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Formulations and characterizations of vegetable oil-based nanoemulsions prepared for use as colloidal delivery systems

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Abstract

Although the food industry has a high potential to benefit from nanotechnology, it is still very limited. However, in recent years, the world food industry has started to search for ways to benefit from this technology and nanotechnology-based techniques have been introduced to the food industry to provide solutions to food safety-related problems. Nanoparticles have great importance in extending the shelf life of foodstuffs due to their antimicrobial properties without causing any change in flavor or color. Nanotechnology is a technology focused on the characterization, production, and processing of biological and non-biological structures smaller than 100 nm. Nanoparticles have great importance in extending the shelf life of foodstuffs due to their antimicrobial properties without causing any change in flavor or color. Recently, there has been increasing interest in the application of innovative nanoscience-based technologies to improve the health, safety, and quality of food products. A major breakthrough in this area has been the use of nanoemulsions. Because nanoemulsions can be easily formulated with existing food ingredients and technologies. In particular, oil-in-water nanoemulsions consisting of small oil droplets (<200nm) dispersed in water are used as delivery systems for various hydrophobic substances in foods, such as nutraceuticals, antioxidants, antimicrobials, colorants, and sweeteners. Encapsulation of hydrophobic substances in nanoemulsions can greatly increase their water dispersibility, chemical stability, bioavailability, and bioactivity. As a result, they have several advantages over traditional emulsions for certain applications, including increased resistance to aggregation and gravitational separation during storage, as well as higher bioavailability after ingestion. For these reasons, nanoemulsions are particularly suitable for encapsulating hydrophobic bioactive substances and subsequently incorporating them into food and beverage products. Therefore, the aim of this project includes the formulation, production, and characterization of nanoemulsion-based delivery systems for use in food products. In current study, characterization studies of nanoemulsions prepared using vegetable oils (sunflower and olive oil) were carried out and their physical properties such as viscosity, thermodynamic stability, droplet size, and surface tension were determined.

Key Words: *Nanoemulsion, formulation, characterization, food products, antimicrobial*

Kolloidal salınım sistemi olarak kullanılmak üzere hazırlanan bitkisel yağ bazlı nanoemülsiyonların formülasyonları ve karakterizasyonları

Özet

Gıda endüstrisinin nanoteknolojiden faydalanma potansiyeli yüksek olmasına rağmen halen çok sınırlı ölçülerdedir. Ancak son yıllarda dünya gıda sanayi bu teknolojiden faydalanma yollarının arayışı içine girmiş ve nanoteknolojiye dayalı teknikler gıda güvenliği ile ilişkili sorunlara çözüm sunmak için gıda endüstrisine tanıtılmıştır. Nanoparçacıklar gıda maddesinde, lezzet ve renk değişimine yol açmadan antimikrobiyal özellikleri sayesinde raf ömrünün uzatılmasında büyük öneme sahiptir. Nanoteknoloji 100 nm' den daha küçük biyolojik ve biyolojik olmayan yapıların karakterizasyonu, yapımı ve işlenmesi üzerinde yoğunlaşmış bir teknolojidir. Nanoparçacıklar gıda maddesinde, lezzet ve renk değişimine yol açmadan antimikrobiyal özellikleri sayesinde raf ömrünün uzatılmasında büyük öneme sahiptir. Son zamanlarda, gıda ürünlerinin sağlığını, güvenliğini ve kalitesini iyileştirmek için yenilikçi nanobilim temelli teknolojilerin uygulanmasına ilgi artmaktadır. Bu alandaki büyük bir hamle, nanoemülsiyonların kullanılması olmuştur. Çünkü nanoemülsiyonlar, mevcut gıda bileşenleri ve teknolojileri ile kolayca formüle edilebilirler. Özellikle, suda dağılmış küçük yağ damlacıklarından (<200nm) oluşan su içinde yağ nanoemülsiyonları nutrasötikler, antioksidanlar, antimikrobiyaller, renklendiriciler ve tatlandırıcılar gibi gıdalardaki çeşitli hidrofobik maddeler için dağıtım sistemleri olarak kullanılmaktadır. Nanoemülsiyonlar içinde hidrofobik maddelerin kapsüllenmesi suda dağılabilirliklerini, kimyasal stabilitelerini, biyoyararlanımlarını ve biyoaktivitelerini büyük ölçüde artırabilir. Sonuç olarak, belirli uygulamalar için geleneksel emülsiyonlara göre bazı avantajlara sahiptirler. Bunlara depolama sırasında topaklaşma ve yer çekimsel ayrılmaya karşı daha fazla direnç ve ayrıca yutulduktan sonra daha yüksek biyoyararlılık dahildir. Bu nedenlerden dolayı, nanoemülsiyonlar hidrofobik biyoaktif maddeyi kapsüllemek ve daha sonra bunları yiyecek ve içecek ürünlerine dahil etmek için özellikle uygundur. Bu nedenle, bu projenin amacı, nanoemülsiyon bazlı dağıtım sistemlerinin gıda ürünlerinde kullanımı amaçlı formülasyonu, üretimi ve karakterizasyonunu içermektedir. Çalışmamızda bitkisel yağlar (ayçiçek ve zeytin yağı) kullanılarak hazırlanan nanoemülsiyonların karakterizasyon çalışmaları yapılmış olup viskozite, termodinamik stabilite, damlacık büyüklüğü ve yüzey gerilimi gibi fiziksel özellikleri belirlenmiştir.

