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Factors influencing the properties of zein nanoparticles encapsulated with fragrances prepared by liquid-liquid dispersion

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ABSTRACT

The duration of the fragrance is one of the factors that influences a customer's choice of fabric softeners. Fragrances, a mixture of various aromatic compounds, usually present low solubility and stability in the environment, so they do not last long. Micro/nanoencapsulation technology of fragrances can be used to solve this problem. This research studied the factors influencing the preparation of zein nanoencapsulation with fragrances. Fruity fragrances were encapsulated in zein nanoparticles (PF-ZNs) by the liquid-liquid dispersion method, using Tween 20 as a surfactant. The effects of zein and ethanol concentrations of 0.4%–0.8% and 70%–85%, respectively, homogenized at 15,000 rpm for 5–15 min on zein encapsulation, were investigated. The fruity fragrance was loaded at 30% of the zein content. Increased zein concentration resulted in increased particle size with decreased zeta potential. Particle agglomeration was detected when the ethanol concentration was decreased from 85% to 75%. Compared to using a vacuum concentrator centrifuge, the zein nanoparticles agglomerated less when freeze-dried. The encapsulation efficiency of the fruity fragrance was 39.7%–68.4%, and the yield percentage was 54.5%–72.3% when freeze-drying was used.

Keywords: fragrances; encapsulation efficiency; liquid-liquid dispersion; nanoencapsulation; zein

1. INTRODUCTION

Zein is a water-insoluble plant protein derived from corn (*Zea mays* L.), which can be described as a prolamin-rich, alcohol-soluble protein (Pascoli et al., 2018; Podaralla and Perumal, 2012). The hydrophobicity of zein results from the prolamin groups and the high content of non-polar amino acids such as alanine, proline, glutamine, asparaginase, and leucine (Elzoghby, 2015). The global corn market was worth 895 million USD in 2022 and is predicted to increase at a compound annual growth rate (CAGR) of 10% from 2023 to 2032 (Global Market Insights, 2023). Zein is soluble in binary solvents composed of lower aliphatic alcohols, such as methanol, isopropanol, and ethanol solution, with concentrations ranging from 55% to 90% (v/v) (Elzoghby, 2015; Zhong and Jin, 2009). Zein is widely used in the food, drug, cosmetics, and textile sectors because it can form potential biomaterials, such as films, plastics, and spheres, that can be easily applied to many products (Guo et al., 2020). Zein can also self-assemble into nano/microparticles that form various mesostructures, which have been used for encapsulation (Zou et al., 2017). Many techniques, such as phase separation techniques (Zou et al., 2017), coacervation (Tiwari et al., 2020), anti-solvent precipitation (Li et al., 2017), and liquid-liquid dispersion (Pithanthanakul et al., 2021), are used for the encapsulation of colloidal particles in a zein base. The generation of these agglomerations depends on the solubility of zein in binary solvent mixtures.

Nanotechnology is defined as the creation and application of nanoscale materials, mechanisms, or systems to overcome the insolubility in water and limited oral bioavailability of bioactive components (Pateiro et al., 2021). Developing nanosized carriers (with sizes from 10 to 1000 nm) can improve the antioxidation, ease of handling, and solubility of the encapsulated compounds (Rungsardthong et al., 2021). Liquid-liquid dispersion is a nano-encapsulation technique for zein nanoparticle (ZN) preparation (Zhong and Jin, 2009). The technique uses the differences in zein solubility in water and ethanol to generate nanoparticles. The interaction of alcohol and water reduces the ethanol concentration and zein solubility, forming nanoparticles (Pascoli et al., 2018; Zou et al., 2017). Zhong and Jin (2009) have reported that the zein concentration and the amount of ethanol in the stock solution are the primary factors influencing the formation of colloidal particles by liquid-liquid dispersion. Several previous works have revealed that the solubility of zein can be used to generate nano or microcapsule zein, which can then be used to encapsulate substances and improve their stability (Hosseini et al., 2021; Pithanthanakul et al., 2021; Rodsuwan et al., 2021; Suwannasang et al., 2021; Tortorella et al., 2021).

A fragrance is a mixture of volatile aromatic compounds (mostly synthetic compounds), essential oils, or complexes of hydrocarbons, esters, alcohols, ketones, and ethers (Manayi and Saeidnia, 2014). Fragrances are widely used in products used daily, such as personal care products, household cleaning products, and textiles (He et al., 2018; Lee et al., 2016). However, fragrances are sensitive to air, light, and high temperatures when stored for long periods (Lopes et al., 2019). Encapsulating fragrances in a polymeric membrane is the best solution to this problem (Ghayempour and Montazer, 2016). Various wall materials have been used for the micro/nanoencapsulation of fragrances, such as chitosan, gelatin, gum arabic, sodium carboxymethyl cellulose, zein, and poly(methyl methacrylate) (Šumiga et al., 2019; Tang et al., 2019; Ban et al., 2020; Liu et al., 2020; Pithanthanakul et al., 2021, 2023). Pithanthanakul et al. (2021) have studied the encapsulation of two types of fragrances using ZNs by liquid-liquid dispersion. The results showed that the average particle size of fragrances encapsulated with the average particle size of ZNs was 200 to 300 nm and had a low encapsulation efficiency (EE), <70%.

