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Investigation and Production of Thyme Essential Oil Nanoemulsion Using Chitosan-Zein Pickering Emulsion Method by Nano Spray Drying Mechanism

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ABSTRACT

Nowadays, emulsion plays a significant role in encapsulating nutrients and volatile essences. One of the new methods for emulsion production involves the use of nanoparticles. A novel approach in producing nanoparticles is through the utilization of spray drying nanoencapsulation. In this study, thyme emulsion produced by three methods (rotary, spray drying, and Tween 80) was investigated. The results of morphological analysis of chitosan-zein particles (produced by rotary and spray drying) using scanning electron microscopy (SEM) revealed that the powder obtained from spray drying exhibited some agglomerated and clumpy regions, while the image of the powder obtained from the rotary method showed a more homogeneous and uniform structure. Particle size measurements indicated that the largest particle size was observed in the emulsion produced using the nano spray drying nanoencapsulation device. The rheological behavior of the emulsions demonstrated an increase in shear stress with an increase in shear rate for all samples in this research.

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INTRODUCTION

Emulsion is a heterogeneous system consisting of two immiscible liquids, where one liquid is dispersed as droplets, usually with a diameter greater than 1 micron, in the other liquid. Nanoemulsions are oil-in-water dispersions, less than a micron in size, which are highly unstable, with particle diameters ranging from 10 to 100 nanometers (McClements, 2004). Depending on the particle size, emulsions are classified into nano (100-10 nanometers), micro (1000-100 nanometers), and macro (100-500 micrometers) categories (Windhab et al., 2005). Some advantages of nanoemulsions, such as high physical stability, high bioavailability, and low turbidity, contribute to their increased use in food, cosmetic, and pharmaceutical industries. Nanoemulsions are used as carriers for bioactive compounds, such as drugs in the pharmaceutical industry and antimicrobial agents in the food industry, solubilizing water-insoluble pesticides in the chemical-agricultural industry, and in personal care and skincare products in the cosmetic and hygiene industries. Ultrasonic emulsification is a high-energy method for developing nanoemulsions, which has been documented as a rapid and effective approach for formulating stable nanoemulsions with very small particle size and low dispersity (Lin & Chen, 2008).

Spray drying is a process that transforms a liquid into dried particles by spraying the liquid into a drying environment. It is a one-step process used to convert various liquid forms, such as aqueous and organic solutions, emulsions, and suspensions, into dry powders (Ahrabi & Ghassemzadeh, 2022; Jafari et al., 2021). In the food industry, spray drying is the most common and oldest method used for encapsulation. Spray drying a solution, suspension, or formulated emulsion is extensively employed to produce powders where its active components are well distributed in the dispersed matrix (Fang & Bhandari, 2010; Madene et al., 2006). In fact, spray drying is the most widely used encapsulation method for food products. Spray drying is applied for food ingredients such as flavors, vitamins, salts, minerals, colors, fats and oils, spices, polyphenols, proteins, carotenoids, antioxidants, live probiotic cells, probiotic bacteria, enzymes, peptides, and many other substances (Mahdavi et al., 2014).

Stabilized emulsions with solid particles instead of surfactants are called Pickering emulsions (Chevalier & Bolzinger, 2013). Due to their wide range of applications, Pickering emulsions have attracted significant attention in the food, pharmaceutical, and cosmetic industries compared to conventional emulsions that use surfactants, which can have toxic effects. In these emulsions, the particles irreversibly adsorb at the water-oil interface due to their high interfacial energy, resulting in long-term stability of the formed Pickering emulsions for months or even years (Binks, 2002). Additionally, Pickering emulsions maintain the primary structure of classical emulsions and can be classified into any type of oil-in-water, water-in-oil, or multiple emulsions (water/oil/water or oil/water/oil). Figure 1 illustrates images of Pickering and conventional emulsions.

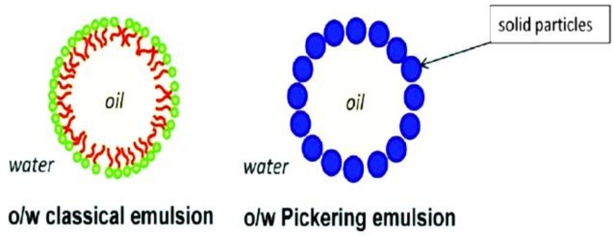

Figure 1. Schematic depiction of a conventional emulsion and a Pickering emulsion (Chevalier & Bolzinger, 2013)

Encapsulation, also known as entrapping, is one of the most important methods used as a suitable process for the protection and increased stability of volatile food compounds and their controlled release in food products. Encapsulation technology, as a unique method for packaging materials in nano-sized particles and as a process of trapping an active substance within another substance, which serves as a wall, is rapidly advancing. In the food industry, phenolics, volatile additives, colors, enzymes, and bacteria are encapsulated in small capsules to create stability and protect them against food-related damages (Mahdavi et al., 2014). Emulsions stabilized with solid particles instead of surfactants are called Pickering emulsions (Chevalier & Bolzinger, 2013).

