The Pharma Innovation Journal 2021; 10(3): 454-458

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2021; 10(3): 454-458

www.thepharmajournal.com
Received: 19-12-2020
Accepted: 29-01-2021

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Application of nanoemulsion technology for development of novel functional foods with essential oils encapsulation: A review

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Abstract
The demand for shelf-stable and nutritious food products is increasing day by day and consumers are ready to pay a higher price for such food products. Some artificial preservatives and antioxidant agents, such as butyrate hydroxanisole (BHA), tertiary butyl hydroquinone (TBHQ), and butyrate hydroxytoluene (BHT), have been widely used for food preservation, but these are harmful for human health and can cause DNA mutation and carcinogenesis. Now-a-days, the latest researches focus on compounds of natural origin as preservatives of novels that can help in the extension of food shelf life without harmful effects on human health. Spices and aromatic herbs with medicinal value have been traditionally applied in phytotherapy due to the presence of a variety of activities of their secondary biomolecules, particularly carminative, antiviral, antimicrobial, spasmolytic, hepatoprotective, anticarcinogenic, etc. Essential oils and extracts from these herbs have bio-preservation actions and a lot of research has been done for the use of these essential oils and extracts in the food sector. Essential oils are aromatic metabolites derived from plants, such as grasses, buds, flowers, leaves, roots, stems, bark, resins, and its secretions, which have excellent antioxidant and antimicrobial properties. Therefore, essential oils from these traditional herbs and spices have excellent potential in the food industries, but have some limitations in its direct incorporation. Due to their hydrophobic nature, they are unstable in aqueous medium, which poses more challenges for incorporating it into food due to the interaction of essential oil with protein and fat that may reduce its antioxidant and antimicrobial potential. In addition, strong taste with a high concentration for effective microbial inhibition would exceed organoleptic levels of acceptance. Therefore, one of the ways to overcome these deficiencies is to encapsulate essential oils before incorporating them into food matrices. Encapsulation provides isolation, penetration, protection, or controlled release of reactive substances from the surrounding matter. Nanoemulsion delivery systems are known to use a low concentration of essential oils to increase their functionality in food.

Keywords: Nanoemulsion, essential oils, functional foods

Introduction

Safety and quality of food is one of the major concerns worldwide. For this reason, researchers are constantly searching for solutions to prevent food contamination by food borne pathogens [1]. Maximum refrigerated food worldwide becomes spoiled during storage, causing adverse biochemical changes (oxidation rancidity) and deterioration due to multiplication of pathogenic or spoilage microorganisms [2]. The excessive use of artificial additives and preservatives to control spoilage activity in food is causing serious health concerns among consumers [3] and due to this; many synthetic preservatives have been banned in developed countries. Nowadays, consumers have become more aware about health and food stuff. Demand for organic and natural additives is increasing, which can increase nutritional value and protect food from spoilage organisms. Among them, essential oils are emerging as potential candidates.

Nanoemulsion is a thermodynamically unstable colloidal dispersion consisting of two immersed liquids, one of which is dispersed below about 100 nm in the form of small spherical droplets, which have two major critical properties i.e. increased biological activity and stability [4]. Due to the small droplet size, it can enhance the antimicrobial and antioxidant action of natural preservatives. Several nanotechnologies have been employed to create nanoemulsion, including various low-energy and high-energy methods. Ultrasonic emulsification is a high-energy method that is increasingly used and is found to be able to efficiently prepare nanoemulsions with small droplet diameters and narrow size distributions [5, 6].

In addition, nanoemulsions with non-ionic surfactants are safe to use, biocompatible and have high grade stability. It turns out that the application of this technique is very limited in food science and is a new field for developing tasty, shelf-stable and functional food products.