The chemical and mechanical properties of ZN can be customized by adjusting the initial zein concentration, alcohol concentration, drying conditions, pH, and ionic strength (Pascoli et al., 2018; Tortorella et al., 2021). Podaralla and Perumal (2012) have demonstrated that the pH and zein concentration are crucial in controlling particle size. Many studies have focused on characterizing colloids prepared from ZNs, while ZNs were preferentially applied in powder form (Anderson and Lamsal, 2011).

Freeze-drying is a drying process that causes minimal physical and chemical disruption to bioactive substances. It is known as the drying method that yields the highest quality of dried materials because they are dried at low temperatures (Suwannasang et al., 2021). Nonetheless, freeze-drying may cause morphological changes in particles, affecting their size, and altering the pH levels of the suspension, which might also affect their morphology (Rodsuwan et al., 2021; Zhong and Jin, 2009). A speed vacuum concentrator centrifuge (vacuum concentrator) is a unique tool for drying biological and non-biological materials or removing solvents from samples to concentrate them. The main advantage of a vacuum concentrator is that only a very small sample volume is required (Irudayam and Malathi, 2005). Very few studies have been reported on the effect of using a vacuum concentrator for drying micro/nanocapsules.

Anarjan et al. (2015) have shown that the duration of homogenization could induce adverse changes in the physicochemical properties of astaxanthin in a dispersion. Furthermore, studies on the effect of preparation factors on the characteristics of ZNs after drying are needed for several potential applications, including those of the pharmaceutical, food packaging, and textile industries. Therefore, this research aimed to enhance encapsulation in ZNs using the liquid-liquid dispersion technique and study the factors influencing the particle size, morphology, EE, and yield percentage of the resulting nanoparticles.

2. MATERIALS AND METHODS

2.1 Materials

Purified zein (98%) was purchased from Sigma-Aldrich (St Louis, MO, USA). The commercial fragrances, i.e., the fruity fragrances (PF), were provided by Thai-China Flavours and Fragrances Industry Co., Ltd. (Ayutthaya, Thailand). The main components of PF were phenyl ethyl alcohol, terpineol, and geraniol, which gave a soft and sweet rose floral odor. Tween 20 was supplied by Union Chemical 1986 Co., Ltd. (Bangkok, Thailand). Citric acid anhydrous ($C_6H_8O_7$) and tri-sodium citrate ($Na_3C_6H_5O_7$) were acquired from Ajax Fine Chem Pty., Ltd. (Auckland, New Zealand). All other chemicals used were of analytical grade.

2.2 Preparation of ZNs by liquid-liquid dispersion

The effects of zein concentration on the particle size and zeta potential of ZNs were investigated. ZNs were prepared by liquid-liquid dispersion following the methods of Pithanthanakul et al. (2021) with slight modifications. Zein solutions at various concentrations, 0.4%, 0.6%, and 0.8% (w/v), were prepared by dissolving zein in 85% ethanol (v/v) and stirring at 400 rpm for 30 min at room temperature. After that, 20 mL of the mixture was poured into 60 mL of 0.1 M pH 8 citrate buffer (aqueous phase containing Tween 20 with a zein:Tween 20 ratio of 5:0.25 w/w). The suspension was sheared using a high-speed homogenizer (Ika Ltd., Staufen, Germany) at 15,000 rpm for 10 min. Then, the ethanol in the dispersion was evaporated using a rotary vacuum evaporator (Buchi Rotavapor R-215, Buchi Ltd., Flawil, Switzerland) with a pressure of 175 mbar, at 60 °C for 30 min.

The colloidal samples were centrifuged at 9,000 ×g for 50 min in a high-speed centrifuge (MX-301, Tomy Ltd., Tokyo, Japan). The supernatant was discarded, and the precipitate was rinsed three times with 5 mL of citrate solution. The samples were frozen before being freeze-dried (Alpha 1-4 LSCplus, Martin Christ, Osterode am Harz, Germany) at a pressure of 0.022 bar for 24 h at −50 °C.

2.3 Effect of ethanol percentage

The 0.8% zein solution (w/v) was dissolved in 70%, 75%, 80%, and 85% (v/v) ethanol and prepared according to the method specified in section 2.2. The morphology of the nanoparticles obtained after freeze-drying was determined using a scanning electron microscope (JSM-6610LV, JEOL, Tokyo, Japan).

2.4 Effect of drying methods

The frozen ZN samples were dried for 24 h using two techniques: a speed vacuum concentrator (Univapo 100 ECH, Uniequip Ltd., Planegg, Germany) at 40 °C and the freeze-drying method described above.

2.5 Preparation of PF-ZNs

PF-ZNs were prepared by liquid-liquid dispersion following the guidelines of Pithanthanakul et al. (2021) with slight modification. ZNs were prepared with 30% PF loading by adding 0.08 µL of PF dropwise into 20 mL of 0.4% zein (w/v) in 85% ethanol and stirring at 400 rpm for 10 min. Then, 20 mL of the mixed zein and fragrances was poured into 60 mL of citrate solution (pH 8.0) and homogenized at 15,000 rpm. The effect of homogenization time was studied at 5, 10, and 15 min. The ethanol in the PF-ZNs containing dispersed fragrances was evaporated at 60 °C for 30 min before centrifugation at 9,000 ×g for 50 min to remove the free fragrance. As described above, the precipitate was dissolved in citrate solution at pH 8 and dried using a freeze-dryer.

