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Encapsulation of lycopene using electrospraying method

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ABSTRACT

In this study encapsulation of lycopene with zein as a biopolymer by using electrospraying method to enhance stability of lycopene was carried out. Effect of the encapsulation on the lycopene stability was investigated after exposure to UV light. The results were compared and discussed in terms of morphology and effectiveness of the encapsulation system for enhanced stability of lycopene. The results showed that encapsulation of lycopene using zein as biopolymer improved the stability of lycopene.

Keywords: *Lycopene, Encapsulation, Electrospraying method, Stability*.

1. INTRODUCTION

With the increasing interest in well living antioxidants become indispensable. By interacting with free radicals antioxidants act as a protector of the biomolecules. Antioxidants in right dosage drastically delays or prevents oxidation of the oxidizable substrate. Lycopene, which has a carotenoid structure, is one of the strongest natural antioxidants. Even though lycopene is chemically carotene, it does not contain vitamin A activity. Lycopene mostly found in tomatoes and other red fruits and vegetables with exception of strawberries and cherries [1].

Due to its strong red color first usage of lycopene has started with food industry for food coloring purposes (registered as E160d) and is approved for usage in the USA and the EU [2]. Several studies investigated to understand effects of lycopene more extensively. And it is found that lycopene have far more important properties. Such as its activity in the prevention of chronic diseases such as atherosclerosis, skin cancer and prostate cancer [3, 4]. After these studies area of usage of lycopene expanded. In medicine its anti cancerogenic activity and reducing effects on low-density lipoprotein (LDL) oxidation which known as a bad cholesterol and it helps reduce cholesterol levels [3, 5]. For its anti-aging properties lycopene have also used in functional cosmetic industry.

For all its valuable qualities working with antioxidants has its own handicaps. One of the biggest challenges working with antioxidants is the stability problems. High antioxidant capacity of lycopene is based on all trans structure, which is linear and long-chained. Interaction with reactive oxygen species happens due to conjugated double bonds of lycopene which provide electron transfer to the free radicals by reducing their energy requirement [6]. Plant produced lycopene consists of trans configuration. However, exposure to the light or heat affects great deal of stability by converting trans configuration to cis configuration. Parallel with stability, antioxidant capacity decreases greatly [7].

To preserve antioxidant capacity, it is necessary to encapsulate the lycopene in carrier systems. Also more controlled release can be achieved with these carrier systems. Electrospraying method can be considered to encapsulate lycopene. There are examples of microencapsulation systems developed for antioxidants, for example; lycopene in gelatin by using a spray-drying technique [8]. In other study a method developed to obtain nanoparticles of caseine and ß-carotene core and dextran shell [9]. In encapsulation method types of carriers are crucial to the intended purpose of encapsulation. The most common carriers are: gelatin, modified starch, maltodextrin and arabic gum. In this study zein is used as a biopolymer carrier system. It has been reported that zein could linked and cover lipid compounds, and decreasing degradation ratio [10]. Zein is a protein which has applications in the food, pharmaceutical and biotechnology industries. Advantages of using zein are its low cost values, commercially feasible, biodegradable, biocompatible and applicable to the food processes. For encapsulation variety of methods have been used; electrospraying method, spray-drying, molecular inclusion, complex coacervation. Inclusion complexes had high moisture content, causing differentiation of granular structure. In complex coacervation gelation might observed and that might led to microstructures to transform into macro scale. Spray-drying is the most conventional method. However the drying process yield strongly depends on the equipment configuration [11]. With electrospraying method micro/nano sphere structures is procured. And it provides a high degree of control over particle characteristics without the use of harsh processing conditions or solvents with poor biocompatibility.

2. EXPERIMENTAL SECTION

2.1. Materials.

Lycopene extract was kindly supplied by Cosis Co., Ltd., Korea. Zein from maize was purchased from Sigma-Aldrich, Germany. Hexane and ethanol were purchased from Sigma-Aldrich.

2.2. Preparation of the Zein Solution with Lycopene.

Zein was dissolved in 70 % ethanol: water (v/v) solution and stirred at room temperature until completely dissolved. Zein concentration was kept constant as 5 % in solvent (w/v) based on the results from preliminary studies [12]. Lycopene-zein solution was prepared in 70 % aqueous ethanol solution at different ratios, (1:5, 1:10, and 1:20 (w/w)).

