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Nanoemulsions based delivery system of antimicrobial essential oils in meat and meat products

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Abstract
The globalisation of food trade and increasing demand of minimally processed food, ready to eat meat products has posed a serious threat to food safety and quality. Natural Antimicrobial agents including essential oils (Eos) have been used as food preservative to inhibit food borne bacteria and extend the shelf life of processed food particularly meat (Yin and Cheng, 2003). Essential oils have potential antimicrobial efficacy and have been used in variety of meat based products. But their direct incorporation in meat products results in immediate and short term reduction of bacterial populations due to destruction of active principle. Also, it possesses strong flavour which results in poor organoleptic acceptability which is not required. So emulsion based delivery system of these essential oils is an alternative way of adding essential oil in meat and meat products.

Keywords: Nanoemulsion, TVC, ultrasonication, essential oil, antimicrobial, antioxidant

Introduction
Meat and meat products are highly vulnerable to microbial contamination and spoilage due to their nutritional content and perishability. This is further enhanced by other intrinsic factors including higher water activity and pH of fresh meat. In general, most raw and fresh meat has a water activity value higher than 0.85 and its pH value falls within the favourable pH range for spoilage microorganism of meat (Dave & Ghaly, 2011) [22]. Hence, deterioration in quality of meat and meat products impose serious public health risk if these products are not properly handled and preserved (Fratianni *et al*., 2010; Solomakos *et al*., 2008) [27, 66].
A significant level of deterioration of quality of fresh meat and meat products takes place every year at different levels of the supply chain including the preparation, storage, transportation and distribution. Along with this fat oxidation, autolytic enzymatic spoilage and microbial spoilage plays a significant role in this deterioration process and leads to considerable financial and Health loss (Dave & Ghaly, 2011) [22].
Refrigeration is the most common method which is used to increase the shelf life of fresh meat and meat products (Solomakos *et al*., 2008) [66] along with this many synthetic preservative are being widely used over the past years. Synthetic preservatives contains carcinogenic and toxicogenic properties. This increased the consumer awareness toward healthier and safer meat and meat products and hence the demand of natural food preservatives has been increased recently (Mariutti *et al*., 2011).These natural additives or preservatives are supposed to enhance meat quality without leaving any harmful residues in the meat and meat product (Bhavaniramya *et al*., 2019; Pinelli *et al*., 2021) [11, 56].
Essential oils are presumed to have potent antimicrobial and antioxidant properties. Therefore, EOs can be used as part of a hurdle system to attain preservative action in meat and meat products. (Mastromatteo *et al*., 2009) [45]. Threshold levels of essential oils having significant amount of antioxidant and antimicrobial properties can be combined along with existing and novel preservation technologies including refrigeration temperature and acidity (Al-Reza *et al*., 2010; Skandamis & Nychas, 2001) [4, 65], modified atmosphere packaging (MAP; Marino *et al*., 1999) [43], high hydrostatic pressure (Devlieghere *et al*., 2004) [23], and low-dose irradiation (Chouliara *et al*., 2005) [18].
Emulsification of essential oils is the new innovative approach of addition of essential oils into meat and meat products. Food emulsions are manufactured by homogenization of oil and aqueous phase together by various techniques. (Kim *et al*., 2006) [36]. Essential oil emulsions are thermodynamically unstable systems but can be made kinetically stable by use of suitable stabilizers or emulsifiers (Guzey and McClements, 2007) [30].

Emulsion based delivery has been adopted for various food components like fatty acids (Averina and Allemann, 2013) [6], vitamins (Chen *et al*., 2006) [17] and phenolic compounds for variety of food systems. Very limited work has been done in area of meat science therefore it is a new area of extension of shelf life of meat products.

Generally, an emulsion is made up of at least two immiscible liquids (usually oil and water, with one of the liquids being dispersed in the other with variable droplet size. In general, the mean droplets size within food emulsions is somewhere between 100nm and 100μm, but in some systems the droplet size may be either higher or smaller than these values. Emulsions can be classified as per spatial distribution of the oil and water phases.

