Nano Particles of Blueberry in Inulin and β-Cyclodextrin Microencapsules

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Abstract:
In this study, blueberry essential oil as a core material was microencapsulated with Inulin and β-cyclodextrin at a ratio of 1:5 (core: wall). Oil in water nano-emulsions were prepared by ultrasonic liquid processors and then transformed to encapsulate powder in spray dryer. Dried powder was stored at 4 ˚C in refrigerator for determining moisture, encapsulation efficiency and morphology properties of encapsulated powders by Scanning electron microscopy. Results showed that the best encapsulation efficiency was found where Inulin was used as wall material. Also, on the basis of observations with Scanning electron microscopy, it was indicated that particles size varied from the range of 140 nm to 20 µm. Accordingly, there is a direct relation between emulsion droplet size and surface blueberry content which affects the oxidation. On the other hand, there is a revise relationship between the emulsion droplet size and encapsulation efficiency.
Keywords: *Blueberry, Nano-emulsion, Ultrasound, Spray dryer, Inulin, β-Cyclodextrin.*

1. INTRODUCTION

Encapsulation is a rapidly expanding technology with a lot of potential in different areas including pharmaceutical and food industries. It is a process by which small particles of core materials are packaged within a wall material to form microcapsules [1]. Application of encapsulation has become widely accepted in the flavor industry, because most flavors are volatile and chemically unstable in the presence of oxygen, light, water and heat [2, 3-4].

Blueberries are one of the few fruit crops that are native to North America and, next to strawberries, are the second most important berry in the U.S [5]. Among berry fruits, blueberries (Vaccinium corymbosum L.) are considered to be a good source of phenolic compounds and praised for their high antioxidant activity scores [6].

Inulin is a carbohydrate built up from β (2,1)-linked fructosyl residues mostly ending with a glucose residue and it is present as storage carbohydrate in a large number of plants. From nutritional point of view inulin is a dietary fiber and exhibits a prebiotic function of stimulating the growth of Bifidobacterium sp. in large intestine. Inulin also enhances calcium absorption and reduces the level of cholesterol [7-8].

Cyclodextrins are cyclic oligosaccharides composed of glucose units [9-10]. The best-characterized forms are α, β and β-Cyclodextrin consisting of six, seven and eight D-glucose units, respectively. Cyclodextrins have found numerous applications in food industry. They are used for the removal and masking of undesirable components and control release of desired food constituents [11]. Cyclodextrins are used in food formulations for flavor protection or flavor delivery. Most natural and artificial flavors are volatile oils or liquids, and complexation with cyclodextrins provides a promising alternative to the conventional encapsulation technologies for flavor protection. β-Cyclodextrin as a molecular encapsulant allows the flavor quality and quantity to be preserved to a greater extent and longer period compared to other encapsulants and provides longevity to the food item [12].

It has been well documented that emulsion droplet size (EDS) plays an important role in the retention of volatiles and surface oil content of encapsulated powders during spray drying [13,14,15-16]. It has been proved that the lower the emulsion size, the higher the encapsulation efficiency. Accordingly, many emulsion properties such as stability, rheology, and color, depend on the EDS and size distributions [17-18].

Based on EDS, emulsions can be divided into micro - (10–100 nm), mini (nano) - (100–1000 nm) and macro-emulsions (0.5–100 μm) [1]. Nano-(submicron) emulsions are kinetically stable systems that can be transparent (EDS < 200 nm) or ‘‘milky” (EDS - 500 nm) [19-20], and because of their very small EDS and high kinetic stability, they have been applied in various industrial fields, for example, personal care and cosmetics, health care, pharmaceuticals, and agrochemicals [21-22].

Spray drying is the most common methods used for microencapsulation because it is economical. It is also one of the oldest encapsulation methods used originally in the 1930’s to encapsulate flavours using gum acacia [23].

The objective of this study was to produce blueberry essential oil nano-emulsions by ultrasound and to spray dry them in order to investigate the properties of microcapsules .It should mention that a lot of studies have been done for encapsulation of core materials by β-cyclodextrin using inclusion complex. But we used a new method for encapsulation of core materials by β-cyclodextrin in this study.