In a study, the researchers investigated the encapsulation of various phenolic compounds in nano-sized particles coated with chitosan/alginate (ali/chiZN). Ferulic acid, glycosylated flavonoids, and aglycone flavonoids were successfully encapsulated in three nano-particles. The encapsulation efficiency of the zein nano-particles coated with chitosan/alginate was reported as 98.5-95%. Developed systems have the potential to encapsulate various phenolic compounds and improve their bioaccessibility (Carrasco-Sandoval et al., 2021).

In another research, the development of zein nano-particles coated with carboxymethyl chitosan (CMCS) for encapsulation and controlled release of vitamin D3 was examined. The nano-particles coated with CMCS had a spherical structure with particle sizes ranging from 86 to 200 nanometers. The encapsulation efficiency after CMCS coating improved to 87% compared to 52% when zein was used as a single capsule. The results showed that encapsulating water-insoluble nutrients in zein nano-particles with CMCS coatings is a promising approach to enhance chemical stability and controlled release properties (Luo et al., 2012).

Veneranda et al. (2018) conducted a study on the formation and identification of complex zein nanoparticles, caseinates, and pectin for encapsulating Eugenol. The smallest and most homogeneous complex nanoparticles were obtained when zein nanoparticles were heated at a pH of 6.6, the isoelectric point of zein. Sodium caseinates played an important role in achieving desired stability during storage and spray drying. Innovative spray drying of nano-suspensions to convert colloidal nanoparticles into fine powder particles was experimented with, providing long-term storage capability in powder form and therefore greater potential for applications in the food industry. Under optimized preparation and formulation conditions, complex nanoparticles loaded with Eugenol were obtained with a size of 140 nanometers, spherical shape, and uniform size distribution, and their structure was confirmed using both scanning and transmission electron microscopy (Veneranda et al., 2018).

Loureiro et al. (2022) investigated the effect of zein/hydroxypropyl methylcellulose (HPMC) blends and water content on their properties. The addition of HPMC in the blend resulted in increased particle size and polydispersity index (PDI) values. The interaction between zein and HPMC affected the particle size and PDI values significantly. Zein/HPMC blends with suitable moisture content showed good flowability. The PDI values of zein/HPMC blends were found to be pH-dependent, with pH 3 exhibiting the highest dispersibility (Loureiro et al., 2022).

Shi et al. (2016) conducted a study on the effect of applying different amounts of organic and inorganic fertilizers on corn growth. The results showed that the application of 100 kg of organic fertilizer and 20% of inorganic fertilizer significantly improved the growth of corn particles. The study also used CLSM (Confocal Laser Scanning Microscopy) to examine the internal structure of corn particles and revealed that the addition of inorganic fertilizer at a rate of 20% resulted in the formation of larger particles. Furthermore, the study found that the addition of organic fertilizer alone increased the growth of corn particles by 5 to 20%, depending on the dosage. These findings suggest that the combination of organic and inorganic fertilizers can enhance the growth of corn particles (Shi et al., 2016).

Another study investigated the effect of different treatments on the growth and yield of corn. The study evaluated the impact of applying different amounts of organic and inorganic fertilizers on the growth of corn particles and the yield of corn kernels. The results indicated that the application of organic and inorganic fertilizers significantly improved the growth and yield of corn. The study also found that the addition of organic and inorganic fertilizers for seven consecutive years resulted in a significant increase in yield. Overall, the findings demonstrated the importance of using a combination of organic and inorganic fertilizers for sustainable corn production (Wang et al., 2016).

The aim of this research was to form a nano-emulsion containing thyme essential oil using the emulsion encapsulation method, to preserve the properties of the essential oil. Additionally, it was intended to add this nano-emulsion to food industry as an effective ingredient in edible films, thereby improving the properties of the films.