A system that consists of oil as dispersed phase and water as dispersing phase is called an oil-in-water or o/w emulsion, whereas an emulsion in which water droplets dispersed in an oil phase is called a water-in-oil or w/o emulsion. The material inside the emulsion droplets is generally referred as thediscontinuous or dispersed phase, whereas the material surrounding it usually referred to as the continuous or dispersing phase.

Recently, nanotechnology or nanoemulsions is the new area of research and investigation by researchers in food industry as Nanoemulsions or nanoparticles possess some distinct characteristics which can be very useful in food systems (Dammak *et al*., 2020) [21].

Essential oil (Eos)

Essential oils (EOs), are generally extracted from aromatic and medicinal plant materials, including flowers, buds, roots, bark, and leaves by means of extraction, fermentation, and steam distillation (Burt, 2004) [12]. Essential oils are volatile and oily extract and are quite popular in flavour and fragrance markets. Due to their important biological properties and flavour characteristics, these essential oils have been used for centuries in food products. Essential oils from lemongrass, cinnamon, oregano, lime, thyme, rosemary, has been widely used in many meat and meat products as potent antimicrobial and antioxidant agents by many researchers.

Besides antibacterial properties (Song *et al.*, 2021) [67], EOs have been shown to exhibit antiviral (Duschatzky *et al*., 2005) [25], antimycotic (Pawar and Thaker, 2006) [54], antitoxigenic (Ultee and Smid, 2001) [70], antiparasitic (Moon *et al*., 2006) [48], and insecticidal (Chaiyasit *et al*., 2006) [14] properties. It has been stated that that the biological properties (antimicrobial & antioxidant properties) of a particular EO are predominantly related to its major compounds (Bakkali *et al.*, 2008) [7]. In addition, minor compounds of a particular EO may possess synergistic effects with other components resulting in antibacterial properties (Burt, 2004; Marino *et al.*, 2001) [12, 41]. In addition, Eos and oleoresins are chosen over crude spices in meat and meat products because of microbial safety, better stability during storage, low concentration requirement due to high concentration of flavor components, ease of handling, and standardization (Tipsrisukond *et al.*, 1998) [69].

Need of encapsulation emulsification of Essential oils

Meat structure is heterogeneous and complex system and can influence the interaction of essential oil with its targets (Gutierrez *et al*., 2008) [29]. Various bioactive constituents of EO can interact with muscle or meat proteins through various electrostatic or hydrophobic forces and can lead to reduced bioactivity (Weiss *et al*., 2015) [72]. The pH of the meat matrix can also the interactions of EO constituents. Along with this, essential oils possess strong organoleptic properties and can change the sensory properties of meat so they can not added directly into the meat and meat products (Hyldgaard *et al*., 2012) [32]. Along with this active bioactive components present in essential oils are unstable compounds and are prone to oxidation and volatilization (Hyldgaard, Mygind, & Meyer, 2012) [32] can react with other food components. (Hyldgaard *et al*., 2012; Prakash *et al*., 2018) [32, 58]. Encapsulation is a viable alternative to enhance the stability of these bioactive compounds. Encapsulation reduces the strong flavour of these essential oils which will minimally effect the qualitative and sensory properties of food. Essential oils are less soluble in water, so essential oils need to be encapsulated in a suitable delivery system like emulsions so that there activity can be assured in those areas of food where microorganism generally grow and proliferate I.e. water rich phase and solid liquid interface (Weiss *et al*., 2009) [72]. Application of Nanotechnology has emerged as possible solution of adding essential oil in meat matrices by preparation of Nanoemulsions as they have better and distinct properties as compared to conventional emulsions (Salvia-Trujillo *et al.*, 2017) [63].