Essential oils

Essential oil is an aromatic concentrated hydrophobic liquid containing volatile chemical compounds. The aromatic liquids are achieved from oil stored in secretary cells of plant oil glands, cavities, canals, glandular trichomes, or epidermis cells and can be retrieved from various plant components like flowers, leaves, berries, stems, grass, flowers, roots, barks, fruits, peels, buds, resins and woods [7]. Generally, essential oils are extracted by steam distillation, solvent extraction, resin tapping, cold pressing of a plant and its components [8]. In majority, they are rich in aromatic and volatiles properties and are frequently used in perfumes, cosmetics, flavoring drinks, food products and pharmaceutical industries because of their good antioxidant and antibacterial activities [9, 10]. The activity of essential oil differs due to their active compounds, type, compositions, concentration of oil, structure of the substrate, storage conditions, and treatment processes [11, 12]. These oils are chemically complex mixture contains around 300 different components [13]. Chemically these volatile compounds are alcohols, amides, phenols, heterocyclic, ethers or oxides, aldehydes, ketones, esters, and mainly the terpenes. Due to presence of such active constituents, these essential oils have several biological properties [14] including antimicrobial [15], antimycotic [16] and antioxidant [17].

Essential oils action mechanism

Essential oils are reported to have biological activities such as broad-spectrum inhibitory action against gram-positive and gram-negative bacteria. Various essential oils derived from various sources including oregano, rosemary, thyme, sage, basil etc. are used alone or in combination with other essential oils or other preservation methods, which are used to improve sensory characteristics and shelf life of food products [18]. Primarily these essential oils contain volatile components such as monoterpenes and sesquiterpene hydrocarbons and their oxygenated derivatives. Non-volatile parts that mainly contain hydrocarbons, fatty acids, sterols, carotenoids, waxes and flavonoids also perform protective functions [19]. Flavonoids, terpenoids and phenolic components of essential oil are found to exhibit significant antioxidant effects [20]. The antimicrobial potential of these oils is attributed to the lipophilic nature of the hydrocarbon skeletons and the hydrophilic nature of the functional groups. This induces changes in cell membrane permeability, interfering with enzymes involved in energy production and disrupting the protein driving force that ultimately leads to cell death [21].

No single mechanism can be tagged as a cause of antimicrobial action because the sites of action of these essential oils are variable, however, some result in degradation of the cell wall, damage to the cytoplasmic membrane, damage to membrane proteins and coagulation of the cytoplasm [22]. The antimicrobial activity of terpenes is attributed to their ability to inhibit respiratory and other energy dependent processes [23], whereas the action of terpenoids is partly due to a disturbance of the fatty acid composition of bacterial cell membranes resulting in alterations of membrane permeability and leakage of intracellular materials [24].

Antimicrobial and Antioxidant action of essential oils

In recent times, essential oils have gained popularity as powerful antimicrobial and antioxidant agents in food systems. A lot of work and research has been done to assess the antimicrobial and antioxidant activities of essential oils. Twenty-four essential oils producing families were examined [25] and it was found that oils containing eugenol, thymol and carvacrol had marked antioxidant activity. Three organum essential oils demonstrated high levels of antimicrobial activity against eight strains of gram-positive and gram-negative bacteria [26]. Among the major components of the three oils, carvacrol and thymol exhibited the highest levels of antimicrobial activity.

Baratta *et al*. [27] investigated the chemical composition, antimicrobial and antioxidant activity of laurel, Sage, Rosemary, parsley and coriander essential oils. They reported that all oils showed a high level of inhibition against all 25 microorganisms tested, leading to the essential oil of oregano with the highest and broadest antimicrobial and antioxidant activity. *In vitro* antimicrobial and antioxidant activities of essential oil of Salvia tomentosa (Miller) evaluated [28] and reported strong antimicrobial activity attributed to its main components i.e. pin-pinene (39.7%), α-pinene (10.9%) and camphor (9.7%). Essential oil of black cumin oil showed complete zone of inhibition against *Penicillium citrinum* and complete growth inhibition against *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus, Pseudomonas aeruginosa* [29]. This essential oil had good antioxidant activity as compared with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The essential oils were having good antimicrobial activity and they can act as bio preservatives in food matrices [30]. Essential oils from Lemongrass (*Cymbogon citratus*), Cinnamon (*Cinnamomum verum*) and Clove (*Syzygium aromaticum*) were investigated *in-vitro* for antimicrobial and antioxidant potential [31] and found that all these essential oils possess potent antimicrobial and antioxidant activity and can be used as a natural alternative for preservative in food industry.

