO components affects their precise mode of action and antibacterial activity. Generally, the EOs possess the strong antibacterial properties against food borne bacteria because phenolic compounds containing hydroxyl group such as carvacrol, eugenol and thymol, which are responsible for disrupting the cell membrane and inhibiting the functional properties of the cell. It appears that the type of alkyl group has influence on antimicrobial activity of non-phenolic components of EOs (*Burt*, 2004; *Bakkali et al*., 2008; *Lv et al*., 2011; *Bajpai et al*., 2012, *Velebit et al*., 2012). There are some indication that EOs act on the enzymes involved in the energy regulation or synthesis of structural components, which is explained by the fact that some EOs stimulate the growth of pseudomycelia (a series of cells adhering end-to-end as a result of incomplete separation of newly formed cells) in certain yeasts (*Burt*, 2004).

Antibacterial properties of EOs depend not only on EOs chemical characteristics, but also on type of bacteria. Essential oils are more effective against Gram-positive bacteria rather than Gram-negative bacteria. Gram-negative bacteria are less susceptible because their membrane contains hydrophilic lipopolysaccharides (LPS), which create a barrier toward macromolecules and hydrophobic compounds (*Hyldgaard et al*., 2012). Gram-negative bacteria, *Pseudomonas spp.*, in particular *P. aeruginosa*, appear to be least sensitive to the action of EOs, and exception of the rule is *Aeromonas hydrophila*, which appears to be one of the most sensitive species (*Burt*, 2004).

Essential oils have not only antibacterial properties, but their application in meat can affect some meat characteristics as well. Oxidation by free radicals is one of the primary mechanisms of quality deterioration in foods, and especially in meat products. Some secondary products of oxidation, like short-chain aldehydes, ketones and other oxygenated compounds may adversely affect quality of meat by causing loss of color and nutritive value, limiting shelf-life and making meat potentially dangerous for consumer health (*Simitzis et al.,* 2010). Active essential oil compounds, such as phenolic diterpenes, derivates of hydroxycinnamic acid, flavonoides and triterpenes found in rosemary, oregano, borage and sage have high antioxidant activity (*Sanchez-Escalante et al*., 2003; *Oberdieck,* 2004; *Fasseas et al*., 2007; *Ryan et al.,* 2009; *Weiss et al.,* 2010). Some EOs, for example clove essential oil, have been reported to have higher antioxidant activity than some synthetic antioxidants, like BHT or butylated hydroxyanisole, which is why EOs may present natural alternatives to synthetic antioxidants, without leaving residues in the product or the environment (*Yanishlieva-Maslarova,* 2001; *Simitzis et al*., 2010; *Teixeira et al*., 2013).

Application of EOs in meat

In many studies it has been experimented with essential oils and their effects on meat pathogens as well as on spoilage flora. These studies have shown that efficiency of essential oils depends not only on type, chemical composition and concentration of essential oils, but also on meat characteristics, type of bacteria, mode of application of EOs in meat and some other physical parameters, such as pH values, water activity, oxygen tension, temperature (*Burt*, 2004; *Chouliara et al*., 2007; *Gutierrez et al*., 2008; *Simitzis et al*., 2008; *Gutierrez et al*., 2009; *Govaris et al.,* 2010, *Emiroğlu et al*., 2010; *Lv et al.,* 2011; *Hsouna et al.*, 2011; *Karabagias et al*., 2011; *Bajpai et al*., 2012; *Awaisheh,* 2013; *Khanjari et al*., 2013; *Teixeira et al*., 2013).