MATHERIALS AND METHODS

The materials used in this study included: chitosan (Sigma Aldrich, USA), pectin (Pectin, Silvateam, Italy), whey protein (Helimar, USA),corn zein (Zarin Zein, Iran), glycerol (Merck, Germany), alcohol (Zakaria Jahrom, Iran), Tween 80 (Merck, Germany), and acetic acid (Merck, Germany).

Zein Purification

For the purification of zein (a by-product of Zarin Zorat factory), initially, 100 grams of impure zein was added to 600 milliliters of 70% alcohol.

Then, the solution was thoroughly mixed using a mixer to ensure proper dissolution of zein. Next, the desired solution was heated on a heater at a temperature of 70 ℃ for 2 hours, stirring continuously. After the specified time elapsed, the clear supernatant was separated using a centrifuge, and the resulting solution was concentrated using a rotary evaporator at a temperature of 40 ℃. Finally, the concentrated solution was poured into large trays and dried at a temperature of 70 ℃ for 24 hours. The remaining substance after the drying process is pure zein (Anderson & Lamsal, 2011). Figure (2) illustrates a depiction of pure zein.

Figure 2. Pure zein

Nano Spray Dryer mechanism

In nano-spray drying, first the liquid is sprayed by a nozzle in hot air with pressure. An electrostatic collector with a maximum voltage of 30kv is used to produce nanoscale powder. In this mechanism, clumping is prevented due to the particles being charged. In this mechanism, the liquid flow rate is controlled by the pump. Also, the pregnancy rate of the particles can be adjusted by changing the voltage. The hot air temperature is controlled and monitored by a heater and temperature sensor (figure 3).

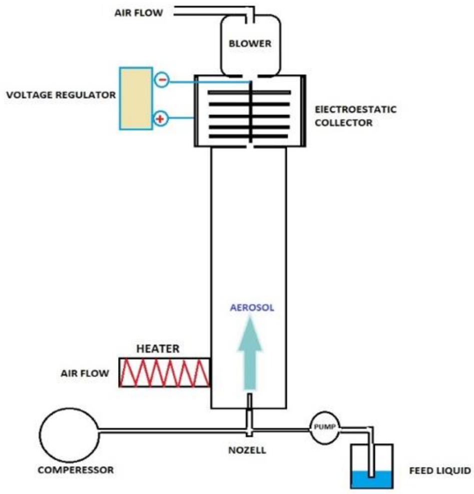

Figure 3. Nano Spray Dryer mechanism

Production of Chitosan-Zein Corn Powder using Spray Drying Nano Technique

In the spray drying method, initially, 0.5 grams of chitosan powder was added to 100 milliliters of distilled water. Then, 2 milliliters of concentrated acetic acid were added to the previous solution. To facilitate better dissolution, the solution was placed on a mixer at a temperature of 35 ℃ for 15 minutes. Simultaneously, 0.5 grams of pure zein powder was added to 100 milliliters of 70% alcohol at room temperature and mixed using a magnetic stirrer. Once the desired solutions were prepared, they were combined and diluted to a volume of 800 milliliters with distilled water. The resulting solution was then subjected to an ultrasonic device with a power of 60 watts for 15 minutes. The desired solution was dried using a spray dryer (Figure 4) at an initial temperature of 64 ℃, converting it into powder. The obtained powder was packaged and stored in a dry and cool place (Silva et al., 2018).

Figure 4. Nano Spray Dryer

Production of Chitosan-Zein Corn Powder using Rotary Method

To produce Chitosan-Zein corn powder using the rotary method, the desired solutions were prepared as in the spray drying method. Then, the combined solution was diluted to a volume of 200 milliliters with distilled water and transferred to the rotary device. After concentrating the solution using the rotary device, the solution was placed in the oven at a temperature of 70 °C for 24 hours to dry. Finally, the dried powder was packaged and kept in a dry and moisture-free place for further testing (Silva et al., 2018).

Analysis of Particle Size and Morphology Using SEM Device

To investigate the particle size and morphology of the particles produced using the nano spray drying method and solvent evaporation (vacuum concentrator), the SEM (Scanning Electron Microscope) method was employed. In this method, after obtaining the chitosan-zein powders using the nano spray drying and vacuum concentrator methods, a portion of the powder was taken and directly placed on a sample holder and transferred to the SEM device for the desired tests. In this method, the samples were initially coated with gold using a desktop sputtering device (DSRI-Nano-structured coatings, made in Iran), and then imaging was performed (Almasi et al., 2020).