2.6 Characterization of ZNs and PF-ZNs

The particle size distribution, zeta potential, and polydispersity index (PDI) of the ZNs in the suspensions were analyzed using a Zetasizer Nano (Delsa™ Nano C, Beckman Coulter Ltd., Tokyo, Japan). All measurements were performed in triplicate. The morphology of the ZNs and PF-ZNs was observed using a scanning electron microscope (SEM, JSM-6610LV, JEOL Ltd., Tokyo, Japan) with an acceleration voltage of 15 kV. All samples were mounted on specimen stubs and coated with thin (20 nm) conductive gold using a sputter coater before investigation (JFC-1200 Fine Coater, JEOL Ltd., Tokyo, Japan). Digital images of the samples were obtained and representative images presented. The average particle size was measured from the SEM images (n<300) using the ImageJ software (Schneider et al., 2012).

2.7 Determining EE and yield percentage of PF-ZNs

Gas chromatography was used to examine the EE of the PF-ZNs. First, 10 mL of each sample was mixed with 20 mL of ethanol, and the ZNs were extracted by centrifugation at 5,000 rpm at 4 °C. Then, 1 µL of the supernatant was injected into the gas chromatograph (Agilent Hewlett Packard, Model G1530A, USA) with an FID using an HP-5MS fused silica capillary column (I.D. = 0.32 mm, length = 25 m, and 0.5 m film thickness with dimethylpolysiloxane). The carrier gas was N_2 at a constant flow rate of 1 mL/min, with the column temperature starting at 60 °C and rising to 250 °C at 10 °C/min, and held at 250 °C for 10 min. The fragrance calculation was based on the area of the peak of the main component of the fragrance, phenyl ethyl alcohol, which had a retention time of 10.45 min. The EE and particle yield were calculated using Equation 1 and 2:

$$\text{EE}\ (\%) = \left(\frac{A}{B}\right) \times 100\% \qquad (1)$$

where A is the total amount of PF in nanoparticles and B is the total amount of PF used for nanoparticle preparation.

$$Particle\ yield(\%) = \left(\frac{C}{D}\right) \times 100\% \qquad (2)$$

Where C is the mass of the nanoparticles, and D is the total PF and zein used for nanoparticle preparation.

2.8 Statistical analysis

The statistical analysis was performed using analysis of variance (ANOVA) with SPSS 11.6 for Windows (SPSS Inc., Chicago, Ill, USA). The differences between the mean values were determined with Duncan's multiple range test at p<0.05. All parameters were measured in triplicate.

3. RESULTS AND DISCUSSION

3.1 Effect of zein concentration

Figure 1 shows the particle size distribution and zeta potential of ZNs prepared at 0.4%, 0.6%, and 0.8% (w/v) zein dissolved in 85% ethanol and citrate solution (pH 8). Figure 1A shows that with increasing zein concentration, the particle size of ZNs, determined using dynamic light scattering, increased from 123.4±5.6 to 154.6±3.9 and 204.1±0.8 nm, respectively. Thus, the zein concentration is one of the main factors contributing to the particle size distribution. The dispersion and sedimentation of zein were affected by the viscosity of the solution, which increases with increasing zein concentration. Increasing the viscosity makes it hard to form small droplets during liquid-liquid dispersion. Increasing the viscosity of the zein solution in the dispersion phase was expected to reduce its solubility in the solvent, as the dissolution from the colloidal particles to form the homogeneous substance decreased, resulting in the precipitation of zein protein and rapid formation of larger particles during the process (Alargova et al., 2006; Zhong and Jin, 2009).

Figure 1B shows the decrease in the negative zeta potential of ZNs observed with increased zein concentration. The zeta potential of ZNs decreased from −21.8±0.3 to −19.5±0.2 mV, respectively. The values of the zeta potential (positive or negative) can be used to evaluate the charge stability of a particle suspension and predict susceptibility to aggregate formation (Honary and Zahir, 2013; Vigneshkumar et al., 2022). A greater electric charge on the surface of the nanoparticles inhibits aggregation in a buffer solution because of the strong repelling forces between the particles. Zeta potential values between −30 and +30 mV indicate instability or aggregate. However, values in the range of −20 to −20 mV represent short-term stability, and those in the range of −5 to +5 mV are associated with the rapid aggregation of particles (Honary and Zahir, 2013). The ZNs were rather unstable, demonstrated by a zeta potential of −20 mV. This instability could be attributed to the use of pure Tween 80 as a surfactant. A high zeta potential (i.e., over −30 mV) was observed in this study, compared to those reported by Suwannasang et al. (2021) and Rodsuwan et al. (2021), who encapsulated bioactive compounds with ZNs in liquid-liquid dispersion using a mixed surfactant of Tween 80 and lecithin. The mixed surfactant prevented particle aggregation by increasing the electrostatic interactions between the ZNs because the lecithin acted on the phospholipid monolayer as an anionic surfactant (Podaralla and Perumal, 2012).

3.2 Mechanical properties of bubble shells

The effect of the ethanol–water ratio on particle morphology was studied using 0.8% (w/v) zein solution prepared with a homogenizer at 5,000 rpm. Figure 2 shows the distribution curves and that the average hydrodynamic sizes of ZNs prepared with 70%, 75%, 80%, and 85% (v/v) ethanol were 2.55±0.7, 2.24±0.9, 2.19±0.6, and 2.19±0.5 µm, respectively.