Based on antibacterial properties of EOs and type of affected pathogen, some essential oils are better than others in meat applications. Eugenol and coriander, clove, oregano and thyme oils were found to be effective at levels of 5–20 μl/g in inhibiting *L. monocytogenes*, *A. hydrophila* and spoilage flora in meat products, whilst mustard, cilantro, mint and sage oils were less effective or ineffective (*Burt*, 2004). *L. monocytogenes* also exhibited to be sensitive on combination of EOs and nisin (*Tajkarimi et al.,* 2010). Several studies were performed in order to confirm efficacy of EOs against *Salmonella spp*. in food. Results have shown that oregano and thyme EOs, and EOs extracted from *Salvia officinalis* and *Salvia molle* inhibit the growth of *Salmonella* bacteria up to a significant reduction in the CFU levels. However, cinnamon bark EO (7000 mg/kg^{-1}) exerted the strongest antibacterial efficacy against *Salmonella* spp., while rosemary EO showed low antibacterial efficacy (*Hayouni et al*., 2008; *Bajpai et al.,* 2012). Addition of nisin at 500 or 1000 IU/g in minced sheep meat did not show any antibacterial activity against *S.* Enteritidis, but combination with oregano EO at 0.6%, showed to be efficient (*Govaris et al.,* 2010). Experiment in which oregano EOs and sodium nitrite were used against *Clostridium botulinum* spores, has shown that EOs in combination with low concentration of sodium nitrite inhibits or slows growth of bacteria more than the sodium nitrite alone, depending on the number of inoculated spores (*Burt*, 2004; *Tajkarimi et al*., 2010).

Concentration of essential oils, which should be added to meat in order to prevent oxidation, foodborne pathogens, or to extend shelf-life, is usually higher than one used in *in vitro* conditions because of interaction with meat components (*Burt,* 2004; *Hyldgaard et al*., 2012). An exception to this phenomenon is *A. hydrophila* where no higher concentration of EO was needed in experiments to inhibit these bacteria on cooked pork in comparison to tests *in vitro* (*Burt*, 2004).

The high levels of fat or protein in meat and in food generally, appear to reduce the effectiveness of antibacterial EOs. If the EO dissolves in the lipid phase of the food it will be relatively less available to act on bacteria present in the aqueous phase, while the other suggestion is that the lower water content of meat compared to laboratory media may slow down the progress of antibacterial agents to the target site in the bacterial cell. For example, mint and cilantro EOs were not effective in products with a high level of fat, such as pâté and a coating for ham containing canola oil (*Burt*, 2004). However, some studies on beef extract culture medium have shown that efficacy of oregano and thyme oil was greater at higher concentrations of protein, which may have displayed hydrophobic properties with consequent interactions with EOs to facilitate their dissolution in the medium (*Gutierrez et al*., 2008). Also it has been reported that proteins usually possess a high binding capacity for flavor volatile compounds. General opinion is that carbohydrates do not protect bacteria from the action of EOs as much as fat and protein do and some other components of meat, such as water and salt in higher level assist the action of EOs (*Burt*, 2004).

Essential oils can be applied directly in meat or PEO (polyethylene oxide)-based antimicrobial packaging can be used (*Bajpai et al*., 2012; *Hyldgaard et al.,* 2012; *Velebit and Petrović*, 2012). This is one of the many antimicrobial packaging technologies which improve the quality of the meat products, mainly by reducing spoilage flora and extending shelf life, but also provide information about food quality during storage (*Lončina et al.,* 2013). Essential oils can be encapsulated in polymers of edible and biodegradable coatings or sachets that provide a slow release to the food surface or to the headspace of packages of meat (*Hyldgaard et al.,* 2012). Depending on the concentration, after application in meat, some essential oils may alter the qualitative properties of the product. A way to minimize negative organoleptic effects of essential oils added to the matrix of a meat is to encapsulate essential oils into nanoemulsions. Nanoencapsulation of bioactive compounds represents a viable and efficient approach to increasing the physical stability of the active substances, protecting them from the interactions with the food ingredients and, because of the subcellular size, increasing their bioactivity (*Donsí et al*., 2011; *Hyldgaard et al.,* 2012).