Kaur and Kaushal [32] investigated the antibacterial action of Harsingar (*Nyctanthes arbortristis*) essential oil. The essential oils, eugenol and its derivatives showed maximum antibacterial potential with diameter of inhibition zone of 23.8 and 26.3 mm at 1000 µg/ml against *K. pneumoniae* and *P. aeruginosa*, respectively. They concluded that Harsingar essential oil may be used as a promising antimicrobial agent in biological systems. Several studies have already proven the role of essential oil in inhibiting microbial growth, reducing lipid oxidation, and therefore increasing the shelf-life of foods. But, there is still a lack of technical support in line with the hydrophilic, volatile, reactive nature of the active components of essential oils in direct incorporation into foods.

Need of encapsulation of essential oils

Due to the hydrophobic nature of essential oils, apart from easy degradation in the presence of light, air, and high temperatures, their use as food preservatives is not economical and viable [33]. To maintain their biological activity as well as to minimize their adverse effects on the organoleptic properties of foods, these essential oils need to be encapsulated in the delivery system, which are compatible with food applications [34]. Emulsion-based delivery systems can be prepared with food-grade materials and can be easily dispersed in areas of food, such as water-rich phases or liquidsolid interfaces where microorganisms grow and propagate. Due to this, encapsulation of essential oils with the help of food grade emulsifiers at the nanoscale represents a viable and novel technique to improve the physical and chemical stability of bioactive compounds [35, 36]. In addition, nanoencapsulation efficiently improves its diffusion capacity in aqueous solutions, prevents interaction with foods and improves its bioavailability and absorption [37], therefore, to fight bacterial resistance phenomena can be useful. Encapsulated delivery systems of essential oils can serve as promising strategies to achieve high antimicrobial efficiency against spoilage caused by microorganisms and food borne pathogens [38]. Among the nanoencapsulation systems currently used for the delivery of bioactive components, nanoemulsion have been described as suitable for use in food products due to their ease and desirable functional characteristic [39, 40].

Essential oils delivery by nanoemulsion

Nanoemulsions are emulsions that have a small size range of 20 nm to 200 nm, consisting basically of the lipid phase, dispersed in an aqueous continuous phase, and each oil droplet is surrounded by a thin interfacial layer consisting of emulsifier molecules [41, 42]. They are seen as promising delivery vehicle systems for essential oils, which have some unique advantages. Small particle size has two benefits. Primarily, it increases the possibility of enhancing physicochemical properties and stability and secondly enhances the ability to increase the biological activity of lipophilic compounds by increasing the surface area per unit [39]. Nanoemulsion-based delivery systems allow improving the bioavailability of encapsulated compounds and the use of low doses of active ingredients. Several studies have shown high antibacterial activity of nanoemulsions containing essential oils [34, 43, 44].

Acevedo *et al*. [45] developed nano-sized dispersions of essential oils to improve water dispersion and protect them from erosion. Nanoemulsions containing essential oils and polysaccharides were used to make edible films with functional properties. The average droplet size of nanoemulsions was reduced after micro-fluidization treatment exhibiting multimodal size distribution. They concluded that by using essential oil nanoemulsions as active ingredients for the manufacture of edible films, an improvement in physical and functional properties with antibacterial action is observed. With the increase in concentrations of nanoemulsions, the moisture in the chitosan film decreased as well as the antimicrobial activity of the Nanoemulsion-Chitosan film against *Staphylococcus aureus* was found to be higher than the chitosan film [46, 47].

Antibacterial activity of *Thymus daenensi* essential oil in both pure and nanoemulsion forms against *Escherichia coli,* an important food-borne pathogen bacterium, was investigated [48]. The antibacterial activity of the essential oil against *E. coli* was enhanced considerably when it was converted into a nanoemulsion, which was attributed to easier access of the essential oils to the bacterial cells. The characteristics and antimicrobial properties of citral essential oil nanoemulsion using an ultrasonic processor were documented [49]. Significant antimicrobial activity was demonstrated by the formulated citral oil nanoemulsion. Considering the relationship between formulation and activity, they suggested that nanoemulsion-based delivery systems may be a rational design for antimicrobial-based essential oils in the food, cosmetics and agrochemical industries.