The ZNs prepared with 80% ethanol (Figure 2C) had the same average particle size as those prepared with 85% ethanol (Figure 2D). In comparison, the average hydrodynamic size distribution and SEM image of ZNs at 85% ethanol display fewer particle agglomerations compared with the ZNs from 80% ethanol. The SEM image also shows that the particle diameter decreased with increased ethanol concentration. This observation might be due to the formation of particles during the homogenization of systems with low ethanol concentrations. Larger particles and aggregates occur at low ethanol concentrations because of the high concentration of zein protein in solutions (Zhong and Jin, 2009).

In addition, the highest ethanol percentage (85% ethanol) reduced the coalescence of particles. The morphology of the ZNs observed using SEM agreed with the hydrodynamic size distribution presented in Figure 2. These results show that the ethanol–water ratio plays a crucial role in the aggregation and disaggregation processes of the zein solution.

3.3 Effect of drying method on ZN morphology

The surfaces of nanoparticles prepared with a citrate solution (pH 8) after drying with a freeze-dryer and speed vacuum concentrator for 24 h are shown in Figure 3. These SEM images reveal that after freeze-drying, the particles were ZN powder because they were frozen to around −45 °C, and the sublimation of spherical particles with diameters of 50 to 150 nm prevented agglomeration between the particles. The samples were dried on ice under vacuum conditions. The particles obtained from freeze-drying were spherical with less agglomeration than those obtained using the speed vacuum concentrator. The water from the sample was evaporated using a speed vacuum concentrator at a temperature of about 40 °C (Correia et al., 2009), resulting in more homogeneous particles, as shown in Figure 3B. Protein denaturation generally occurs more frequently in a speed vacuum concentrator (Lepock et al., 1993).

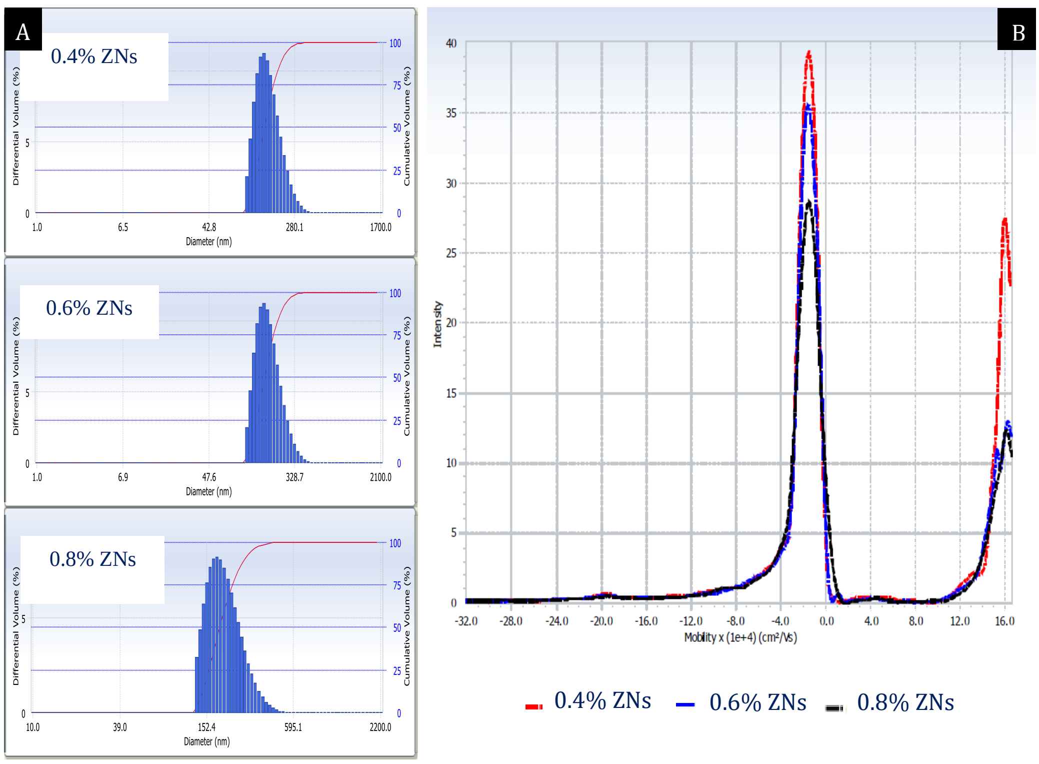

Figure 1. (A) Particle size distribution and (B) zeta potential of zein nanoparticles (ZN) prepared at 0.4%, 0.6%, and 0.8% zein