Safety aspect of the use of EOs

Although essential oils possess antibacterial properties and may improve taste and some other characteristics of the meat, they should be used with care, because EOs may cause some side effects (*Burt*, 2004; *Bakkali et al.,* 2008). Some essential oils, such as menthol, eugenol and thymol, depending on concentration, may cause irritation of mucous membranes, probably as a result of membrane lysis and surface activity, while cinnamaldehyde, carvacrol, carvone and thymol *in vitro* appear to have mild to moderate toxic effects at the cellular level (*Burt*, 2004). Some essential oils contain components which cause allergic contact dermatitis in people who use them frequently and the other essential oils contain photoactive molecules like furocoumarins, which cause phototoxic reactions (*Burt*, 2004; *Bakkali et al*., 2008). Several EOs which have been used in phytomedicine and aromatherapy have exhibited spasmolytic or spasmogenic properties, but these effects were not associated with a particular component of EOs (*Burt*, 2004). EOs mostly have no carcinogenic properties, but some of them may be considered as secondary carcinogens after metabolic activation (*Guba*, 2001; *Bakkali et al.,* 2008). For example, some EOs provoke estrogen secretions which can induce estrogen-dependent cancers, while some photosensitizing molecules found in EOs, such as flavins, cyanin, porphyrins can cause cancer (*Bakkali et al*., 2008). However, many studies showed that essential oils have anti-tumoral potentials (*Ferraz et al.,* 2013; *Bostancıoğlu et al.*, 2011; *Sharma et al*., 2009). Because of genotoxicity and other potential sideeffects, use of essential oils as flavorings and their maximum allowed concentration in food products have been controlled by regulations and laws. A number of EO components such as carvacrol, carvone, cinnamaldehyde, citral, p-cymene, eugenol, limonene, menthol and thymol have been registered by the European Commission and considered to present no risk to the health of the consumer. United States Food and Drug Administration (FDA) has classified the substances as generally recognized as safe (GRAS) or as approved food additives, and it is mostly based on the EU registered flavorings list with some modifications (for example, estragole, specifically prohibited as flavoring in the EU, is on the EAFUS list), (*Burt,* 2004; *Bajpai et al.,* 2012).

New flavorings might be considered for registration only after toxicological and metabolic studies proving later not to be dangerous for human health (Commission Decision of 23 February, 1999; Commission Regulation (EC) No 1565/2000 of 18 July, 1565/ 2000; Commission Regulation (EC) No. 622/2002 and Regulation (EC) No 2232/96; *Burt,* 2004).

Conclusion

As the food industry is facing great challenges to produce safe, and at the same time food without synthetic chemical preservatives, essential oils make their way into the scientific focus. Due to their antibacterial, antifungal and antiviral activity, as well as antioxidant properties, they are used to prevent foodborne diseases, to extend shelf-life, and to improve some meat characteristics. Their efficiency against pathogens and spoilage flora depends on many different factors, and their implementation in practice faces some obstacles. Essential oils are recognized to be used, not only as food additives, but also in aromatherapy, antitumor therapy, as potential antimicrobial agents against multi-resistant bacteria, and in other purposes in medical and nonmedical fields. Yet, benefits of their use remain to be confirmed.

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Upotreba eteričnih ulja u cilju prevencije bolesti prenosivih hranom uzrokovane patogenima iz mesa

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R e z i m e: Iako je industrija hrane unapredila način i tehnologiju proizvodnje, kao i higijenu klanja, patogeni mikroorganizmi prenosivi hranom koji se mogu naći u mesu, kao što su Salmonella spp., Campylobacter spp. i E. coli, odgovorni su za milione oboljenja svake godine. Prevelika upotreba antibiotika i dezinficijenasa, kako u veterinarskoj, tako i u humanoj medicini rezultiralo je fenomenom bakterijske rezistencije, zbog čega se javila potreba za novim antimikrobnim sredstvima. Jedna od mogućih alternativa je upotreba eteričnih ulja koja predstavljaju aromatične tečnosti uljane konzistencije, koje se različitim metodama, ekstrahuju iz skoro svih delova biljaka. Dokazano je da eterična ulja, u različitom stepenu, imaju antibakterijsku aktivnost koja zavisi od vrste bakterije kao i vrste i hemijskog sastava ulja koje se koristi. Eterična ulja imaju i antioksidativnu ulogu i inhibiraju rast patogenih, ali i mikroorganizama kvara, utičući na taj način, na kvalitet i održivost mesa u cilju stvaranja bezbednijeg proizvoda.

***Ključne reči**: biljna eterična ulja, antibakterijska svojstva, patogeni prenosivi hranom, meso.*

Paper received: 13.09.2013.
Paper accepted: 19.09.2013.