2.3. Encapsulation of Lycopene Using Electrospraying.

Lycopene-zein mixture solutions were used in electrospraying process with a blunt end steel needle syringe which was attached to the positive electrode of a direct current of (DC) power supply. The solution was fed to the syringe needle using an injection pump (New Era, Model NE-1000, Programmable Single Syringe Pump, U.S.A.).

Aluminum foil covered collector plate was fixed at a distance of 10 cm away from the needle tip and connected to the counter electrode of the power supply. Solution was fed to process with plastic syringe and to control the flow rate of solution a syringe pump was used. Voltage adjusted to 14 kV and flow rate was changed between 0.3 ml/h and 1ml/h. For different set of experiments, flow rate and ratio of lycopene-zein in the solution were changed. The flow rates were 0.3 and 1 ml/h. And lycopene-zein mixture solutions were prepared in 70 % aqueous ethanol, ranging from 1:5, 1:10 and 1:20 (w/w).

2.4. Scanning Electron Microscopy (SEM) Analysis.

The morphology of sphere was observed using a Scanning Electron Microscopy, Philips XL30 SFEG (FEI Company, Oregon, USA) and Quanta FL ESEM unit. The samples were collected from the aluminum sample holder which was covered the surface of the collector plate.

Before analysis, samples were treated with gold-palladium. All SEM evaluations were conducted at an accelerating voltage of 5-7 kV.

2.5. Fourier Transform Infrared (FT-IR) Analysis.

Encapsulated lycopene was powdered and mixed with dry potassium bromide (KBr) (Sigma-Aldrich, USA) with ratio of 1:100 in a mortar. The mixture was pressed into transparent disc by using high pressure. The liquid samples were dropped on blank KBr discs.

2.6. Photo-stability Analysis.

Both free and encapsulated lycopene were dissolved in hexane in order to see degradation of lycopene under UV light. Samples were subjected to UV lamp at 365 nm for four hours. Every 30 minute time interval, samples were taken and UV-absorbance measurements were carried out between 200-800 nm using a spectrophotometer (Thermo Scientific Geneysis 10S UV-Vis).

3. RESULTS SECTION

3.1. Morphology of Encapsulated Lycopene.

SEM images in Figure 1 and 2 show the effect of flow rate on the size and shape of microstructures. Except the flow rate, the microstructures observed in Figure 1 and 2 were obtained with electrospraying using the same parameters. These parameters were; same ratio of lycopene-zein solution (1:5 w/w), voltage (14 kV) and distance from needle to collector (10 cm). In Figure 1 microstructures were almost spherical. However the spheres were piled up and formed an agglomerated structure. Also the surfaces of the particles were uneven and rough. Mean size of the particles were determined as 0.36 μm.

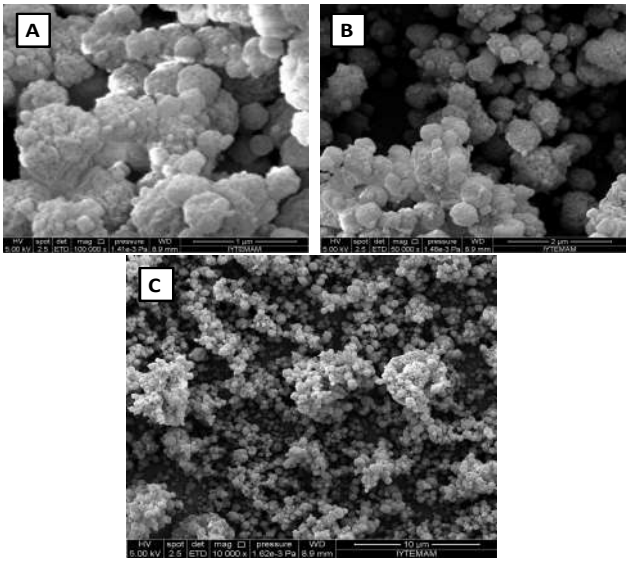

Figure 1. SEM images of encapsulated lycopene. Electrospraying conditions: 1:5 (lycopene: zein, w/w); Flow rate (1 ml/h). Magnifications; A: 100000X; B: 50000X; C: 10000X.