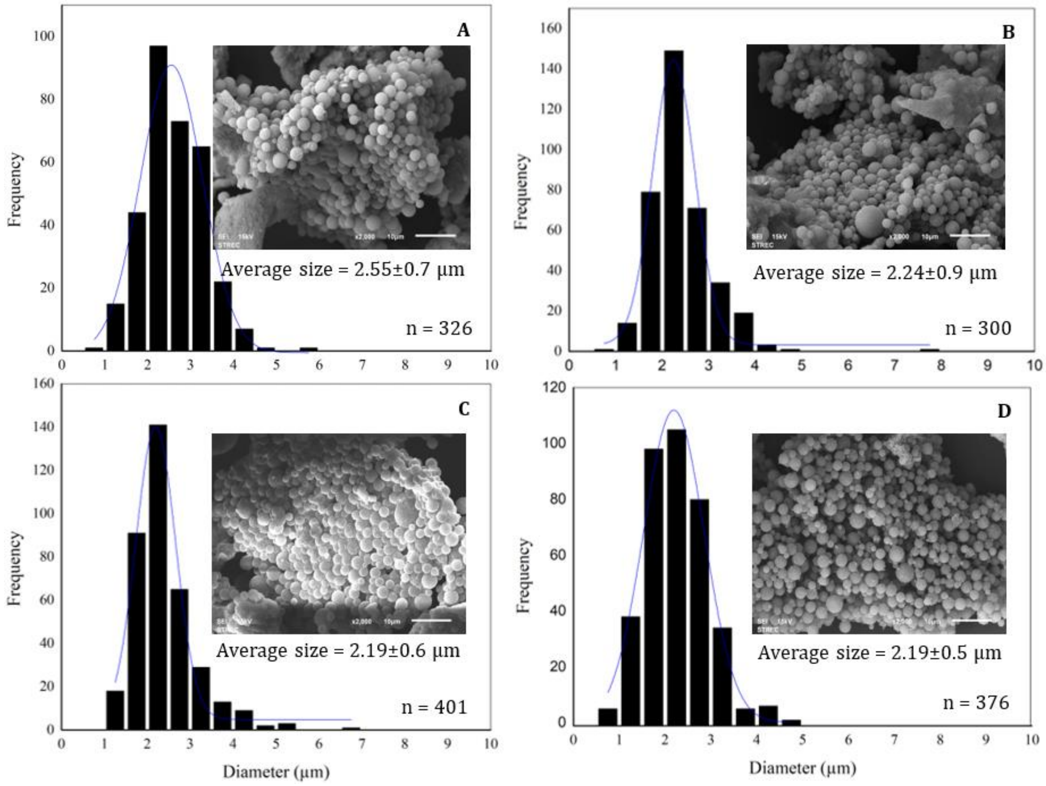

Figure 2. Hydrodynamic size distribution of zein nanoparticles prepared from (A) 70%, (B) 75%, (C) 80%, and (D) 85% ethanol and their SEM images (2000×)

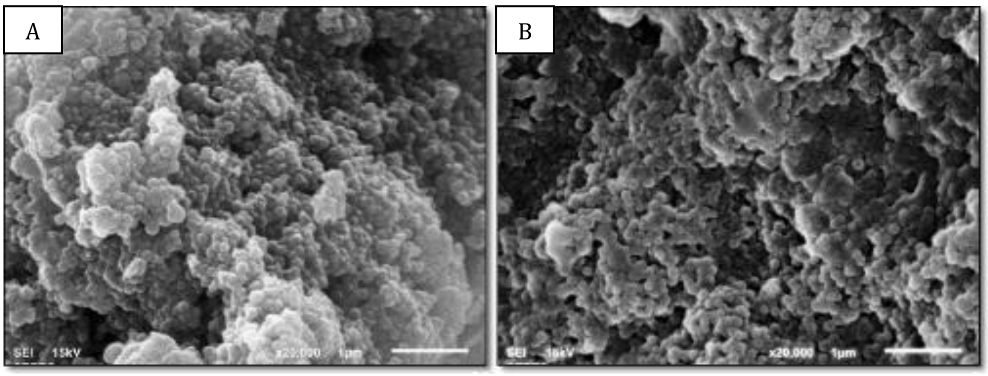

Figure 3. SEM images of zein nanoparticles at pH 8 after (A) freeze-drying and (B) centrifugal vacuum concentration (20000×)

3.4 Characterization of PF-ZNs

The effect of homogenizing at 15,000 rpm for 5, 10, and 15 min on the emulsion stability, surface morphology, particle size, PDI, EE, and yield of PF-ZN formation was studied.

3.4.1 Surface morphology

SEM images of PF-ZNs homogenized for 5, 10, and 15 minutes are presented in Figure 4. In these three samples, most particles were spherical with diameters of around 200 nm. These results reveal that shorter-duration homogenization influenced the aggregation of the PF-ZNs. Zhong and Jin (2009) have reported the effect of the shear rate due to homogenization at 5,000, 10,000, and 15,000 rpm on the formation of ZNs. The particle size decreased with increased shear rate, resulting in a particle diameter of less than 200 nm. However, the agglomerated ZNs prepared at 15,000 rpm might be due to the high shear rate of homogenization for 2 min (Namira et al., 2021). This study showed that the number of connected particles decreased with an increased homogenization time. The results support the theory that increasing the homogenization time increases homogeneity, leading to higher stability emulsions (Namira et al., 2021).

3.4.2 Particle size and PDI

The dynamic light scattering (DLS) technique was used to estimate the mean particle size of freshly prepared PF-ZNs in solvent before freeze-drying. As observed in Table 1, the duration of homogenization slightly influenced the particle size. Increasing homogenization time from 5 to 15 min significantly decreased the mean particle size and PDI ($p < 0.05$) from 344.5±3.6 to 158.2±3.7 nm and 0.35±0.1 to 0.06±0.0, respectively. The mean particle size of the PF-ZNs was larger than the particle size of the ZNs (100–150 nm), indicating that the PF was encapsulated in the ZNs. These results agree with Anarjan et al. (2015), who reported that the mean particle size of astaxanthin nanodispersions increased with homogenization time.