2. MATERIALS AND METHODS

2.1. Materials

In this study, blueberry essential oil (Fermotec, Holland) was used as the core material. The wall materials were Inulin (Sigma-Aldrich, USA) and β-cyclodextrin (Sigma-Aldrich, USA). N-Hexane, Isopropanol and Tween 80 were purchased from Merck Company (Germany). Distilled water was used for the preparation of all solutions. All general chemicals used in this study were of analytical grade.

2.2. Preparation of emulsions

20% of wall materials were dissolved in distilled water by magnetic stirring at 60˚C according to composition listed in Table 1. They were kept overnight in ambient temperature in order to warrant a full saturation of wall materials.

Table 1: *Different ratios of wall material*

Formulation	Inulin (%)	β-cyclodextrin (%)
1	100	0
2	0	100
3	50	50
4	25	75
5	75	25

Blueberry essential oil in the ratio of 1:5 (core: wall) and 1% of Tween 80 were added to emulsions .After that, they were stirred by magnetic stirring for pre-emulsion preparation.

2.3. Ultrasonication

An Ultrasonic Liquid Processor (Model S-4000-010, USA) was used in this study for transforming pre-emulsion to nano-emulsion that is equipped with an ultrasound probe with 4.8 mm in diameter. It was operated at 24 KHz with 600 W high-intensity for 130s.

2.4. Emulsion droplet size analysis

The size distribution of emulsion droplets was determined by Stabisizer (Model PMX200C, Germany). Nano-emulsions were diluted by distilled water at a ratio of 1:40.

2.5. Spray draying

The emulsions were immediately dried by pilot-plant spray drier (Model B-191, Buchi, Switzerland). The spray drier was operated at 120˚C inlet temperature and 65˚C outlet temperature. The nozzle air pressure was 6 bar. Ultimately, dried powders were collected and stored at 4˚C for further analysis.

2.6. Encapsulation efficiency analysis

One of the important parameters for encapsulated powders is the encapsulation efficiency (EE) during the process. By definition, it is the amount of core material encapsulated inside the powder particles [1]. Encapsulation efficiency was calculated according to the following formula:

$$EE = \frac{\text{Total oil content} - \text{surface oil content}}{\text{Total oil content}} \times 100$$

2.6.1. Total oil content

0.5 g of encapsulated powder was mixed with 10 ml of distilled water and then stirred by magnetic agitator for 2 min. Next, Isopropanol and N-hexane were added in ratio of 1:3, respectively and stirred for 5 min. Then, they were centrifuged at 8000 rpm for 20 min. After that, they were filtered by Whatman filter paper. Water bath was used at 70˚C for solvent evaporation. Finally, amount of oil was weighed.

2.6.2. Surface oil content

Encapsulated powder (1 g) was mixed with N-hexane (8 ml) and shaken by magnetic agitator. Then, it was centrifuged at 8000 rpm for 20 min and filtered with Whatman filter paper. After evaporating the solvent, the amount of oil was weighed.

2.7. Moisture content

Moisture content of the encapsulated powders was determined by oven drying (Model Vord-460-D, Australia) at 70˚C to constant weight. 1 g of powder was widened inside glass plates and the vacuum drying time was 72 h. The results were reported on the wet basis as (weight loss/sample weight) × 100.

2.8. Scanning electron microscopy (SEM) of encapsulated powder

Microstructural properties of the encapsulated powders were evaluated by scanning electron microscopy (Model S360 Mv2300, England). Powders were placed on SEM stubs using a two-sided adhesive tape. The samples were coated with gold using Auto sputter coater (Model E5200, Bio-RAD, England) and they were analyzed at voltage of 15 kV. The images were obtained with instrument's software installed on a PC connected to the system.

2.9. Experimental design & statistical analysis

All the experiments were performed based on fully factorial design. ANOVA test was used for determination of differences between treatments with SPSS 19 program. Also, Pearson correlation was used for determining relation between emulsion droplet size, encapsulation efficiency, total oil and surface oil. Treatments means were considered very significantly different at $p \leq 0.01$. All experiments were done in triplicate and average values reported. All graphs were drawn by Excel.