Soheila *et al*. [50] worked on a nanoemulsion-based edible sodium caseinate coating containing ginger essential oil applied to chicken breast fillet to extend its shelf life. They reported that nanoemulsion-based food coatings with 6% of GEO nanoemulsion caused a significant reduction of the total aerobic psychophilic bacteria of the refrigerated chicken fillet over the course of 12 days. Punya *et al*. [51] designed a study to investigate *in-vitro* antimicrobial and antioxidant efficacy for its potential encapsulation in nano-delivery systems and further application in meat food products. In general, there was a stronger inhibitory effect with zone sizes ranging from 27.5 mm to 45 mm compared to the test organisms; however, the effect was higher against gram positive bacteria than gram negative bacteria. It was concluded that thyme essential oil has significant antimicrobial and antioxidant activity and can be encapsulated in nano-delivery systems for potential application in any food matrices.

The feasibility of nanoemulsification to improve the efficiency of chitosan-*Ferro Sago angulata* essential oil coating (CH + EO) when storing the of Rainbow trout fillets for 16 days at 4 ^{0}C was explored by Sajad *et al.* [52]. The prepared nanoemulsion (3% EO) treatment demonstrated a significantly better inhibitory effect on the growth of bacteria of refrigerated stored Rainbow trout fillets. In addition, nanoemulsification reinforced the efficacy of CH + EO in inhibiting the increase of TVB-N and lipid peroxidation in fish fillets. The texture, color, and overall acceptability of the CH + EO nanoemulsion treated samples were better. The results suggested that nanoemulsification active coating is a potential approach to increase its efficacy.

Conclusion

Essential oils from traditional herbs and spices have excellent potential in the food industries, but have some limitations in its direct incorporation. Due to their hydrophobic nature, they are unstable in aqueous medium, which poses more challenges for incorporating it into food due to the interaction of essential oil with protein and fat that may reduce its antioxidant and antimicrobial potential. In addition, strong taste with a high concentration for effective microbial inhibition would exceed organoleptic levels of acceptance. Therefore, one of the ways to overcome these deficiencies is to encapsulate essential oils before incorporating them into food matrices. Encapsulation provides isolation, penetration, protection, or controlled release of reactive substances from the surrounding matter. Nanoemulsion delivery systems are known to use a low concentration of essential oils to increase their functionality in food.

References

1. Barbara N, Colle MJ, Ünlü G. Meat safety and quality: a biological approach." Int. J. Food Sci. & Technol 2020;56:39-51.
2. Devlieghere F, Vermeiren L, Debevere J. New preservation technologies: Possibilities and linitations. Int. Dairy J 2004;14:273-85.
3. Olmedo RH, Nepote V, Grosso NR. Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano and rosemary essential oils. LWT-Food Sci. Technol 2013;53(2):409-17.
4. Guzey D, McClements DJ. Impact of electrostatic interactions on formation and stability of emulsions

