Essential oils as natural additives in dry-fermented sausages

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Abstract. The usage of food additives is one of the key methods for inhibiting microorganisms’ growth and delaying oxidative changes in dry-fermented sausages (DFS). However, they have numerous negative health effects, so the novel meat industry is oriented towards different natural alternatives while focusing on plant extracts, including essential oils (EOs). EOs are interesting since they are generally recognized as safe (marked as GRAS) and have a broad acceptance from the consumers. Their activity depends on numerous parameters including the method of extraction, concentrations and possible synergistic effects of their bioactive compounds. Various conventional (hydrodistillation and organic solvent extraction) and novel (microwave-assisted, ultrasound-assisted and supercritical fluid extraction) extraction techniques are being applied for EO recovery, and optimization of these process is an essential step towards cost-effective production of high-quality extracts. Generally, it can be concluded that EOs are added into DFS to delay or prevent lipid oxidation, retard microorganisms’ growth, improve colour stability and extend the shelf-life.

1. Introduction
Dry-fermented sausages (DFS) have been produced in countless countries all around the world. Nowadays, consumers are becoming increasingly aware of these meat products for their significant health benefits and unique odour and flavour [1]. DFS are manufactured using meat (e.g. pork, beef) and fat, salts, spices, starter cultures and food additives [2,3]. Due to the relatively high fat content (20-30%), various raw resources and absence of high temperature during processing, DFS are very disposed to quality deterioration [2,3].

Lipid oxidation is one of the main non-microbial factors of quality deterioration in meat processing [4]. It should be noticed that lipid oxidation leads to loss of colour, rancidity and accumulation of volatile compounds (e.g. aldehydes, ketones) with negative implication for human health [4,5]. Spoilage and pathogenic microorganisms induce numerous problems in food [6]. The spoilage microorganisms could produce slime and gas, affects to discoloration, off-odours and off-flavours. Moreover, pathogenic bacteria are mainly responsible for foodborne diseases and food poisoning via meat and meat products. Hence, lipid oxidation and microbiological deterioration of meat and meat products can be marked as major limitations in the modern meat industry [7].

The usage of food additives is one the best solution in order to slow down the quality deterioration of and prolong their shelf lives of DFS. Sodium and potassium nitrite are the most common food additives used in DFS processing, mainly as an inhibitor of pathogenic bacteria (e.g. Clostridium botulinum and Listeria monocytogenes). Additionally, these additives possess a strong antioxidative potential and contribute to development of typical reddish-pink colour and flavour in DFS [8]. However, these additives are deemed as unhealthy due to potential formation of nitrosamines, compounds with strong
totoxic and carcinogenic potential. It is well known that nitrosamines could be shaped as a consequence of interaction among nitrites and secondary amines [8-10].

Subsequently, the interest of many scientists is motivated on discovery substitutes for these additives from natural resources [11-20]. It is noteworthy that several studies suggested different plant extracts, including essential oils (EOs), which could contribute to customers’ health status and might even have stronger antioxidant and antimicrobial capacity than synthetic antioxidants [21]. EOs are defined as volatile oils with high percentage of terpenoids with strong antioxidative and antimicrobial potential [21].

In addition, several studies showed that EOs isolated from diverse plants and vegetables could be used as novel antioxidants and antimicrobials in meat processing [2,4,7, 9-11].

2. Essential oils (EOs)
EOs contain complex mixtures of terpenoid compounds, which can be present in different parts of herbs, (e.g. waxy channels, glands and trichomes). These compounds present very complex mixtures of organic compounds such as terpenoids, aldehydes, esters, ketones, acids and alcohols, where the major constituents commonly compose up to 85% of the EO, while the minor compounds and trace elements constitute up to 15%. The chemical status of EOs highly depends on several factors: climate, geographical origin, plant cultivar, development stage, cultivation conditions, post-harvest processing of plant material and applied technique for their isolation [22].

Terpenoids are the most important class of organic compounds present in the EOs. They are recognized as isoprene (5 carbon atoms) oligomers and depending on the number of carbon atoms they can be classified as monoterpenes (10 carbon atoms), sesquiterpenes (15 carbon atoms) and diterpenes (20 carbon atoms). Terpenoids with more than 25 carbon atoms (sesterpenes, triterpenes and tetraterpenes) are mainly present in fatty oils rather than EOs. Terpenoids from EOs could also have different degrees of oxidation and they can be divided in hydrocarbon and oxygenated compounds, while nitrogen and sulphur are rarely present in EO constituents [22].