Preparation of Thyme Essential Oil Emulsion Using Chitosan-Zein Powder Obtained from Spray Dryer

To prepare the emulsion, initially, 0.91 grams of powder obtained from the spray dryer was mixed with 45 milliliters of distilled water, and the resulting solution was heated on a heater at a temperature of 50 °C for 20 minutes. Then, 0.227 grams of thyme essential oil was combined with 20 milliliters of water and stirred for 5 minutes at room temperature (25 °C). The two prepared solutions were mixed and placed on a heater for 2 minutes and then subjected to an ultrasonic bath for 5 minutes (Noori et al., 2018).

Preparation of Thyme Essential Oil Emulsion Using Chitosan-Zein Powder Obtained from Rotary Method

In this method, initially, 0.455 grams of corn zein powder was added to a specified amount of 70% alcohol (30 milliliters) and mixed until fully dissolved. Then, 0.455 grams of chitosan was taken and dissolved in 90 milliliters of distilled water (it should be noted that 1 milliliter of acetic acid was added to facilitate better dissolution of chitosan in distilled water). Finally, the two prepared solutions were combined and transferred to the rotary device for concentration. After the rotary process, 20 milliliters of thyme essential oil was combined with the solution obtained from the rotary (0.227 grams) and placed on a heater for 2 minutes for proper mixing. Ultimately, the desired solution was exposed to ultrasound waves for 5 minutes (Noori et al., 2018).

Preparation of Essential Oil Emulsion with Tween 80

To prepare the Tween 80 emulsion, a similar method as the previous ones was used. However, in this method, 0.227 grams of thyme essential oil were combined with 0.068 grams of Tween 80. Then, the mixture was diluted with 65 milliliters of distilled water (Noori et al., 2018).

Measurement of Emulsion Particle Size Using DLS

The particle size (droplet size) of the emulsion was measured using a DLS device (ZEN3600, England). To perform this task, a few drops of the sample were diluted with distilled water at a ratio of one to one thousand and added to a special cell up to the marked line. Then, the cell was placed inside the device, and the corresponding graphs and values were obtained using the software installed on a computer connected to the laser sizer (Noori et al., 2018).

Rheology Tests of the Emulsion

Rheology tests were conducted using a rheometer device (Modular Compact Rheometer, Austria) equipped with coaxial cylinder geometry (with a height of 40 millimeters) at a temperature of 20 ℃. In this test, the relationship between shear stress and viscosity was evaluated. The shear rate also increases linearly from 1 to 100 s^{-1} (Salehi & Kashaninejad, 2015).

RESULTS AND DISCUTION

Morphology analysis of particles produced using SEM

The results related to the morphology analysis of chitosan-zein corn particles produced using a vacuum concentrator and a spray dryer nano device using scanning electron microscopy (SEM) are shown in Figure (4). The images obtained from the scanning electron microscope provide valuable information about the interaction and dispersion between the constituent components of the particles. As shown in Figure (4), the image corresponds to the powder obtained from the spray dryer nano. In some areas, the particles obtained from the spray dryer nano have been reduced to the size of a small nano particle and are easily distinguishable. However, in the image corresponding to the powder obtained from the vacuum concentrator, no separation is shown, and a homogeneous and uniform structure is formed. Considering that chitosan particles have a high adhesive property and cause agglomeration in the powder (Aranaz et al., 2021), Alizade et al. (2017) evaluated the physical and antimicrobial properties