Characteristics and advantages of Nanoemulsions

Size of droplets in nano emulsions varies from 20 to 200nm where properties of nano emulsions (physiochemical and biological) depends on the droplet size, zeta potential, poly dispersibility index and aggregation state of emulsion (Barradas & Silva, 2020) [9].

With the decrease in droplet size physiochemical properties of emulsion changes, which differentiate them from macroemulsions as Nano emulsions are more optically clear, kinetically stable and chemically reactive system as compared to conventional emulsion. (Pratap-Singh *et al*., 2021; Sheth *et al.*, 2020) [59, 64].

Nanoemulsions are optically transparent and minimally changes the sensory quality of foods, making them more useful for application in foods. (Dammak *et al*., 2020; McClements *et al*., 2021) [21, 47]. Nanoemulsion have greater antimicrobial and antioxidant potential due to reduction in droplet size and increased surface area, which facilitate the transport of bioactive constituent across the microbial cell membrane (Moraes-Lovison *et al.*, 2017) [49], and also favours the interaction of active constituents with free radicals and reactive oxygen species (Balasubramani *et al.*, 2017) [8].

Methods of Preparation of nanoemulsions

Different emulsification techniques used to prepare Nano emulsions are generally categorized as low-energy or high-energy approaches. Frequently used low-energy emulsification techniques phase inversion temperature (PIT) method, emulsion inversion point (EIP) method and spontaneous emulsification. These techniques facilitate the spontaneous formation of Nanoemulsions and are dependent on composition and surrounding environment of the emulsion system. High-energy techniques are high pressure homogenization, microfluidization andultrasonication.These methods rely on mechanical devices for the generation of intense disruptive forces needed for the breakdown of macroscopic phases.

High energy methods rely on supplying mechanical energy to produce disruptive forces enable to reduce the size of dispersed phase droplets at the oil-water interface (Karthik *et al*., 2017) [33]. Basically two steps are involved in production of Nanoemulsions by these methods obtaining nanoemulsions by these methods. Firstly, coarse emulsions (500–1000 nm) are prepared with the help of homogenisers such as Ultraturrax® by mixing the aqueous phase, oil, and surfactant (Ghani *et al*., 2018; Keykhosravy *et al*., 2020) [28, 35]. Then, further droplet size is reduced by application of high-intensity mechanical energy equipment such as high-pressure homogenizers, ultrasound, or microfluidizers (Barradas & Silva, 2020) [9].

Ultrasound (US) based preparation methods involve generating ultrasonic waves from a device with a rod or piston that oscillates at high frequencies in the range of 20–40 kHz (Oca-A´valos *et al*., 2017; Rosario *et al*., 2020) [52, 62]. Cavitation process governs the formation of Nano emulsions in such a way that rapid formation and collapse of micro-bubbles occurs at the interface of two immiscible (continuous and dispersed) phases under the influence of high-intensity acoustic field (Mason *et al*., 2006) [44], and the implosions due to collapsing bubbles result in an intense wave which result in the rupture of oil and water interface and hence size of droplets reduces to nano scale (Barradas and Silva, 2020; Donsì and Ferrari, 2016) [9, 24].

Factors effecting Stability of Nanoemulsions

Emulsions are thermodynamically unstable systems which have tendency to break down over time due to various of physicochemical mechanisms, like gravitational separation, flocculation, coalescence, and Ostwald ripening (Sheth *et al*., 2020; McClements 2005a) [64, 5]. Gravitational seperation is the most common phenomenon for instability in food emulsions, and it occurs either in the form of sedimentation or creaming depends on relative densities of dispersed and continuous phases. Creaming occurs when the droplets moves upward due to relatively lower density and in case of sedimentation movement is downward due to higher density. Generally, creaming and sedimentation is more prevalent in case of oil-in-water and water-in-oil emulsions respectively (Helgeson, 2016; McClements *et al*., 2021) [31, 47]. Droplet concentration in emulsion effect the gravitational separation, higher the droplet concentration less will be the gravitational separation due to resistance of movement by surrounding droplets.