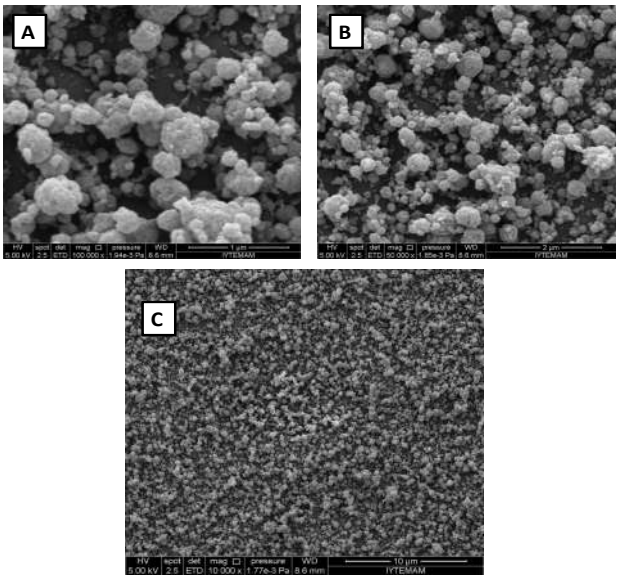

Figure 2. SEM images of encapsulated lycopene. Electrospraying conditions: 1:5 (lycopene: zein, w/w); Flow rate (0.3 ml/h). Magnifications; A: 100000X; B: 50000X; C: 10000X.

As shown in Figure 2 particles formed were more uniform and spherical and separated from each other. For evaluating the effect of flow rate on the morphologies of the microstructures Figure 1 and Figure 2 can be compared.

With lower flow rate (0.3 ml/h) it was seen that structures were more spherical and separated from each other. Mean size of the spheres were determined as 0.23 μm at flow rate of 0.3 ml/h which indicated that decreasing the flow rate decreased the mean size of the particles. Also with lower flow rate particles were

provided more time in the electric field which led to evaporation of the solvent efficiently. With evaporation of the solvent, particles were dried better and separated evenly.

The lower flow rate resulted in better encapsulation. The other parameters affecting the elctrospraying process were also studied, effect of lycopene:zein ratio on encapsulation was investigated at a consant flow rate of 0.3 ml/h. SEM images of particles obtained for two different lycopene:zein ratios were given in Figure 3 and 4.

As seen in Figure 3 with increasing the lycopene: zein ratio (1:10 w/w) structures of the spheres were smoother, and size distributions were more evenly than those for the 1:5 lycopene-zein ratio. Also with increasing lycopene-zein ratio particle size was increased as well. The mean size was determined as 0.35 µm.

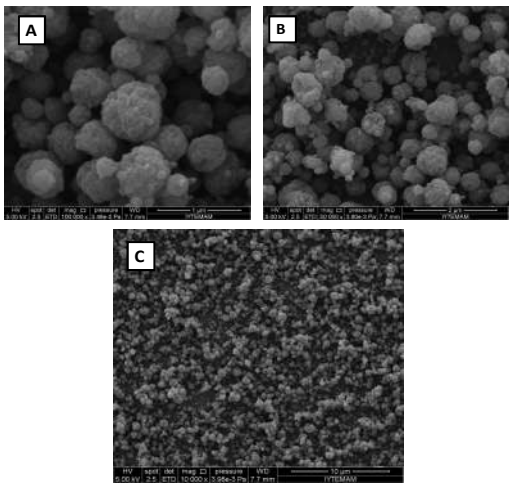

Figure 3. SEM images of encapsulated lycopene. Electrospraying conditions: 1:10 (lycopene: zein, w/w); Flow rate (0.3 ml/h). Magnifications; A: 100000X; B: 50000X; C: 10000X.

As seen in Figure 4 with further increasing the lycopene: zein ratio (1:20 w/w) surface of the spheres stared to become smoother. And the spheres were more separated and uniformly distributed. The mean size was determined as 0.32 µm.

When all the SEM images were compared increasing ratio of zein in the electrospraying solution caused particles to have smoother surfaces and uniform distribution.

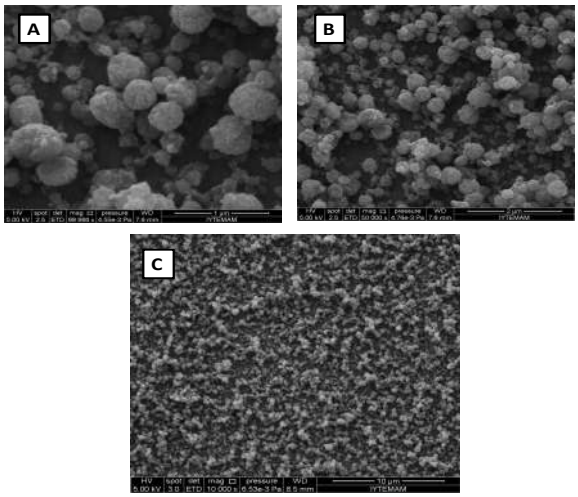

Figure 4. SEM images of encapsulated lycopene. Electrospraying conditions: 1:20 (lycopene: zein, w/w); Flow rate (0.3 ml/h). Magnifications; A: 100000X; B: 50000X; C: 10000X.