Anahtar Kelimeler: *Nanoemülsiyon, formülasyon, karakterizasyon, gıda ürünler, antimikrobiyal*

Introduction

Although the food industry has a high potential to benefit from nanotechnology, it is still very limited. However, in recent years, the world food industry has begun to seek ways to benefit from this technology and nanotechnology-based techniques have been introduced to the food industry to provide solutions to food safety-related problems. Nanoparticles have great importance in extending the shelf life of food products due to their antimicrobial properties without causing any change in flavor or color (Josef & Morrison, 2006; Chaudhry et al., 2008).

In the food industry, despite the use of various preservation techniques, microbiological spoilage and food poisoning can occur in foods due to the effect of microorganisms. This situation constitutes a significant problem for the food industry. Synthetic or non-synthetic substances such as natural antioxidants and antimicrobials are widely used to prevent or control the formation of these unwanted microorganisms. In terms of the negative effects of synthetic preservatives on food and human health and the development of antibiotic-resistant strains, there is an increase in studies on the use of natural additives, which directs researchers to new searches. Nanotechnology is a technology focused on the characterization, production and processing of biological and non-biological structures smaller than 100 nm. Nanoparticles are of great importance in extending the shelf life of foodstuffs thanks to their antimicrobial properties without causing flavor and color changes. Recently, interest in the application of innovative nanoscience-based technologies to improve the health, safety and quality of food products has increased. A major breakthrough in this area has been the use of nanoemulsions. Because nanoemulsions can be easily formulated with existing food ingredients and technologies. In particular, oil-in-water nanoemulsions consisting of small oil droplets (<200 nm) dispersed in water are used as delivery systems for various hydrophobic substances in foods such as nutraceuticals, antioxidants, antimicrobials, colorants and sweeteners. As is known, the consumption of meat products is important for consumers. However, especially fish meat spoils quite quickly due to its high moisture content and weak connective tissue compared to the connective tissue of land animals. In addition to fish meat, the consumption of red meat and chicken meat also plays a key role in a balanced diet. Despite being composed of very important nutrients, these meat products can also spoil easily. In particular, the growth of total aerobic bacteria as well as total aerobic psychrophilic bacteria can be observed during the cold storage period. In this respect, nanoemulsion is considered as a new approach as a colloidal dispersion system of material rich in phenolic compounds, fatty acids and vitamins, which are of economic importance to the food industry.

In general, many different types of food-grade bioactive substances, including fat-soluble vitamins, antioxidants, antimicrobials, nutraceuticals, triglyceride oils, flavor oils, and essential oils, can be encapsulated within nanotechnology-based delivery systems. Nanoemulsions are one of the most widely used delivery systems for this purpose, as they can be prepared using existing processing technologies and their properties can be easily manipulated (McClements & Rao, 2011; Salvia-Trujillo et al., 2015).

Encapsulation of hydrophobic substances in nanoemulsions can greatly increase their water dispersibility, chemical stability, bioavailability, and bioactivity (Nakajima, 2005). Nanoemulsions differ from conventional emulsions by their smaller droplet diameters (d>200 nm) (McClements & Rao, 2011; Salvia-Trujillo et al., 2015). As a result, they have some advantages over conventional emulsions for certain applications, including greater resistance to agglomeration and gravitational separation during storage, as well as higher bioavailability after ingestion (Salvia-Trujillo et al., 2015). For these reasons, nanoemulsions are particularly suitable for encapsulating hydrophobic bioactive substance and subsequently incorporating them into food and beverage products (McClements et al., 2007; Sanguansri & Augustin, 2006; Weiss et al., 2008). Recently, a number of comprehensive reviews have been conducted on the potential applications of nanoemulsions in the food and beverage industry (Acevedo-Fani et al. 2017a, 2017b; Dasgupta et al., 2019; McClements & Rao, 2011). However, there is a lack of information regarding the characterization of nanoemulsions prepared using sunflower and olive oils. Therefore, the aim of this study is to include a series of studies that will form the basis for the production of nanoemulsion-based delivery systems for formulation into food products.

Material and Method

Various vegetable oils (sunflower and olive oil) used in the study were obtained from commercial markets and nanoemulsions were prepared from vegetable oils in two different stages as oil and water phases according to the method used by Hammouda et al. (1999). 14% oil, 3% ethanol, 3% biosurfactant (tween 80) and 1% carvacrol and thymol were mixed and kept in an oven at 86 ºC for 1 hour. Then, pure water (79%) was added to this mixture and mixed in an ice-filled bathtub in an ultrasonic homogenizer for 15 minutes. The viscosity, thermodynamic stability,

nanoemulsion droplet size and surface tension parameters of these nanoemulsions were determined. These analyses were carried out from the central laboratories of Middle East Technical University (METU).