Importantly, terpenoids possess specific physico-chemical properties in terms of odour, polarity, volatility and bioactivity which directly reflect the approach for their isolation, purification and further application mainly in food, cosmetics and pharmaceutical sectors. EOs are commonly screened for their in vitro antibacterial and antifungal activity [21]. This suggests their potential application as direct natural antimicrobials for human and animal pathogens. This effect also ensures that EOs might be used as additives in foods and cosmetics and could be used as alternative for synthetic preservatives. The specific odour of EOs is responsible for their wide use in the perfume and fragrance industry, and for their ability to improve aromas in food, beverages or drugs [6,9,10]. EOs are often recognized as potent antioxidants, which additionally enables their utilization as natural additives together with their antimicrobial effects. Large variations in their chemical profiles further influence their bioactivity, and it has been reported that certain EOs might exhibit antiparasitic, allelopathic, anticarcinogenic, anti-obesity, immunomodulatory, anti-inflammatory, and antispasmodic effects along with some other biological activities [21,22].

Since various factors can strongly affect the chemical profile of EOs, which further alters their bioactivity, standardization is one of the main issues with these natural products, and their legislation is of great importance. EOs are permitted for use in food packaging in the EU, while EOs are also listed as flavouring ingredients by the Food and Drug Administration and have generally marked as safe in the United States [21,22].

2.1. Essential oil extraction technique
EOs and plant extracts could be isolated using diverse extraction techniques. Generally, there are two types of extraction techniques: conventional (e.g. Soxhlet - SX, hydrodistillation - HD) and emerging (e.g. supercritical fluid extraction - SFE, microwave-assisted - MA, ultrasound-assisted – UA, subcritical
water - SW extraction and high-pressure assisted extractions - HPAE). HD and SFE have been commonly used for lipophilic fractions, while diverse emerging techniques such as MA, UA and SW extraction have been used for polyphenolic fractions recovery [20-22].

It is well known that SX with organic solvents and HD extraction techniques have some disadvantages. High temperatures during process of HD affect to degradation of thermosensitive compounds, which outcomes in difference of the chemical shape. Moreover, HD demands huge energy consumption. The high toxicity of solvent residue and its poor selectivity are the main drawbacks of SX technique. Consequently, new trends of “green” chemistry demand application of “green” extraction techniques. Moreover, it has been highlighted that green extraction techniques, such as SFE provided some advantages in relation to chemical shape and selectivity over conventional extraction [5,20-22].

2.1.1. Supercritical fluid extraction (SFE)

SFE is a progressive and environmentally friendly alternative to conventional solid-liquid or liquid-liquid solvent extraction both for analytical sample preparation and for production scale applications. In this technique a clean solvent such as carbon dioxide is used instead of toxic organic solvents. The advantages of SFE are the opportunity of tuning the solvent power of the fluid by changes in pressure and temperature, while modifying, at the same time, other physico-chemical properties such as diffusivity, viscosity and density. In general, transport properties are favoured under supercritical conditions, and therefore, extraction processes are faster and provide higher extraction yields [20-22].

Yousefi et al. [25] suggested the resulting SFEs are clean and pure, of high quality, and have a great similarity to the aroma of the original plant before the extraction process. The temperatures used in SFE (around 35 °C) allow it to be used for thermally sensitive compounds, maintaining the quality of the final product.

3. Application of EOs in DFS

Recently, many scientific researchers observed the usage of EOs as emerging antioxidants and antimicrobials in DFS [7, 9, 26-33]. EOs were isolated from different pharmaceutical plants and spices (e.g. basil, caraway, juniper, oregano, sage, thyme) and applied as emerging additives in order to extend the shelf-life and improve quality of DFS [7-9].