containing oil droplets coated by β-lactoglobulin-pectin complexes. J. Agri. Food Chem 2007;55(2):475-85.
5. Ghosh V, Mukherjee A, Chandrasekaran N. Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. Ultrasonic Sonochemistry 2013;20(1):338-44.
6. Manchun S, Dass CR, Sriamornsak P. Designing nanoemulsion templates for fabrication of dextrin nanoparticles via emulsion cross-linking techniques. Carbohydrate Polymers 2014;101:650-55.
7. Franz C, Novak J. Sources of essential oils. Handbook of essential oils science, technology, and applications 2010, 39-81.
8. Rubiolo P, Sgorbini B, Liberto E, Cordero C, Bicchi C. Essential oils and volatiles: sample preparation and analysis- A review. Flavour and Fragrance J 2010;25(5):282-90.
9. Mahmoudzadeh M, Hosseini H, Shahraz F, Akhondzadeh BA, Khaneghah AM, Azizkhani M, *et al.* Essential oil composition and antioxidant capacity of Carum copticum and its antibacterial effect on Staphylococcus aureus, Enterococcus faecalis and Escherichia coli O157: H7. *J.* Food Processing and Preservation 2017;41(3):e12938.
10. Bakhtiary F, Sayevand HR, Khaneghah AM, Haslberger AG, Hosseini H. Antibacterial efficacy of essential oils and sodium nitrite in vacuum processed beef fillet. Applied food biotech 2018;5(1):1-10.
11. Farag RS, Daw ZY, Hewedi FM, El-Baroty GSA. Antimicrobial activity of some Egyptian spice essential oils. J. food protection 1989;52(9):665-67.
12. Pandit VA, Shelef LA. Sensitivity of Listeria monocytogenes to rosemary (Rosmarinus officinalis L.). Food microbial 1994;11(1):57-63.
13. Charles Sell. The chemistry of fragrances: from perfumer to consumer. Royal Society of Chemistry 2006.
14. Deans SG, Waterman PG, Hay RKM. Volatile Oil Crops: their biology, biochemistry and production. Their Biology, Biochemistry and Production Essex, UK: Longman Scientific and Techical 1993, 97-111
15. Deans SG, Kennedy AI, Gundidza MG, Mavi S, Waterman PG, Gray AI. Antimicrobial activities of the volatile oil of Heteromorpha trifoliata (Wendl.) Eckl. & Zeyh.(Apiaceae). Flavour and Fragrance J. 1994;9(5):245-48.
16. Carlton RR, Waterman PG, Gray AI, Deans SG. The antifungal activity of the leaf gland volatile oil of sweet gale (Myrica gale) (Myricaceae). *Chemoecology* 1992;3(1):55-59.
17. Dorman HJD, Deans SG, Noble RC, Surai P. Evaluation *in vitro* of plant essential oils as natural antioxidants. J. Essential Oil Res 1995;7(6):645-51.
18. Goulas AE, Kontominas MG. Combined effect of light salting, modified atmosphere packaging and oregano essential oil on the shelf-life of sea bream (Sparus aurata): Biochemical and sensory attributes. Food chem 2007;100(1):287-96.
19. Castro AP, Adamowicz WL, Hall ER. Life cycle assessment for sustainable forest management. Sustainable Forest Management Network 1999.
20. Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A, *et al.* Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food chem 2005;89(4):549-54.
21. Basim H, Yegen O, Zeller W. Antibacterial effect of essential oil of Thymbra spicata L. var. spicata on some plant pathogenic bacteria. J. Plant Diseases and Protection 2000, 279-84.
22. Burt S. Essential oils: Their antibacterial properties and potential applications in foods- A review. Int. J. Food Microbiol 2004;94(3):223-53.
23. Griffin SG, Wyllie SG, Markham JL, Leach DN. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flavour and Fragrance J 1999;14(5):322-32.
24. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, *et al*. Mechanisms of antibacterial action of three monoterpenes. Antimicrobial agents and chemotherapy 2005;49(6):2474-78.
25. Lagouri V, Boskou D. Screening for antioxidant activity of essential oils obtained from spices. Developments Food Sci 1995;37:869-79.
26. Sivropoulou, Afroditi, *et al*. Antimicrobial and cytotoxic activities of Origanum essential oils. J. Agri. Food Chem 1996;44(5):1202-205.
27. Baratta MT, Dorman HD, Deans SG, Biondi DM, Ruberto G. Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils. J. Essential Oil Res 1998;10(6):618-27.
28. Tepe B, Daferera D, Sokmen A, Sokmen M. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa Miller* (Lamiaceae). Food chem 2005;90(3):333-40.
29. Singh G, Marimuthu P, de Heluani CS. Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of Nigella sativa seeds. J. Sci. Food & Agri 2005;85(13):2297-306.
30. Solórzano-Santos F, Miranda-Novales MG. Essential oils from aromatic herbs as antimicrobial agents. Current Opinion in Biotechnol 2012;23(2):136-41.
31. Kumar D, Mehta N, Chatli MK, Kaur G, Malav OP, Kumar P. *In-vitro* assessment of antimicrobial and antioxidant potential of essential oils from Lemongrass (*Cymbopogon citratus*), Cinnamon (*Cinnamomum verum*) and Clove (*Syzygium aromaticum*). J. Anim. Res 2017;7(6):1099-105.
32. Kaur J, Kaushal S. Chemical Analysis, Antimicrobial and Antioxidant Activities of Harsingar (*Nyctanthes arbortristis*) essential oil. J. Essential Oil-Bearing Plants 2020, 1-16.
33. Salvia-Trujillo, Laura, *et al*. Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils. Food hydrocolloids 2015;43:547-56.
34. Buranasuksombat U, Kwon YJ, Turner M, Bhandari B. Influence of emulsion droplet size on antimicrobial properties. Food Sci. Biotechnol 2011;20(3):793-800.
35. Weiss J, Gaysinsky S, Davidson M, McClements J. Nanostructured encapsulation systems: food antimicrobials. Global Issues in Food Sci. Technol 2009, 425-79.
36. Donsi F, Annunziata M, Sessa M, Ferrari G. Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. LWT-Food Sci. Technol 2011;44(9):1908-14.
37. Selim S. Antimicrobial activity of essential oils against Vancomycin-Resistant enterococci (VRE) and Escherichia coli O157: H7 in feta soft cheese and minced