Flocculation and coalescence are another type of instability in emulsions where droplets get aggregated, to form a larger droplet and the nanoemulsion with high Polydispersity index are more prone to these phenomenon due to heterogeneity in suspended droplet size (Karthik *et al*., 2017) [33].

Ostwald ripening is an instability phenomenon in the emulsions in which due to high Polydispersibility index, there is difference in interfacial energy of the droplets and the smaller droplets tends to move in the larger droplets through the continuous phase (Nakashima *et al*., 2021; Zwicker *et al*., 2015) [50, 75]. The increase in PDI favors Ostwald ripening phenomenon. The Polydispersity index (PDI) value shows the uniformity in droplet size distribution in the emulsion and can be measured using light scattering analysis.

Stability of Nanoemulsions is very important and it need to be stable during storage, transport, and processing conditions. Droplet size, PDI, and zeta potential of Nanoemulsions are very important parameters for stability of Nanoemulsions. PDI values ranges from 0.10 to 0.25 shows uniform particle size distribution and above 0.50 shows heterogeneity or broader size distribution.

Zeta potential of the Nanoemulsions depends on type of surfactant, pH and ionic strength, of the solution (Cardoso-Ugarte *et al*., 2016) [13]. Zeta potential values greater than +30 mV/- 30 mV, provide stability to the Nanoemulsions through electrostatic repulsion and prevent aggregation of the droplets (Ferreira and Nunes, 2019; Liu & Liu, 2020) [266]. The addition of non ionic surfactants into the emulsion make bulky interfacial film around the droplets capable of providing steric repulsion and in result provide stability to the emulsion (Helgeson, 2016) [31].

Use of essential oil Nanoemulsions in meat and meat Products

Microbial contamination in meat are commonly caused by various pathogens like *Salmonella enterica*, *Campylobacter jejuni*, *Staphylococcus aureus*, and *Escherichia coli*,*Listeria monocytogenes* and *Clostridium botulinum* with variable incidence and source of contamination. mycotoxins can also produced into meat products with high salt content from the fungal contamination with genus *Aspergillus* and *Penicillium* (Perrone *et al*., 2019; Pizzolato *et al*., 2018) [55, 57]. Food born disease can be caused by microbial and fungal contamination which can cause potential public health hazard and these pathogens can enter into meat at processing and supply chain (Lianou *et al*., 2017) [37].

Oxidative changes can also occurs in meat during storage which can include off flavour development, colour changes, deterioration of sensory quality and the nutritional value (Coombs *et al*., 2018; Cunha *et al*., 2018; Pateiro *et al*., 2018) [19, 20, 53]. Oxidative changes in the meat and meat products occurs due to free radical development during storage.

Bioactivity (antimicrobial and antioxidant potential) of essential oils depends on its constituents and the activity may vary due to source of extraction, climate and storage conditions. (Rahim-malek *et al*., 2017) [60]. Constituents may act as synergistically or antagonistically depends on their interaction with each other (Raut and Karuppayil, 2014) [61].

Sun *et al* (2021) [68] investigated effect of Foeniculum vulgare nano emulsion in pork patties and compared the effect with pure oil at the same concentration and found that by the use of Nanoemulsions shelf life of pork patties get increased from 6 to 10 days with lower TVC, yeast and mould count.

Kazemeini *et al* (2021) [34] studied the effect of Trachyspermum ammi on turkey fillets and found that alginate and Nanoemulsions coated fillets have significantly lower *L. monocytogenes* counts as compared to the control samples (1.99 lo CFU/g). NEO also showed more antimicrobial activity than conventional emulsions at the same concentrations, with differences of 0.86 log CFU/g.

Abbasi *et al*. (2020) [1] observed that starch coating containing Zataria multiflora essential oil Nanoemulsions over chicken meat has better antimicrobial activity at the same concentration against pure oil during 20 days storage period at refrigeration temperature.