Table 2: *Influence of wall materials on emulsion size, encapsulation efficiency and surface blueberry*

Treatments		Emulsion size (nm)	Encapsulation efficiency(%)	Surface blueberry (gr/100gr powder)
1	Blueberry + Inulin(100%)	97.12 ± 4.2 ^{d}	99.45 ± 0.05 ^{a}	0.004 ± 0.001 ^{f}
2	Blueberry+ β-CD(100%)	8.28 ± 0.11 ^{g}	98.82 ± 0.03 ^{c}	0.02 ± 0.002 ^{d}
3	Blueberry+ β-CD(50%)+ Inulin(50%)	206.12 ± 5.08 ^{a}	97.19 ± 0.05 ^{e}	0.01 ± 0.0006 ^{e}
4	Blueberry+ β-CD(75%)+ Inulin(25%)	72.25 ± 1.81 ^{e}	98.73 ± 0.02 ^{d}	0.004 ± 0.001 ^{f}
5	Blueberry+ β-CD(25%)+ Inulin(75%)	70.63 ± 2.55 ^{e}	99.13 ± 0.06 ^{b}	0.003 ± 0.001 ^{f}

a–g Means within the same column fallowed by different letters are very significantly (p<0.01) different.

3. RESULTS AND DISCUSSION

3.1. Emulsion droplets size analysis

Results revealed that amount of wall materials had very significant difference ($p < 0.01$) with emulsion droplets size (Table 2). Using β-Cyclodextrin as wall material singly produced the smallest emulsion size. While Combining Inulin and β-Cyclodextrin led to increase emulsion droplets size. It could be related to changing spatial structure or coalescing emulsion droplets.

Klaypradit and Huang (2008) encapsulated fish oil with different concentration of wall materials (chitosan, maltodextrin and whey protein isolate) by ultrasonic atomizer. They found that with increasing the amount of chitosan, emulsion droplets size decreased. Our results were similar to their results.

3.2. Encapsulation efficiency

When blueberry essential oil was encapsulated by Inulin and β-Cyclodextrin, results showed that samples containing 75% of Inulin and 25% of β-Cyclodextrin had the highest encapsulation efficiency (Figure 1). This result could be due to the interaction between Inulin and β-Cyclodextrin that had the best capacity for preventing instability of emulsions and it was the most resistant sample. Also, it was found that Inulin covered blueberry essential oil better that β-Cyclodextrin.

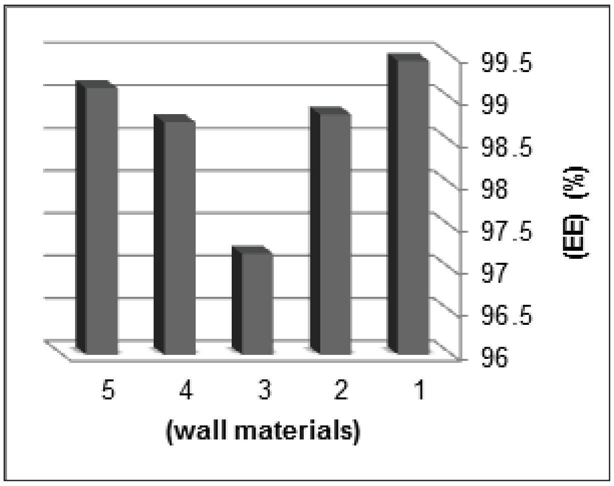

Figure 1. *Effect of wall materials with different ratios on encapsulation efficiency*

On the other hand, there is an inverse relationship between encapsulation efficiency and emulsion droplets size. Namely, increasing emulsion droplets size leads to decrease encapsulation efficiency.

Kaushik and Roos (2007) were studied encapsulation of limonene in freeze drying by various matrices containing of gum Arabic, sucrose and gelatin. They found that increasing emulsion droplets size decreased encapsulation efficiency. In this study, powders containing encapsulated limonene by gum Arabic had the highest encapsulation efficiency.

3.3. Surface oil content

According to the results, there is a direct relationship between droplets size and surface oil (blueberry) content. Decreasing emulsion droplets size reduced surface blueberry content (Figure 2). It is obvious that small blueberry essential oil droplets will be surrounded more efficiently with the wall matrix of the microcapsules, so acquired emulsion will be more stable during spray drying process; thus, encapsulation efficiency will be increased and prevented from leaking blueberry essential oil to the surface of encapsulsated particles.

