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Preservation of meat and meat products using nanoencapsulated thyme and oregano essential oils

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Abstract. Among other plants, thyme and oregano are commonly used in Mediterranean cuisine, especially in meat dishes. Although the essential oils of these two plants possess great antimicrobial and antioxidative properties, their application as natural meat preservatives are limited due to hydrophobicity, sensitivity to external factors and interaction with food components. Furthermore, essential oils can have adverse impacts on meat's organoleptic properties. A possible way to overcome these barriers is by incorporating essential oils into nanometric delivery systems. Nano-sizing essential oils increases their stability, protects them, and allows their controlled release. This enhances the bioavailability of the essential oils and reduces their possible adverse impact on meat products' organoleptic properties by preventing their unwanted interactions with food components. The antibacterial and antioxidative effect of nanoencapsulated essential oils is confirmed in numerous studies, and some of them show that in this form, essential oils were potent in food models e.g. beef burgers, pâté and rainbow trout. However, a more promising way to introduce nano forms of essential oils into foods is incorporating them in packaging systems.

1. Introduction

Microbial contamination and lipid oxidation are the main causes of meat deterioration. Moreover, meat and meat products are common sources of foodborne pathogens and the consumption of contaminated products are frequently linked to *Salmonella*, *Campylobacter*, *Listeria*, *Escherichia coli* and *Staphylococcus aureus* outbreaks [1, 2]. Another safety issue for meat production is the use of synthetic additives, which can have potential toxic and negative side effects [3]. Thus, the meat industry has to meet safety, hygienic and quality criteria as well as consumers demand for healthy, natural and minimally processed food.

Among other herbs, oregano and thyme belonging to the *Lamiaceae* family are the most commonly consumed, almost essential components of Mediterranean cuisine [4, 5]. Essential oils (EOs) obtained from these plants are of great interest in the food industry as in the other fields, due to their significant antimicrobial and antioxidative activities [6]. Moreover, these EOs and their main constituents, the phenolic compounds carvacrol and thymol, do not have a mutagenic effect on human lymphocytes [6, 7], and are categorized as generally recognized as safe (GRAS), which makes them suitable candidates for food preservation.

However, application of EOs in their free (original) form is limited because of their hydrophobicity, which prevents them from dissolving in food's aqueous phase, and their volatility, causing losses during food processing and consequently leading to increased cost of production processes [8]. Interaction with meat components can reduce or completely inhibit the activity of EOs



[9]. Phenolic compounds can attach to food proteins, decreasing the amount of phenolics available for bacterial growth inhibition [10]. EOs are affected by numerous factors including pH, water activity, enzymes, temperature, relative humidity, and storage conditions [11]. Furthermore, some EOs have negative effects on sensory properties of food products, depending on the main compounds of the EO, concentration, and the type of food [12].

Nanoencapsulating EOs is one way to overcome these barriers and allow EOs to exhibit their full preservative potential in food [11]. Nanoencapsulation protects the EOs against unfavorable conditions during processing, storage, and transport [13, 14]. The advantages of nanoencapsulated EOs are their stability and controlled release, which enhances their bioavailability and reduces possible adverse impact on meat products' organoleptic properties [11, 15].

2. Nanoencapsulation

Nanoencapsulation is a new technology and refers to coating the active agent (in this case, EO) within another material at sizes on the nano scale [16]. Materials for nanoencapsulation need to meet a wide range of requirements to be approved by the food sector [17]. All coating materials used for this purpose should be categorized as GRAS [18], should protect the EO and preserve its activity, be nonreactive with the EO or with food components and be biodegradable. The coating materials are mainly polysaccharides of plant origin such as starch and cellulose and their derivatives, different types of gums, galactomannans, pectins, soluble soybean polysaccharides, and polysaccharides of microbial and animal origin including dextran, chitosan, xanthan, and gellan. Moreover, some lipids (fatty acids, fatty alcohols, waxes, glycerides, and phospholipids) and proteins (whey proteins, caseins, gelatin, gluten, zein, albumin, globulin, and silk fibroin) can be used for this purpose [11, 17, 19].

In general, nanoencapsulation techniques use two main approaches: top-down and bottom-up [20]. Top-down approaches induce particle size decreases during the encapsulation process and are high-energy, while bottom-up approaches are low-energy and refer to construction of nano-sized materials *via* self-assembly and self-organization [20, 21]. Although only some nanoencapsulation methods can be used to encapsulate hydrophilic substances, all methods can be used for encapsulating lipophilic compounds (i.e. EOs) [17, 22].