3.2. Size Distribution of Encapsulated Lycopene

After the encapsulation, the size distribution of 1:5 and 1:20 ratios of lycopene:zein mixtures were given in Figure 5 and 6, respectively.

Particle diameter was measured with image j software and after that particle size distribution was evaluated with software. In Figure 5, size of the obtained microstructures changed between 0.1-0.45 µm. And the mean size was determined as 0.231 µm.

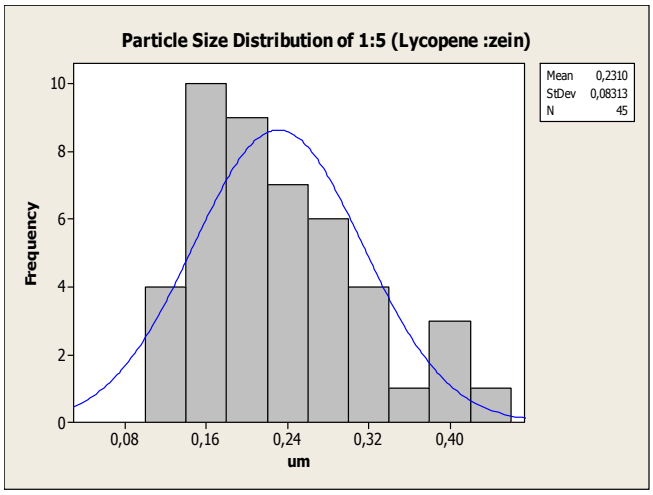

Figure 5. The particle size distribution of encapsulated lycopene Electrospraying conditions: 1:5 (lycopene: zein, w/w). Flow rate (0.3 ml/h)

In Figure 6 size of the obtained microstructures changed between 0.1-0.75 µm and the mean size was determined as 0.32 µm. By increasing ratio of zein in the solution mixture the mean size of the particles were clearly increased.

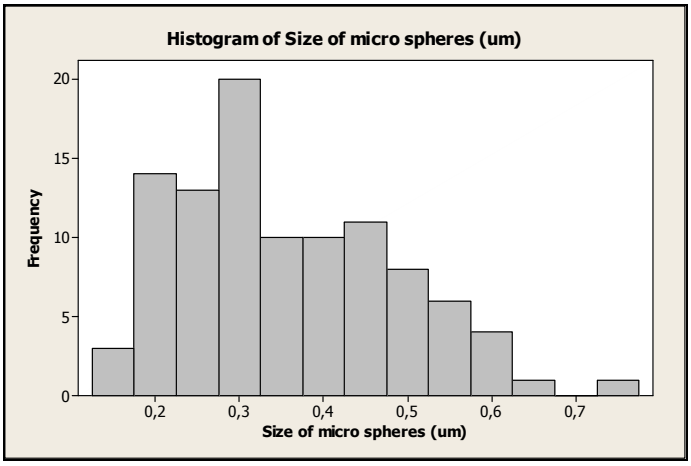

Figure 6. The particle size distribution of encapsulated lycopene Electrospraying conditions: 1:20 (lycopene: zein, w/w). Flow rate (0.3 ml/h)

3.3. Photostability of Lycopene and Encapsulated Lycopene.

In Figure 7, UV spectra of lycopene were given. Initial data was obtained after the lycopene solution was prepared. And absorbance values between 200-800 nm measured with a UV spectrophotometer. In Figure 7, specific three peaks for trans-lycopene peaks were observed between 432-572 nm [13]. After the initial absorbance values were determined, lycopene solutions were exposed to the UV light for four hours. Every half an hour, samples were taken and absorbance values were measured with UV spectrophotometer. With increasing time of exposure to the

UV light, it can be seen that the intensity of individual peaks representing the trans structure were decreased. Indicating the degradation or conversion of trans structure into the cis structure. Obtained data showed that with exposure to the light stability of lycopene decrease significantly.