The homogenization time is the time spent mixing the aqueous and organic phases of the emulsion. Therefore, longer homogenization times increase the residence time, which could increase the total energy applied to the system. As a result, nanodispersions with large particle sizes can be produced (Anarjan et al., 2015). The PDI, calculated from the molecular weight distribution, describes particle agglomeration or aggregation. For the DLS technique, PDI values greater than 0.7 indicate that the particle size distribution in the sample is multimodal, whereas PDI values of less than 0.05 imply monodisperse particles (Pithanthanakul et al., 2021). The results of this study show that the sample prepared with short homogenization time had a high PDI and more aggregated particles.

Table 1. Effect of homogenization duration on characteristics of fruity fragrance encapsulated in zein nanoparticles (PF-ZNs)

Homogenization time (min)	Particle size (nm)	PDI	Encapsulation efficiency (%)	Yield (%)
5	344.5 ± 3.6^{c}	0.35 ± 0.1^{c}	39.7 ± 2.1^{a}	54.5 ± 3.3^{a}
10	219.1 ± 7.7^{b}	0.17 ± 0.0^{b}	68.4 ± 3.8^{b}	72.4 ± 2.4^{b}
15	158.2 ± 3.7^{a}	0.06 ± 0.0^{a}	72.5 ± 2.2^{c}	80.2 ± 2.2^{c}

Note: Letters in columns denote significant differences ($p < 0.05$)

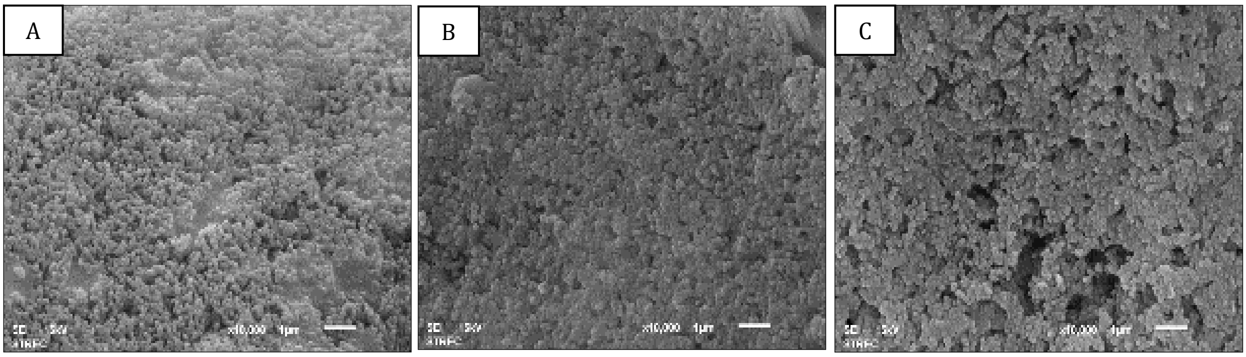

Figure 4. SEM images of fruity fragrance encapsulated in zein nanoparticles prepared by homogenization at (A) 5, (B) 10, and (C) 15 minutes

3.4.3 EE and yield percentage

The effects of homogenization time on the EE and yield of PF-ZNs are presented in Table 1. Increasing the homogenization time increased the EE and yield from 39.7% to 72.5% and 54.5% to 80.2%, respectively. These results might be due to the surface morphology of PF-ZNs, which required more time for aggregates to form. As a result, the particle surfaces present a film-like appearance after freeze-drying, as illustrated in its SEM image (Figure 2). Zein has a unique solubility and excellent film-forming properties because it has a high proportion of hydrophobic amino acids, such as leucine (19.3%), proline (9.0%), alanine (8.3%), and phenylalanine (6.8%) (Guo et al., 2020; Zhang et al., 2011).

Several previous studies on the encapsulation of volatile and non-volatile compounds with zein by liquid-liquid dispersion have shown high EEs, 80%–90% encapsulation of non-volatile compounds (Rodsuwan et al., 2021; Suwannasang et al., 2021; Zhong and Jin, 2009), whereas the EEs of volatile compounds have been only 55%–75% (Parris et al., 2005; Pithanthanakul et al., 2021; Rungsardthong et al., 2021). The volatile compounds quickly evaporated during encapsulation, which used a high temperature (60 °C) (Pithanthanakul et al., 2021; Wu et al., 2012). In addition, an EE lower than 80% might be because a high ethanol concentration (85%) could assist in dissolving the PF in ethanol. However, the morphology of the PF-ZNs showed that more agglomeration occurred when lower ethanol concentrations were used, as shown in Figure 2. This result corresponded with that of Chulurks et al. (2021), who reported that increasing ethanol concentration from 0% to 10% (v/v) resulted in decreased encapsulation efficiency, from 59.9% to 49.9% and 63.7% to 60%, for the encapsulation of nicotine with β-cyclodextrin and methyl-β-cyclodextrin, respectively. These results suggest that the ethanol concentration of the system influenced EE because nicotine is very soluble in ethanol. The presence of ethanol causes nicotine to stay in a free form in the ethanol rather than forming an inclusion complex.