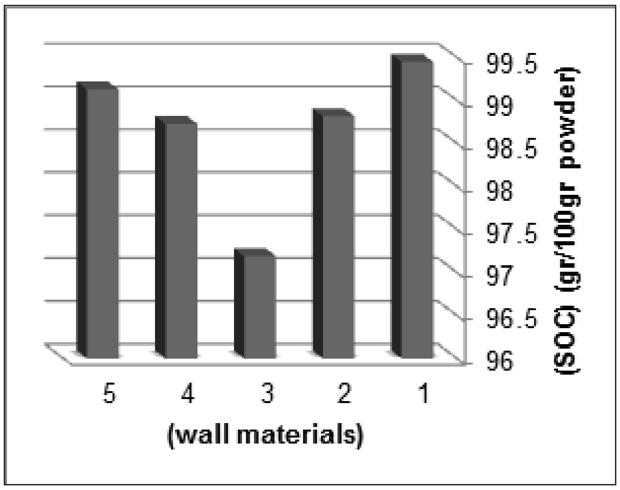

Figure 2. *Effect of wall materials with different ratios on surface blueberry content*

Jafari *et al*. (2008) microencapsulated fish oil (as a core) by maltodextrin, modified starch and whey protein concentrate at a ratio of 3:1 (wall: core). They investigated amount of surface oil. Their results showed that decreasing emulsion droplets