Xiong *et al*. (2020) [73] studied the effect of *Origanum vulgare*in pork loin muscle and found the sample with essential oil Nanoemulsions have significantly lower values of TVC as compare to conventional emulsion of same essential oil.

Noori *et al*. (2018) [51] investigated the effect of Zingiber officinale in chicken breast fillets and found that the Nanoemulsions coated samples were having significantly lower psychrotrophic bacteria, molds, and yeasts count as compare to control and conventional emulsion coated samples after 12 days of storage at refrigeration temperature.

Moraes-Lovison *et al* (2017) [49] studied the effect Origanum vulgare Nanoemulsions over pure oil in chicken patties over 8 days of storage period and found that the Nanoemulsions was quite effective in inhibiting the E. coli concentration over pure oil.

Abdou *et al*. (2018) [2] uses Allium sativum Nanoemulsions in coated Chicken fillets and observed lower TBARS value as compared to control samples during 12 days of storage period at refrigeration temperature.

Amiri *et al*. (2019) [5] studied the effect of Zataria multiflora Nanoemulsions in coated ground beef patties and observed lower TBARS values and slower amino acid degradation in case of NEO coated ground beef patties as compared to control and pure oil coated patties after 12 days of storage period at refrigeration temperature.

Limitation of Nanoemulsions to use in foods

Nanotechnology is newer and emerging area of research in food industry, and researchers are viewing it as new technological tool for preservation of foods due to its promising results. The new technology faces social resistance in acceptance, and need to cross regulatory and technological barrier especially when applied in foods (Chaudhry *et al.*, 2017) [15]. Essential oils are chemically diversified and still standardisation of acceptable daily and intake and safety levels is a challenging issue (Donsì and Ferrari, 2016) [24]. Essential oil consumption can be toxic when taken orally beyond safer limits or consumed in higher concentration than prescribed limits (Bhardwaj *et al.*, 2020) [10]. Maisanaba *et al.* (2017) [39] reviewed the studies conducted on toxicity of essential oils constituents in in-vitro cell death induction in human liver and intestinal cells. It is hypothesised that nanoemulsions made with essential oils due to their particle size can easily be absorbed inside human cells, and can lead to toxicity however the enough research has not been done so far to study the effect of NEO on human cells (Abd-Rabou and Edris, 2021; Marchese *et al*., 2020) [3, 40]. Another possible limitation is the cost of production of Nanoemulsions because high energy approach like high pressure homogenisation and microfluidizer is needed to produce the Nanoemulsions. So currently, these technologies are more used as laboratory scale than at industry scale. Low energy method are unfeasible and require greater amount of surfactants to produce Nanoemulsions (Chemat *et al*., 2017) [16].

Conclusions and future perspectives of nanotechnology

Meat and meat products are highly prone to microbial deterioration and spoilage due to their perishable nature and can potentially lead to safety and quality issues if not properly handled and preserved. Essential oil. Synthetic preservative in meat and meat products can cause potential public health hazards due to their toxicological nature. Plant based natural essential oil can be a natural and healthy alternative to synthetic food preservative due to their potential antimicrobial and antioxidant potential. Nanotechnology has helped to overcome the limitation of low solubility in aqueous phase and volatility of essential oil. Encapsulation through formation of nanoemulsion has helped to mask the strong flavour of essential oils which could potentially effect the sensory qualities of meat and meat products. Addition of nano emulsion in edible coating over meat and meat products has shown promising results in extension of shelf life of meat and meat products without or minimally compromising the sensory score of the products as compared to direct or pure oil. However, many challenges need to be addressed and a detailed studies need to be conducted to examine the stability of nano emulsion, toxicological aspects associated with particle size, and standardisation of safe intake level of essential oils. Interaction of essential oil nanoemulsions with food matrix or meat matrix need to be examined for standardisation of legislation in food application for preservation purpose.so that, essential oil can be commercially used as natural preservative.

Declaration of conflict of interest

The authors declare no conflict of interest.

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