The techniques used for nanoencapsulating EOs can be also classified as producing lipid-based nanoparticles or polymer-based nanoparticles. EOs are lipophilic compounds and, therefore, lipid-based systems mostly produce nanoscale EOs for food preservation purposes [11]. Lipid-based nanoparticles are nanoemulsions, nanoliposomes, solid lipid nanoparticles (SLN), or nanostructured lipid carriers (NLC) [17].

Nanoemulsions are liquid-in-liquid dispersions with sizes in the order of 100 nm [23]. There are two possible means of nanoemulsion preparation: top-down approaches which include high pressure homogenization, micro fluidization, or ultrasonication and bottom-up approaches such as phase inversion and spontaneous emulsification methods [20].

Nanoliposomes, spherical lipid vesicles with an aqueous core and amphiphilic lipid bilayer [24], are another form of nanoscale delivery system for EOs. The advantage of nanoliposomes is that they easily fuse with bacterial membranes and enter the bacterial cell [20], while their main disadvantage is their stability. However, coating nanoliposomes improves their stability, thereby prolonging their half-life [20, 25].

Hot homogenization and cold homogenization are techniques used to prepared SLNs and NLCs [17]. As the name suggests, SLNs are made from solid lipids such as fatty acids, triglycerides, steroids, partial glycerides, and waxes [24].

Polymeric nanoparticles are classified as nanocapsules and nanospheres. Nanocapsules consist of a polymeric shell, a core(s) and active agents, in this case EOs, which can be placed inside the core or adsorbed on the surface, producing a reservoir-type nanomaterial. Nanospheres are matrix-type systems in which the EO is homogeneously dispersed in the structure [17]. Moreover, reservoir-type and matrix-type encapsulations are classified according to the mechanism by which the active agent is released. While the reservoir-type has a capsule surrounding the active agent, which is placed in one or

multiple cores, in the matrix-type, the active agent is dispersed in the polymer phase or carrier material and it is also present at the surface. Release of the encapsulated active agent from the reservoir-type is due to pressure, whereas in the matrix-type, the active agent is released *via* diffusion of active agents or erosion of the matrix. In both types of nanomaterial, release is influenced by solubility, diffusion, biodegradation of the shell and matrix materials and the size of encapsulate [17, 26, 27].

3. Antibacterial mechanisms of free form and nanoencapsulated EOs

The antibacterial effect of free form EOs is attributed to several mechanisms. EOs interact with lipids in microbial cell and mitochondrial membranes, increase cell permeability, change membrane potential, cause ion loss and collapse of the proton pump, and disturb microbial metabolism leading to lysis and microbial death [12, 28, 29]. However, the exact mode of action of nanoencapsulated EOs is still not completely elucidated. It is supposed that nanoencapsulation enhances EO activity due to the reduced size, allowing nano-EOs to interact more efficiently with cell membranes [8] by increasing the surface area per unit of mass [29]. Consequently, lower doses of EOs can be used [30]. Apart from the active agent, some carriers used in nanomaterial production also possess antimicrobial activity, change membrane potential, generate reactive oxygen species and affect microbial metabolism [31]. The antimicrobial activity of thyme EO (TEO) and oregano EO (OEO) is attributed to their main phenolic components, thymol and carvacrol, but it is supposed that a synergistic effect is achieved with minor components, including the monoterpene hydrocarbons *p*-cymene and γ -terpinene [6]. Furthermore, some authors suggest that the nano-EOs could act synergistically with the carrier used for nanoencapsulation. Materials used for encapsulation protect EOs from reacting with the food matrix and transfer nano-EOs to specific targeted sites like water-rich phases [11].

4. Practical application

Numerous studies reported *in vitro* antibacterial and antioxidative effect of nanoencapsulated TEO and OEO and their predominant components, thymol and carvacrol.

Sotelo-Boyás *et al.* [32] reported that nano-TEO, composed mainly of thymol and carvacrol encapsulated in chitosan and with an average size of 9.1 nm, exhibited activity against *S. aureus*, *L. monocytogenes*, *Bacillus cereus*, *Salmonella* Typhi, *Shigella dysenteriae* and *E. coli*. However, the highest inhibitory activity was observed against *B. cereus* (inhibition halo 1.9 cm) for 40 μ L of MIV. Furthermore, Moghimi *et al.* [33] investigated the antibacterial effect of *Thymus daenensis* EO in both free and nanoemulsion forms by measuring the minimum inhibitory concentration (MIC) and minimum bactericidal concentration. The antibacterial activity of the TEO against *E. coli* was significantly greater when it was converted into a nanoemulsion.