4. CONCLUSION

The number of possible applications for zein has grown dramatically due to its unique properties, such as solubility and filming ability. This biodegradable and inexpensive material has received strong endorsements in several fields. Liquid-liquid dispersion is a simple method for producing ZNs. In this study, zein concentration and homogenization time played a vital role in controlling the size of ZNs. ZNs and PF-ZNs have an average size of less than 300 nm. The surface morphology determined by SEM revealed that short homogenization times and a high ethanol concentration in the system could reduce the coalescence of PF-ZNs. The highest EE and yield for PF-ZNs were 68.4% and 72.39%, respectively. This information can be used for further industrial applications.

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REFERENCES

Alargova, R. G., Paunov, V. N., and Velev, O. D. (2006). Formation of polymer microrods in shear flow by emulsification - solvent attrition mechanism. *Langmuir*, 22(2), 765–774.

Anarjan, N., Jafarizadeh-Malmiri, H., Nehdi, I. A., Sbihi, H. M., Al-Resayes, S. I., and Tan, C. P. (2015). Effects of homogenization process parameters on physicochemical properties of astaxanthin nanodispersions prepared using a solvent-diffusion technique. *International Journal of Nanomedicine*, 10(1), 1109–1118.

Anderson, T. J., and Lamsal, B. P. (2011). Review: Zein extraction from corn, corn products, and coproducts and modifications for various applications: A review. *Cereal Chemistry*, 88(2), 159–173.

Ban, Z., Zhang, J., Li, L., Luo, Z., Wang, Y., Yuan, Q., Zhou, B., and Liu, H. (2020). Ginger essential oil-based microencapsulation as an efficient delivery system for the improvement of Jujube (*Ziziphus jujuba* Mill.) fruit quality. *Food Chemistry*, 306, 125628.

Chulurks, S., Jitapunkul, K., Katanyutanon, S., Toochinda, P., and Lawtrakul, L. (2021), Stability enhancement and skin permeation application of nicotine by forming inclusion complex with β-cyclodextrin and methyl-β-cyclodextrin. *Scientia Pharmaceutica*, 89(4), 43.

Correia, P., Leitão, A., and Beirão-da-Costa, M. L. (2009). The effect of drying temperatures on morphological and chemical properties of dried chestnuts flours. *Journal of Food Engineering*, 90(3), 325–332.

Elzoghby, A. O. (2015). Protein and peptide nanoparticles for drug delivery. In *Advances in Protein Chemistry and Structural Biology (Vol. 98)* (Donev, R., Ed.), pp. 169-221. Cambridge: Academic Press.

Ghayempour, S., and Montazer, M. (2016). Micro/nanoencapsulation of essential oils and fragrances: Focus on perfumed, antimicrobial, mosquito-repellent and medical textiles. *Journal of Microencapsulation*, 33(6), 497–510.

Global Market Insights. (2023). *Corn fiber market size competitive market share & forecast, 2023 – 2032*. ASDReports premium market research. [Online URL: https://www.asdreports.com/market-research-report-616711/corn-fiber-market-size-competitive-market-share-forecast] accessed on May 31, 2023.

Guo, X., Ren, C., Zhang, Y., Cui, H., and Shi, C. (2020). Stability of zein-based films and their mechanism of change during storage at different temperatures and relative humidity. *Journal of Food Processing and Preservation*, 44(9), e14671.

He, L., Hu, J., and Deng, W. (2018). Preparation and application of flavor and fragrance capsules. *Polymer Chemistry*, 9(40), 4926–4946.

Honary, S., and Zahir, F. (2013). Effect of zeta potential on the properties of nano-drug delivery systems-a review (part 2). *Tropical Journal of Pharmaceutical Research*, 12(2), 265–273.

Hosseini, F., Miri, M. A., Najafi, M., Soleimanifard, S., and Aran, M. (2021). Encapsulation of rosemary essential oil in zein by electrospinning technique. *Journal of Food Science*, 86(9), 4070–4086.

Irudayam, M. J., and Malathi, R. (2005). Methylxanthines binding with yeast total RNA in the presence of Mg^{2+}: A speed vac-centrifugation-facilitated solid-state Fourier transform infrared analysis. *Analytical Biochemistry*, 339(1), 179–181.

Lee, H., Choi, C.-H., Abbaspourrad, A., Wesner, C., Caggioni, M., Zhu, T., and Weitz, D. A. (2016). Encapsulation and enhanced retention of fragrance in polymer microcapsules. *ACS Applied Materials & Interfaces*, 8(6), 4007–4013.

Lepock, J. R., Frey, H. E., and Ritchie, K. P. (1993). Protein denaturation in intact hepatocytes and isolated cellular organelles during heat shock. *Journal of Cell Biology*, 122(6), 1267–1276.

Li, F., Chen, Y., Liu, S., Qi, J., Wang, W., Wang, C., Zhong, R., Chen, Z., Li, X., Guan, Y., Kong, W., and Zhang, Y. (2017). Size-controlled fabrication of zein nano/microparticles by modified anti-solvent precipitation with/without sodium caseinate. *International Journal of Nanomedicine*, 12, 8197–8209.

Liu, X., Huang, L., Chen, H., Qian, M. C., and Ji, H. (2020). Pore size matching up: A novel insight into cotton textile aromatic finishing. *Flavour and Fragrance Journal*, 35(2), 149–156.

Lopes, S., Afonso, C., Fernandes, I., Barreiro, M.-F., Costa, P., and Rodrigues, A. E. (2019). Chitosan-cellulose particles as delivery vehicles for limonene fragrance. *Industrial Crops and Products*, 139, 111407.