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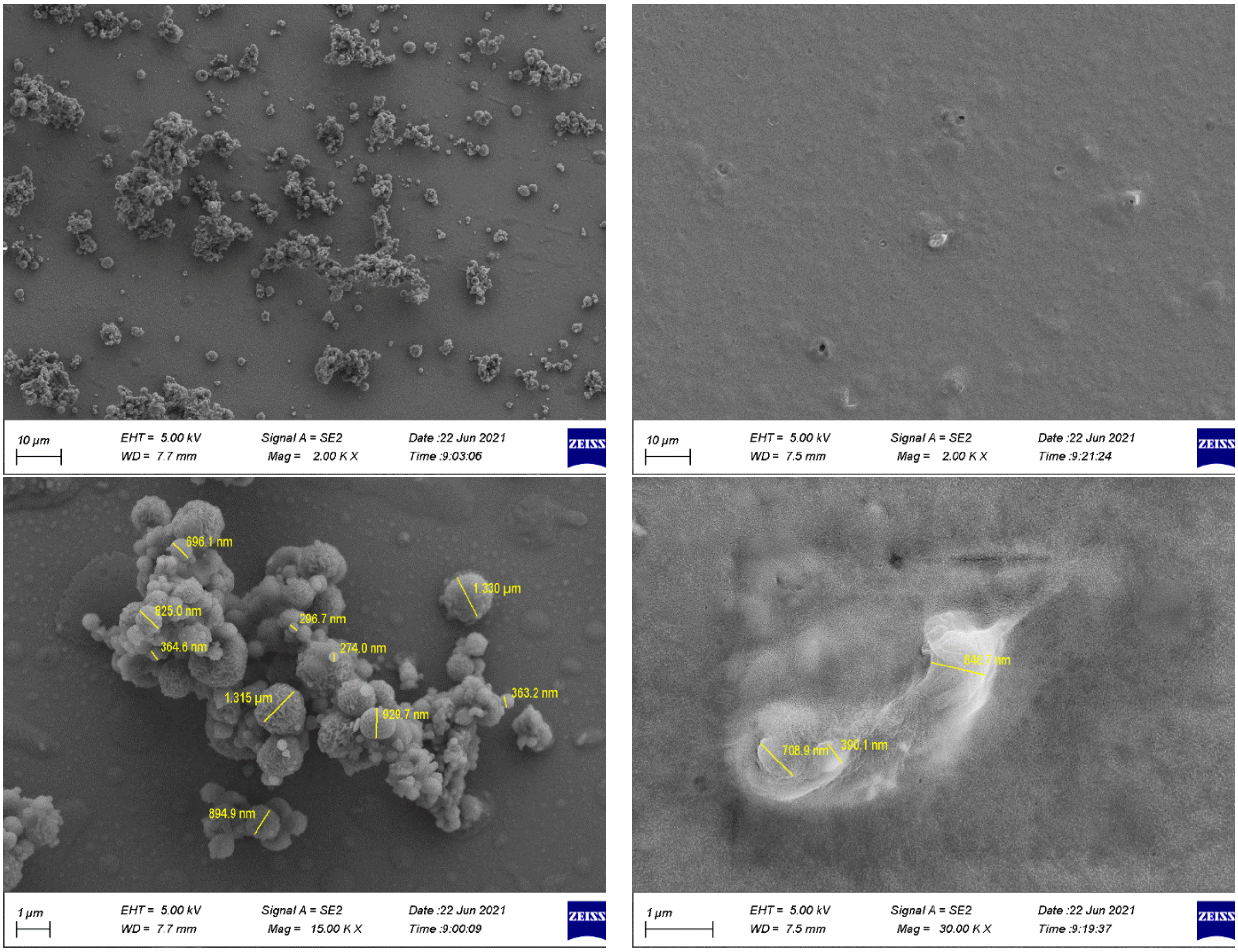
of chitosan edible films containing ben gum essential oil. They stated in their research that adding ben gum essential oil to the produced edible films resulted in reduced cohesion and increased roughness of the chitosan films. Jouki et al. (2014) stated that adding essential oil to edible films leads to the heterogeneity of the film structure. Mahdizadeh et al. (2020) evaluated the antioxidant properties of chitosan-zein edible films containing free and encapsulated hydroalcoholic extract of desert truffle. The results of their research showed that the chitosan-zein structure is smooth and uniform, and there is no significant accumulation of nanoparticles in the microstructure. Albadarin et al. (2017) and Baldrick (2010) stated in their research that chitosan particles are irregular, with an average size of approximately 30 micrometers and a rough surface with pores. The table related to the percentage of constituent elements of the powders obtained from the spray dryer nano and the vacuum concentrator, along with the Avon, is presented in Table (1). These elements are part of the SEM data. The results showed that the highest element in both particles was carbon, but the amount of this element was higher in the powder obtained from the vacuum concentrator than from the spray dryer nano. The amount of oxygen present in the powder obtained from the spray dryer nano was higher than that in the powder obtained from the vacuum concentrator. Furthermore, the results showed that phosphorus, potassium, and chlorine exist in the powder obtained from the spray dryer nano, but they are absent in the powder obtained from the vacuum concentrator. In the powder obtained from the vacuum concentrator, since chitosan is in solution form, it is placed on the surface after drying, and since the SEM measures surface elements, the phosphorus, potassium, and chlorine elements present in zein are not visible. However, in the powder obtained from the spray dryer nano, a compound is formed between zein and chitosan, and the above elements are visible on the surface. The presence of these elements is also due to the incomplete purity of zein



a b

Figure 5. The surface cross-sectional image of chitosan-zein corn particles produced using a spray dryer nano device (a) and a rotary device (b) is shown in the scanning electron microscopy (SEM) image