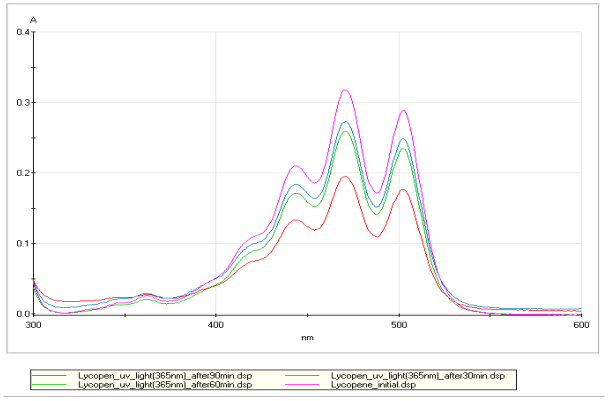

Figure 7. UV spectra of lycopene after exposure the UV light in 30 minutes time intervals.

After proven the lycopene stability decrease with light, efficiency of encapsulation of lycopene was tested. Both free and encapsulated lycopene were subjected to the UV light and the samples were taken in to FT-IR analysis. Firstly free lycopene was measured using FT-IR. Then free lycopene was exposed to the UV light and measured using FT-IR. In Figure 8 free lycopene and UV light exposed free lycopene FT-IR results were given. After exposure to the UV light at 365 nm for 4 hours transmittance of lycopene was increased.

Trans isomers give lower transmittance peaks due to the long chain structure of molecule comparing to the cis isomers of lycopene. With higher transmittance peaks were proven that the degradation of trans form lycopene into the cis form lycopene.

In Figure 9 encapsulated lycopene and UV light exposed encapsulated lycopene FTIR results were given.

Consisted with literature encapsulated lycopene was reached lower transmittance value when compared to the UV exposed encapsulated lycopene.

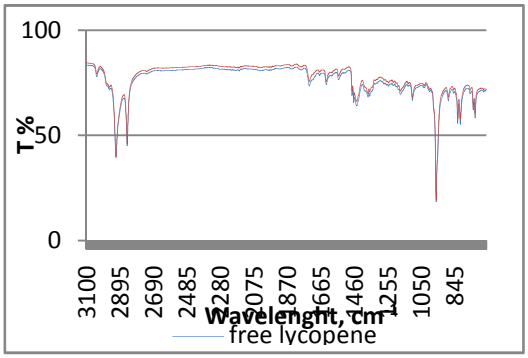

Figure 8. FTIR results of both free lycopene and UV exposed free lycopene

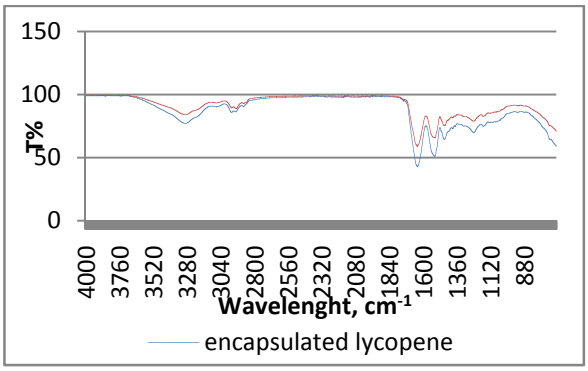

Figure 9. FTIR results of both encapsulated lycopene and UV exposed encapsulated lycopene

According to Bunghez et al, band between 850 and 1200 cm^{-1} were correlated with the lycopene concentration. The spectral signal obtained at a frequency of 957 cm^{-1} can be assigned to the presence of trans lycopene [14]. Degradation percentage of lycopene was calculated using the difference of characteristic peak areas of lycopene and degraded lycopene. Degradation percentages of free and encapsulated lycopene were calculated as 1.02% and 0.72%, respectively. According to our preliminary results with encapsulation degradation can be lowered and the stability of the lycopene can be enhanced. However, more detailed experiments are required to determine the enhancement in photostability of lycopene.

4. CONCLUSIONS

In this study encapsulation of lycopene by using electrospraying method with different flow rates and different lycopene- zein ratios were investigated. According to the results lower flow rate (0.3 ml/h) was better option for encapsulation resulting with more uniform and separate spheres. Due to lower flow rate allowing more retention time in electric field and more time for the evaporation of the solvent which led to more uniform and separated encapsulated spheres. Lycopene:zein ratio was another effective parameter of this study. The best encapsulation was obtained at a lycopene-zein ratio of 1:20. With increasing amount of zein in the solution mixture led to more uniform spherical structures and smoother surfaces on the encapsulated lycopene. Effect of light on lycopene stability was shown. And efficiency of encapsulation for the lycopene stability was observed. With encapsulation, degradation rate of lycopene was lowered.

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