Basil (Ocimum basilicum L.) is a plant belonging to the Lamiaceae family [26]. This plant contains a relative high content of terpenoid and phenolic compounds (linalool, methylchavicol, eugenol and/or methyl cinnamate) with strong antibacterial potential [26,27]. Hence, the usage of basil and its EO has a significant potential in meat industry [26,27]. Rattanachaikunsopon and Phumkhachorn [26] added basil essential oil (BEO) in order to decline the growth of Salmonella Enteritidis strain in fermented sausages. The results of this study suggested that the usage of BEO at 50 ppm efficiently decreased the population of a clinical Salmonella Enteritidis strain in fermented sausages after two days of refrigerated storage. Moreover, Gaio et al. [27] reported that usage of BEO in similar levels leads to decreased the population of Staphylococcus aureus in DFS.

Caraway (Carum carvi L.) of the Apiaceae family is one of the oldest aromatic and medicinal plants all over the world [28]. The seeds of caraway contain a more than 40% of EOs, with significant nutraceutical, antioxidant and antimicrobial potential [28-29]. Additionally, numerous researches evaluated the effect of caraway EO (CEO) on the shelf-life and quality of DFS [28-30]. Krkić et al. [29] suggested that CEO in mixture with chitosan biopolymer powerfully enhanced the oxidative stability of DFS during five months of storage. In other studies, strong antibacterial [28] and antifungal [30] activity of CEO in preservation of DFS were established.

Juniper (Juniperus communis L.) and its EO (JEO) are broadly applied in different industries: food, cosmetic and pharmaceutical. Regarding high content terpenoids (e.g. α-pinene, limonene and myrcene), JEO shows a strong antioxidant, antibacterial, antifungal, and anti-inflammatory properties [7]. In our earlier study [7], we showed that JEO (0.01 µL/g) enhanced the colour and retarded lipid oxidation in DFS. Also, the results revealed the important antioxidative activity of JEO, and subsequently, its high potential as a partial substitute for nitrates in DFS [7].
Oregano (Origanum vulgare) EO (OEO), comprising two significant active terpenoid compounds (thymol and carvacrol), had antioxidative effects in different meat products [31]. Sage (Salvia officinalis L.) and its EO (SEO) are broadly applied in different food processing, including novel meat industry [10, 32], regarding relatively high content of terpenoid compounds (e.g. eucalyptol, α-thujone and camphor) with significant antibacterial and antioxidant activity. Šojić et al. [10] detected that SEO powerfully decreased the level of lipid oxidation and the inhibited mesophilic bacteria growth in DFS stored at 15 °C for 225 days. Also, the results of aforementioned study showed that SEO (0.05 µL/g) could be applied as effective replacement for sodium nitrite in DFS.

Wild thyme (Thymus serpyllum L.) EO (TEO) established the presence of various bioactive compounds (e.g. flavonoids, tannins, terpenoids and other compounds) with strong antimicrobial, antioxidant, antitumor, hypoglycaemic and haematological effects [11, 33]. Huang et al. [33] investigated the application of TEO in DFS produced using horse meat. The results suggested that TEO can be considered as a natural additive in DFS production since it inhibits growth of pathogenic bacteria and accumulation of biogenic amines, and this improves the quality of DFS [33].

4. Conclusions

The results present in this review show that the use of EOs obtained from different plants (basil, caraway, juniper, oregano, sage, thyme) can extend the shelf life of DFS, delaying the deterioration (lipid oxidation and microbiological growth) and enhanced the quality of final products. Also, EOs contain different bioactive compounds with strong health benefit for human. Conventional extraction techniques affect to degradation of thermosensitive compounds and have negative ecological impact. Hence, the need to find more sustainable, ecological and viable extraction processes has led researchers to develop alternative techniques permitting to the “green” extraction concept, included SFE, UA, MW and HPAE. Additionally, these techniques allow the valorization of the by-products obtained from agri-food industry, underwriting to the reducing the environmental impact of food production. Along with green” extraction concept, the recommendations of health international organizations to decrease the usage of synthetic additives (e.g. nitrites) are becoming gradually important, forcing meat industry to reformulate meat products to obtain healthier products. Therefore, the combination of these strategies is important to align the production of healthy meat products with sustainable actions. Finally, prior to addition in meat products, it is also essential to determine the optimal level and evaluate the toxicity of EOs, as well as their bioavailability in the human body.

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