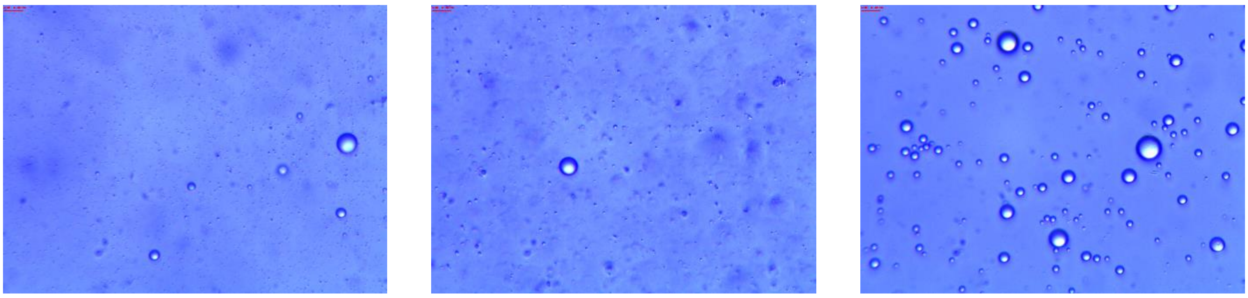
Table 1. The constituent elements of the powders obtained from the spray dryer nano device and the rotary device

Elements	Powders from the nano spray dryer (%)	Rotary (%)
C	69.33	79.62
O	25.44	17.75
Na	1.62	0.68
Si	2.25	1.56
K	0.28	-
Ca	0.28	0.15
S	0.24	0.09
Cl	0.14	-
Mg	0.30	0.14
P	0.13	-

Results of particle measurement in emulsion using DLS

One of the non-destructive physical methods for determining the distribution and size of particles present in solutions and emulsions is the use of dynamic light scattering (DLS) method. This method utilizes visible light radiation. Generally, DLS measures the scattered light intensity present in the suspension. Figure (5) shows a representation of the produced emulsions. Microscopic images depict the conditions of three emulsions. According to Figure 6, observable particles are present in the emulsion prepared from powder, while the concentrated emulsion contains more dissolved particles. Therefore, emulsion characterization is feasible in the powdered sample. In Figure (7a), the particle size of the produced emulsions, using spray drying, vacuum concentrator, and Tween 80 methods is shown. As observed in this figure, the largest particle size belongs to the emulsion produced using the powder obtained from the nano spray dryer, while the smallest particle size belongs to the emulsion produced using Tween 80. Furthermore, no significant difference ($p>0.05$) was observed among the samples. The DLS analysis includes a parameter called polydispersity index (PdI), which indicates the uniformity or non-uniformity of particle dispersion. In Figure (7b), values less than 1.0 indicate lower polydispersity. This parameter signifies that particle dispersion within the solution is uniform and acceptable. As shown in Figure (7b), the minimum polydispersity belongs to the samples produced using the powder obtained from the vacuum concentrator, while the maximum polydispersity belongs to the samples produced using the nano spray dryer. The polydispersity of the emulsion samples produced using the powder from the nano spray dryer and Tween 80 showed no significant difference at the ($p<0.05$) level. However, the polydispersity of the sample produced using the powder from the vacuum concentrator had a significant difference compared to the other samples. Figure (7c) relates to agglomerated particles (particle aggregation). This phenomenon occurs when the polydispersity is greater than 0.5. This parameter indicates poor dispersion of particles in the produced emulsion samples, and the results are unacceptable. As shown in Figure (7c), the highest value of this parameter was observed in the emulsion produced using the nano spray dryer powder, while the lowest value was observed in the sample produced using the vacuum concentrator. Significant differences ($p>0.05$) were observed among the emulsion samples produced from the powder obtained from the vacuum concentrator and the nano spray dryer. Tomazelli Júnior et al. (2017) investigated the microencapsulation of lavender essential oil using a nano spray dryer. The results of their study showed that the average particle size produced using the nano spray dryer was 10.37 microns. Bhati et al. (2021) studied the antibacterial effect of ethanol extracts of lavender and cinnamon in the treatment of vulgaris acne. Their results showed that the vesicle size of lavender and cinnamon measured by DLS was in the range of 290-421 nanometers and 249-325 nanometers, respectively. Razavi Zadeh et al. (2015) examined the physical and chemical properties of rice bran oil microemulsion in water. They initially prepared the rice bran oil emulsion in water using two biopolymers, arabic gum and whey protein, in various ratios, with and without the use of Tween 80, and with or without the application of ultrasound waves. They reported that the size of the emulsion droplets decreased with an increase in the concentration of Tween 80. The results of their study were consistent with the findings of the present study.