beef meat. Brazilian J. Microbiol 2011;42(1):187-96.

38. Xue J, Davidson PM, Zhong Q. Antimicrobial activity of thyme oil co-nanoemulsified with sodium caseinate and lecithin. Int. J. Food Microbiol 2015;210:1-8.
39. McClements DJ, Rao J. Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Critical Reviews in Food Sci. Nutri 2011;51(4):285-330.
40. Caillet S, Cote J, Sylvain JF, Lacroix M. Antimicrobial effects of fractions from cranberry products on the growth of seven pathogenic bacteria**.** Food Control 2012;23(2):419-428
41. McClements DJ, Decker EA, Weiss J. Emulsion based delivery systems for lipophilic bioactive components. J. Food Sci 2007;72(8):R109-24.
42. Acosta E. Bioavailability of nanoparticles in nutrient and nutraceutical delivery. Current Opinion in Colloid & Interface Sci 2009;14(1):3-15.
43. Liang R, Xu S, Shoemarker CF, Li Y, Zhong F, Huang Q. Physical and antimicrobial properties of peppermint oil nanoemulsions J. Agri. Food Chem 2012;60(30):7548-55.
44. Severino R, Ferrari G, Vu KD, Donsi F, Salmieri S, Lacroix M. Antimicrobial effects of modified chitosan-based coating containing nanoemulsion of essential oils, modified atmosphere packaging and gamma irradiation against *Escherichia coli* O157: H7 and *Salmonella typhimurium* on green beans. Food Control 2015;50:215-22.
45. Acevedo-Fani, Alejandra, *et al*. Edible films from essential-oil-loaded nanoemulsions: Physicochemical characterization and antimicrobial properties. Food Hydrocolloids 2015;47:168-77.
46. Saranya S, Mukherjee A, Chandrasekaran N. Eucalyptus oil nanoemulsion-impregnated chitosan film: antibacterial effects against a clinical pathogen, *Staphylococcus aureus*, *in vitro*. Int. J. Nanomedicine 2015;10(1):67.
47. Francesco D, Ferrari G. Essential oil nanoemulsions as antimicrobial agents in food. J. Biotechnol 2016;233:106-20.
48. Moghimi, Royal, *et al*. Superior antibacterial activity of nanoemulsion of *Thymus daenensis* essential oil against E. coli. Food chem 2016;194:410-15.
49. Lu WC, Huang DW, Wang CC, Yeh CH, Tsai JC, Huang YT, *et al*. Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. J. Food and drug analysis 2018;26(1):82-89.
50. Soheila N, Zeynali F, Almasi H. Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets. Food control 2018;84:312-20.
51. Punya SHN, Mehta N, Chatli MK, Wagh RV, Panwar H. In-vitro evaluation of antimicrobial and antioxidant Efficacy of thyme (*Thymus vulgaris L.*) essential oil. J. Anim. Res 2019;9(3):443-49.
52. Sajad S, Parastouei K, Toghdir M, Abbaszadeh S. Application an edible active coating based on chitosan-Ferulago angulata essential oil nanoemulsion to shelf life extension of Rainbow trout fillets stored at 4 ^{0}C. Int. J. Biolo. Macromolecules 2020;153:846-54.