Droplet Size: Nanoemulsions are transparent or translucent emulsions with droplet sizes ranging from 20 to 600 nanometers (Tadros et al., 2004; Fernandez et al., 2004; Zhi et al., 2005). In our study, the nanoemulsions created were sent to the METU central laboratory and their droplet sizes were determined.

Viscosity: Viscosity aids the emulsification process by increasing the resistance of droplets to coagulation. The viscosity of an emulsion slows down the coagulation of droplets by slowing down their diffusion movement, thus affecting the stability of the emulsion and drop size (Cooper & Gunn, 1950). High viscosity emulsions may have low stability, while low viscosity emulsions may have high stability (Martin, 1993).

Thermodynamic stability: The systems with the lowest stability in nanoemulsions are called thermodynamically unstable systems. Therefore, it is important to determine the roles of each of the immiscible phase systems in this system. First of all, since emulsions exhibit a large interfacial area, it is understood that any decrease in interfacial tension will reduce the tendency to coalesce and promote stability.

Surface Tension: Surface tension occurs as a result of the attractive force between molecules that are close to each other on the surface and inside (Martin, 1993). When the tension occurs between two immiscible liquids, it is called interfacial tension. According to surface tension theories; if there is a substance added to the system that reduces the interfacial tension; two immiscible liquids can form an emulsion (Cosgrove, 1963). The substance added to this system that reduces the interfacial tension is usually emulsifiers. Emulsifier molecules are located at the water-oil interface depending on their characteristics. While the hydrophilic part of the emulsifier is directed to water; the hydrophobic part is directed to oil. If the hydrophilic properties of the emulsifier are more dominant than its hydrophobic properties; the emulsifier molecule will be directed at the oil-water interface (Milton, 2004). Therefore, it is necessary to determine the surface tension in the nanoemulsions formed.

Statistical Analysis: In the study, SPSS 22.0 statistical package program was used and the obtained data were evaluated with Duncan multiple comparison test (Duncan, 1955). The significance level was taken as $p<0.05$.

Results and Discussion

Characterization studies of nanoemulsions prepared using vegetable oils were carried out and physical properties such as viscosity, thermodynamic stability, droplet size and surface tension were determined and the results are given in the table below (Table 1).

Table 1. Physical properties of oil-in-water nanoemulsions

	Droplet size (nm)	Viscosity (N s/m^2)	Thermodynamic stability	Surface tension (N/m)
Nanoemulsion based on sunflower oil	375.76±0.12^a	196±1.68^a	+++	40.35±0.38^a
Nanoemulsion based on olive oil	326.36±0.12^b	213±1.26^b	+++	38.19±0.61^b

X±Sx: Mean ; ± Standart deviation; ^{a,b} shows differences ($p<0.05$) between nanoemulsion groups

Statistical differences were observed between the groups in terms of droplet size, viscosity and surface tension of the prepared nanoemulsions. The group with the lowest droplet size was nanoemulsions prepared using olive oil. Özogul et al. (2016) found the droplet sizes of nanoemulsions prepared using sunflower, hazelnut, canola, soybean, corn and olive oils to be 212, 185, 299, 250, 250 and 275 nm, respectively. Durmuş et al. (2020) reported that the group with the lowest droplet size was the olive oil group (O15) prepared with 15% concentration (308.36 nm), and the largest value belonged to the olive oil group (O45) prepared with 45% concentration (541.43 nm). The oils used at 10% in our study were also in similar or close ranges and were supported by the literature.

The thermodynamic stability of nanoemulsions prepared using olive oil was similar to the studies of other researchers. Yazgan et al. (2017) reported that the surface tension of sunflower oil-based nanoemulsions was 41.53 nm, while Özogul et al. (2016) reported that the surface tension of nanoemulsions prepared from olive oil was 30.16 nm. Durmuş et al. (2020) observed the lowest viscosity in the O15 group (2.13 × 10−3 Pas) and the highest viscosity in the O45 group (9.11 × 10−3 Pas).

Yuvka (2014) determined the droplet size of nanoemulsions formed from thyme and rosemary essential oils as 112.82 and 63.02 nm, respectively. Joe et al. (2012) reported that the droplet size of nanoemulsions formed using commercial oils such as sunflower, castor, coconut, peanut, and sesame oils varied between 72.52 and 875.22 nm. Although the physical properties of the nanoemulsion we obtained in our study showed similar results with some researchers, they also showed different results with others. It is thought that the reason for this is the type of oils used in creating the nanoemulsion, the surfactant, and the methods used to obtain the nanoemulsion.

These results were also found to be compatible with the literature and it was concluded that it can be used in foods.

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