Figure 6. Left side: Emulsion produced using powder obtained from the spray dryer. Middle: Emulsion produced using powder obtained from the vacuum concentrator. Right side: Emulsion produced using Tween 80



A B C

Figure 7. A: Emulsion produced using powder obtained from the vacuum concentrator. B: Emulsion produced using powder obtained from the spray dryer. C: Emulsion produced using Tween 80

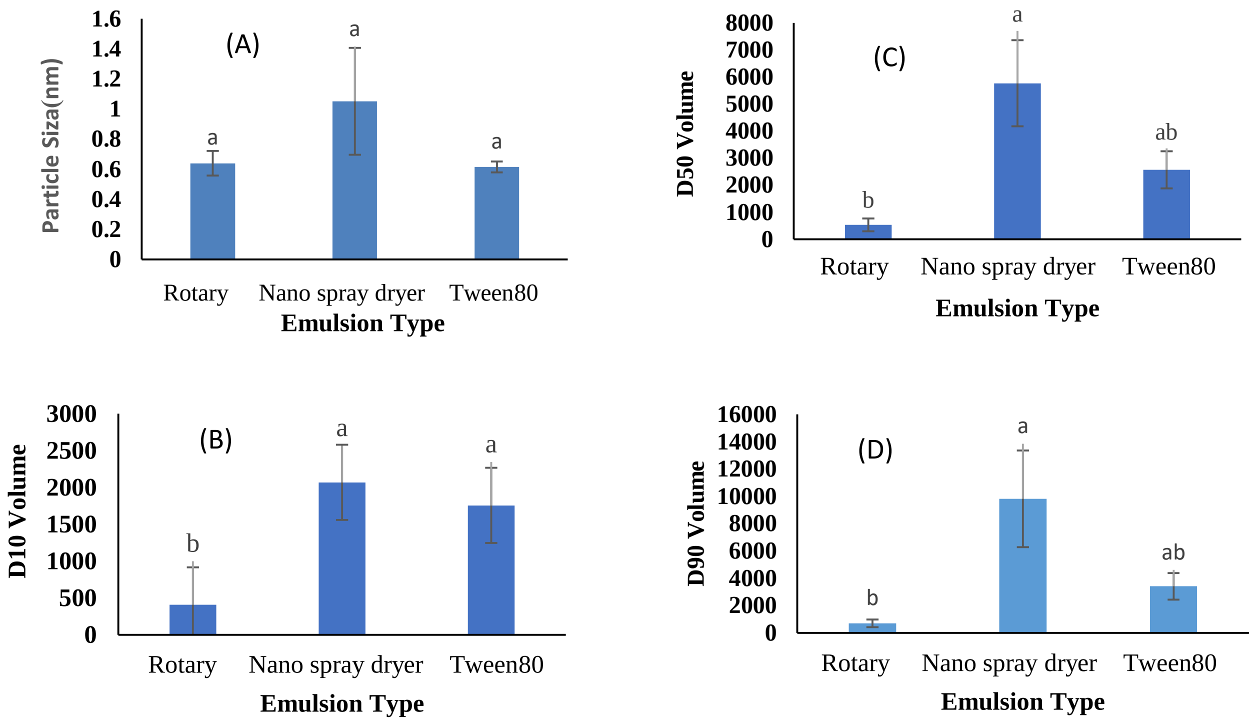


Figure 8. The effect of emulsion type on particle dispersion (particle size (A), D10 Volume (B), D50 Volume (C), D90 Volume (D))

RESULTS OF THE RHEOLOGY TESTS

The results of the rheology tests for the emulsions are generally referred to as colloidal suspensions, which consist of at least two immiscible liquids that cannot mix spontaneously. Emulsions are formed by dispersed droplets of one liquid (the internal phase) in another liquid (the continuous phase) (Gutiérrez et al., 2008). Many properties and characteristics of emulsions, such as stability, rheology, appearance, color, and texture, depend on the droplet size and size distribution of the emulsion (McClements, 2004). In this study, the rheological behavior of the emulsions prepared using a rheometer was determined, and the variations in shear stress and viscosity with shear rate are shown in Figures 8 and 9, respectively. The results indicate that the obtained emulsions exhibit Newtonian-like behavior over a wide range of shear rates. This can be justified based on the changes in shear stress with respect to the linear shear rate, as shown in Figure 8, where viscosity remains nearly constant with increasing shear rate, which is consistent with the findings of previous sections. Viscosity is influenced by xanthan gum and chitosan. In Figure 9, in the concentrated sample obtained from the vacuum concentrator, chitosan chains are dispersed in the interstitial space, and chitosan is present freely in the solution, leading to increased viscosity. However, in the sample obtained from the spray dryer, chitosan is combined with xanthan gum, resulting in a particle-like state and less solubility of chitosan, leading to lower dispersibility compared to the concentrated sample from the vacuum concentrator. In the Tween sample, due to the absence of chitosan and xanthan gum, the viscosity is lower than the other samples.