However, there are very few data available in the literature about the effect of TEO and OEO and/or their nanofoms in the meat and meat products. Thyme EO-loaded chitosan nanoparticles were prepared by a two-step process including oil/water emulsion and ionic gelation and their effect on microbiological, chemical quality and organoleptic properties of beef burgers during 8 days of storage was studied [34]. Nanoencapsulated TEO added at concentrations of 0.1% and 0.05% significantly inhibited microbial growth. The burgers with added nanoencapsulated TEO had lower counts of *Enterobacteriaceae*, *S. aureus*, total mesophilic viable counts, and yeasts and molds than control burgers and burgers with free form TEO. Addition of nanoencapsulated TEO resulted in lower concentrations of thiobarbituric acid reactive substances (TBARS) in the burgers. The burgers containing nanoencapsulated TEO had overall acceptability scores similar to those containing free form TEO and control burgers. Based on the results of this study, encapsulation of TEO in chitosan nanoparticles could potentially control microbial and chemical alterations in the burgers and did not have the strong odor of free form EO, thus improving the sensory quality of the meat products [34].

Similarly, Moraes-Lovison *et al.* [35] reported that nanoemulsions encapsulating OEO could be incorporated into pâtés to prolong their shelf life. MIC and minimum bactericidal concentration (MBC) of OEO nanoemulsions with average droplet diameters of 35 to 55 nm were assessed against *S.*

aureus and *E. coli*. MIC and MBC did not differ significantly during 90 days of storage under refrigeration conditions, indicating the long storage period did not affect stability and antibacterial properties of the nanoemulsions. When added into chicken pâté and stored for 45 days, numbers of *E. coli* and *S. aureus* were reduced. While the antibacterial effect of nano-OEO against *E. coli* was greater than reported for free form OEO, the effect against *S. aureus* was the same for both nanoencapsulated and free form OEO. The incorporation of nanoemulsions in chicken pâté did not change the physicochemical characteristics of the meat product [35].

Moreover, EO nanoemulsions can be used as a preservative for fish meat. Ozogul *et al.* [36] examined the effects of various EO nanoemulsions on microbiological and chemical quality and sensory attributes of rainbow trout (*Oncorhynchus mykiss*) fillets during 24 days storage at 2 °C. TBARS levels were higher in the control than in the fish with nano-TEO, indicating TEO nanoemulsion protected the fish against oxidation. Among the EO nanoemulsions examined, rosemary and thyme nano-EOs slowed the growth of mesophilic bacteria, psychotropic bacteria and *Enterobacteriaceae*. Nano-TEO's main components were carvacrol (71.54%) and *p*-cymene (11.84%), and the nanomaterial extended the shelf life of rainbow trout by approximately 3 days.

Incorporating nano-EOs in packaging systems is a more promising way to introduce nanoencapsulated EOs and their active compounds into food [37]. Moreover, edible films containing active agents such as EOs could be considered as an alternative form of food packaging [30]. Edible films obtained from alginate-based nanoemulsions loaded with TEO exhibited strong antibacterial effects against *E. coli* and reduced the count of these bacteria by 4.71 logs within 12 h [30].

Good hygiene of food contact surfaces in the food chain can help prevent the occurrence of foodborne outbreaks of disease. Microbial adhesion to food contact surfaces and biofilm formation are the main sources of cross-contamination of food [38, 39]. Engel *et al.* [40] examined the effect of 1 and 10 min contact times of liposome-encapsulated thymol and carvacrol with average particle diameter of 270 nm against cocktails of four different strains of *Salmonella* and *S. aureus* adhered to stainless steel. Free form thymol or carvacrol reduced *S. aureus* and *Salmonella* to levels below the limit of detection after 1 min. Thymol and carvacrol nanoliposomes produced similar effects to the free form compounds after 10 minutes. The longer time needed for thymol and carvacrol nanoliposomes to produce antibacterial effects reflects the gradual release of these active agents from the nanoliposomes.

5. Precautionary and research points regarding nano-EOs

Using nano-EOs as preservative agents in meats has numerous advantages over using free form EOs, especially the enhanced antimicrobial activity of nano-EOs and their limited effects on the sensory properties of meat. However, there is a lack of data on the effects of nanomaterials on mechanisms of absorption, metabolism, and human health, while the environmental impact of nano-sized particles remains an issue of great concern. Thus, further studies on the safety of nanomaterials are needed before incorporating nanoencapsulated EOs as natural preservatives in food.

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