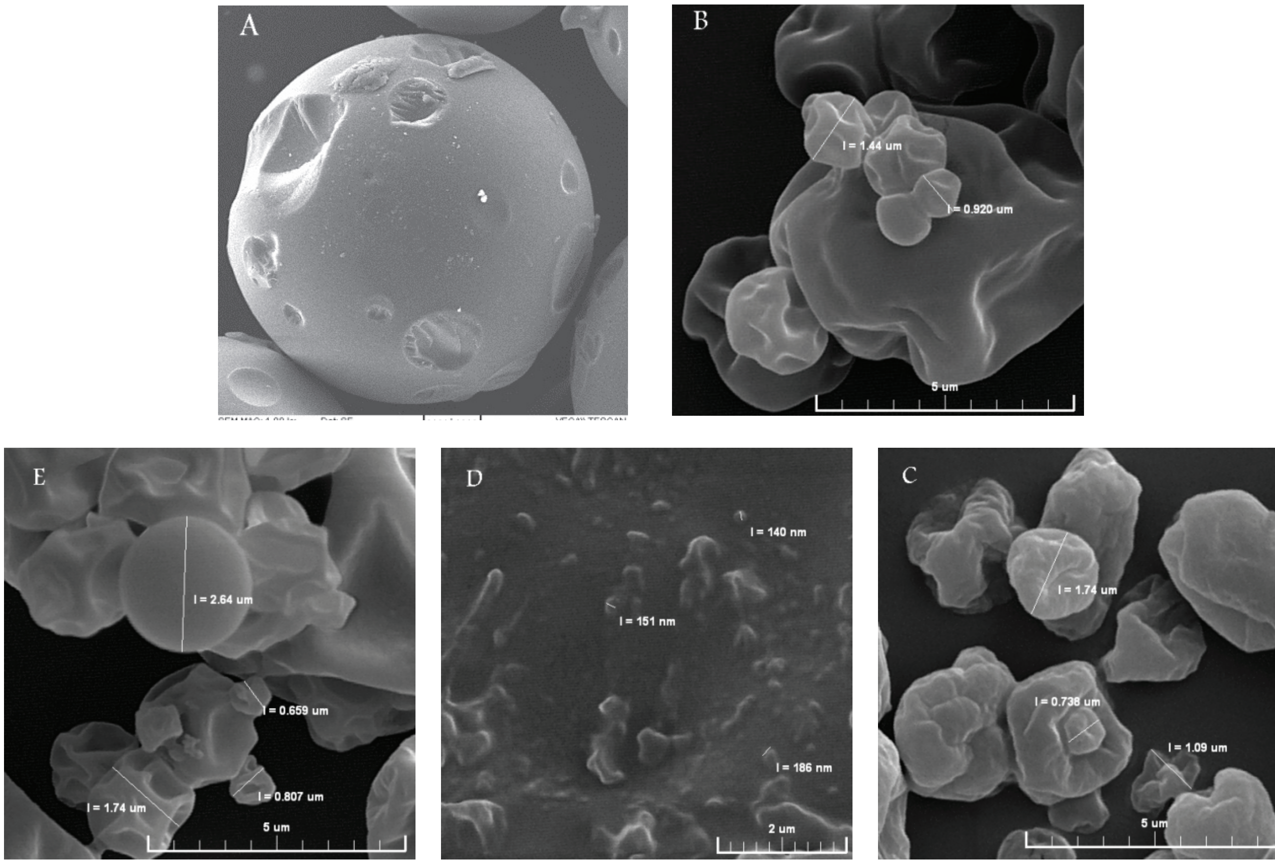

Figure 3: *Scanning electron microscopic photographs of blueberry essential oil encapsulated powders: A: β-cyclodextrin(100%), B: Inulin (100%), C: β-cyclodextrin(50%) and Inulin (50%), D: β-cyclodextrin(75%) and Inulin (25%), E: β-cyclodextrin(25%) and Inulin (75%)*

size reduced surface oil, and increased encapsulation efficiency. Our results coordinated with their results. Danviriyakul, McClements, Decker, Nawar & Chinachoti (2002) studied dried milk fat emulsions with sodium caseinate, corn syrup solids and lecithin by spray dryer. They found that increasing amount of surface oil increased emulsion droplets size. This was due to the instability of emulsions with bigger droplets. Their results were consistent with our results.

On the other hand, our results illustrated that there is an inverse relationship between surface oil (blueberry) content and encapsulation efficiency. Results indicated that the surface oil content increased in samples containing encapsulated blueberry essential oil by β-Cyclodextrin (Table 2). Because crust formation is slow in encapsulated powders containing β- Cyclodextrin and whatever, surface oil (blueberry) content will increase. While surface oil (blueberry) content was lower in encapsulated samples with Inulin due to fast crust formation.

Jafari *et al.* (2008) investigated the influence of encapsulation efficiency on surface oil. Based on their results, encapsulation efficiency had an inverse relationship with surface oil. So that the lowest surface oil belonged to encapsulated fish oil by modified starch which had the highest encapsulation efficiency. Also, surface oil increased in samples containing encapsulated fish oil by whey protein concentrate because of slow crust formation. Their results were similar to our results.

3.4. SEM photos analysis

Figure 3 (A–E) shows morphology of microcapsules. The surface of blueberry essential oil encapsulated by β-Cyclodextrin was smooth, free of shrinkage and with the least dents. While blueberry essential oil encapsulated by Inulin had the surface with the most shrinkage and dents in comparison with powder particles containing β-Cyclodextrin. Also, results indicated that incorporating Inulin with β-Cyclodextrin led to produce powders with a lot of dents and shrinkage in the surface. In other words, Inulin had a profound influence on the structure and surface morphology of encapsulated powders. Photos showed that powder particles containing Inulin (25%) and β-Cyclodextrin (75%) had spherical surface without shrinkage which was due to increasing β-Cyclodextrin in emulsions.

Also, results indicated that there was a fast crust formation in powders containing Inulin that could be related to low levels of surface oil content as there is less opportunity for the blueberry essential oil (core material) to emerge from the surface of particles. While in β-Cyclodextrin samples, crust formation is slower that illustrated more essence droplets could move on the surface.

According to SEM photos, there are different reasons for increasing emulsion droplets size during spray draying: size of spray dryer nozzle, decreasing stability of emulsions during spray drying and type of wall material which needs to further survey.

4. CONCLUSION

The results of this study obviously showed that using ultrasonic waves with 24 KHz intensity for 130 second produced emulsions with diameter range of 10 – 115 nm. Increasing particle size, after drying, can be attributed to instability of emulsions during spray draying. Also, emulsion droplet size affected encapsulation efficiency, surface oil (blueberry) content and total oil (blueberry) content. It’s interesting to know that amount and kind of wall material had an important effect on emulsion droplet size. Considering two different wall materials, we found that Inulin was better than β-Cyclodextrin because it led to encapsulated powder with less surface blueberry content and high encapsulation efficiency.

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