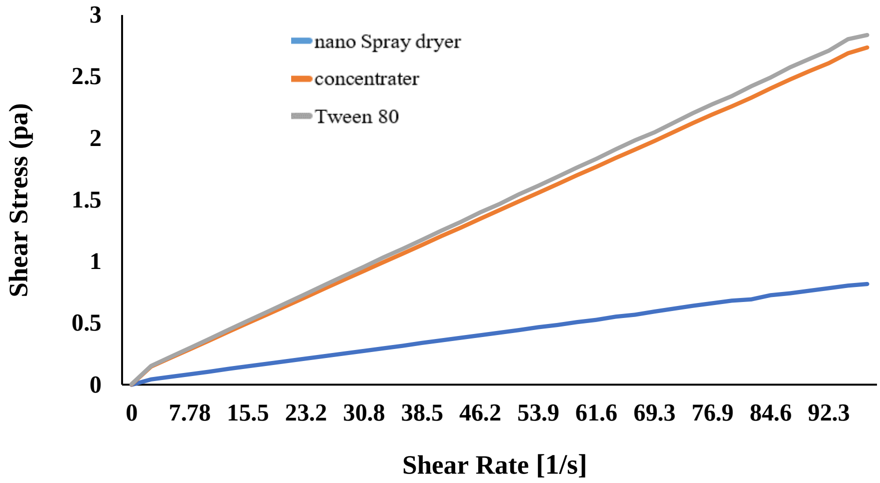


Figure 9. The relationship between shear stress and shear rate for the produced emulsions obtained from Tween 80, rotary, and spray dryer methods

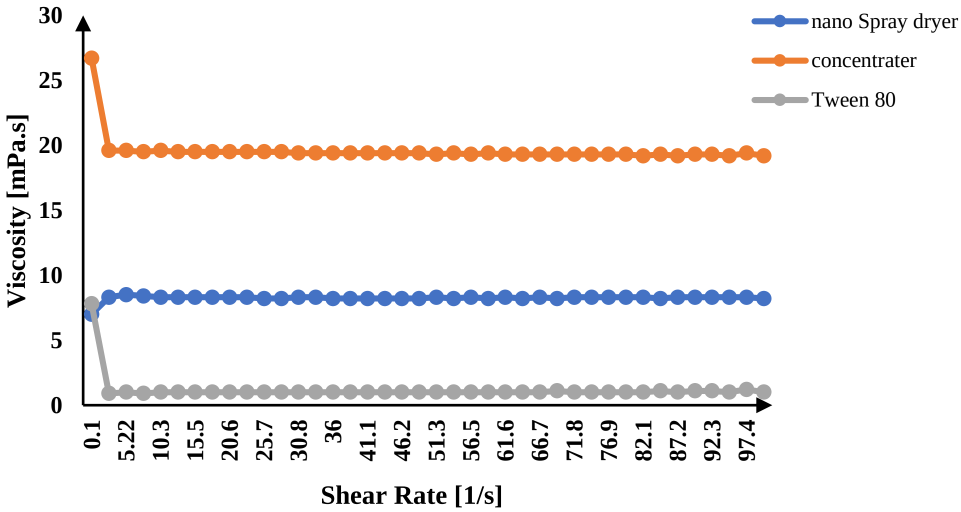


Figure 10. The relationship between viscosity and shear rate for the produced emulsions obtained from Tween 80, vacuum concentrator, and nano spray dryer methods

CONCLUSIONS

The results regarding the morphology of chitosan-zein particles using scanning electron microscopy (SEM) showed that the particles obtained from the nano spray dryer were well-separated, while the particles obtained from the rotary method appeared agglomerated. Particle size measurements indicated that the largest particle size was observed in the emulsion produced using the spray dryer. In this study, the rheological behavior of the prepared emulsions demonstrated an increase in shear stress with an increase in shear rate for all emulsion samples.

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