Manayi, A., and Saeidnia, S. (2014). Cosmetics and personal care products. In *Encyclopedia of Toxicology* (Wexler, P., Ed.), 3rd, pp. 1043–1049. Amsterdam: Elsevier.

Namira, Z. R., Paramita, V., and Kusumayanti, H. (2021). The effect of rotational speed of homogenization on emulsion results obtained using soy lecithin emulsifier. *Journal of Vocational Studies on Applied Research*, 3(1), 14–17.

Parris, N., Cooke, P. H., and Hicks, K. B. (2005). Encapsulation of essential oils in zein nanospherical particles. *Journal of Agricultural and Food Chemistry*, 53(12), 4788–4792.

Pascoli, M., de Lima, R., and Fraceto, L. F. (2018). Zein nanoparticles and strategies to improve colloidal stability: A mini-review. *Frontiers in Chemistry*, 6, 6.

Pateiro, M., Gómez, B., Munekata, P. E. S., Barba, F. J., Putnik, P., Kovačević, D. B., and Lorenzo, J. M. (2021). Nanoencapsulation of promising bioactive compounds to improve their absorption, stability, functionality and the appearance of the final food products. *Molecules*, 26(6), 1547.

Pithanthanakul, U., Rungsardthong, V., Kiatthanakorn, B.-O., Vatanyoopaisarn, S., Thumthanaruk, B., Chaiyasat, A., Uttapap, D., and Ding, Y. (2023). Visible light-based polymerization of poly (methyl methacrylate) encapsulated fragrances used for fabric softener applications. *Industrial Crops and Products*, 194, 116329.

Pithanthanakul, U., Vatanyoopaisarn, S., Thumthanaruk, B., Puttanlek, C., Uttapap, D., Kietthanakorn, B., and Rungsardthong, V. (2021). Encapsulation of fragrances in zein nanoparticles and use as fabric softener for textile application. *Flavour and Fragrance Journal*, 36(3), 365–373.

Podaralla, S., and Perumal, O. (2012). Influence of formulation factors on the preparation of zein nanoparticles. *AAPS PharmSciTech*, 13(3), 919–927.

Rodsuwan, U., Pithanthanakul, U., Thisayakorn, K., Uttapap, D., Boonpisuttinant, K., Vatanyoopaisarn, S., Thumthanaruk, B., and Rungsardthong, V. (2021). Preparation and characterization of gamma oryzanol loaded zein nanoparticles and its improved stability. *Food Science & Nutrition*, 9(2), 616–624.

Rungsardthong, V., Pithanthanakul, U., Puttanlek, C., Uttapap, D., and Boonpisuttinant, K. (2021). Preparation of puerarin-loaded zein nanoparticles: Characterization and stability study. *Journal of Current Science and Technology*, 11(1), 6070.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9(7), 671–675.

Šumiga, B., Šumiga, B., Ravnjak, D., and Podgornik, B. B. (2019). Antimicrobial paper coatings containing microencapsulated *Cymbopogon citratus* oil. *Coatings*, 9(8), 470.

Suwannasang, S., Thumthanaruk, B., Zhong, Q., Uttapap, D., Puttanlek, C., Vatanyoopaisarn, S., and Rungsardthong, V. (2021). The improved properties of zein encapsulating and stabilizing sacha inchi oil by surfactant combination of lecithin and tween 80. *Food and Bioprocess Technology*, 14(11), 2078–2090.

Tang, Y., Zhou, Y., Lan, X., Huang, D., Luo, T., Ji, J., Mafang, Z., Miao, X., Wang, H., and Wang, W. (2019). Electrospun gelatin nanofibers encapsulated with peppermint and chamomile essential oils as potential edible packaging. *Journal of Agricultural and Food Chemistry*, 67(8), 2227–2234.

Tiwari, P., Bharti, I., Bohidar, H. B., Quadir, S., Joshi, M. C., and Arfin, N. (2020). Complex coacervation and overcharging during interaction between hydrophobic zein and hydrophilic laponite in aqueous ethanol solution. *ACS Omega*, 5(51), 33064–33074.

Tortorella, S., Maturi, M., Buratti, V. V., Vozzolo, G., Locatelli, E., Sambri, L., and Franchini, M. C. (2021). Zein as a versatile biopolymer: Different shapes for different biomedical applications. *RSC Advances*, 11(62), 39004–39026.

Vigneshkumar, P. N., George, E., Joseph, J., John, F., and George, J. (2022). Liposomal Bionanomaterials for Nucleic Acid Delivery. In *Fundamentals of Bionanomaterials* (Barhoum, A., Jeevanandam, J., and Danquah M. K., Eds.), pp. 327–362. Amsterdam: Elsevier.

Wu, Y., Luo, Y., and Wang, Q. (2012). Antioxidant and antimicrobial properties of essential oils encapsulated in zein nanoparticles prepared by liquid–liquid dispersion method. *LWT - Food Science and Technology*. 48(2), 283–290.

Zhong, Q., and Jin, M. (2009). Zein nanoparticles produced by liquid–liquid dispersion. *Food Hydrocolloids*, 23(8), 2380–2387.

Zou, Y., Pan, R., Wan, Z., Guo, J., Wang, J., and Yang, X. (2017). Gel-like emulsions prepared with zein nanoparticles produced through phase separation from acetic acid solutions. *International Journal of Food Science & Technology*, 52(12), 2670–2676.