Producing oil in water Nano-emulsion by ultrasonication for spray drying encapsulation

Atena Mazloom *, Nazanin Farhadyar **

* Address: Department of food science and technology, Varamin-pishva Branch, Islamic Azad University, Varamin, Iran
Email: atena.mazloom@yahoo.com
** Address: Department of chemistry, Varamin-pishva Branch, Islamic Azad University, Pishva, Iran
Email: nfarhadyar@gmail.com

Abstract: The purpose of this study was to produce oil in water Nano-emulsion by ultrasonication for spray drying encapsulation. β-cyclodextrin combined with Inulin at two ratios of 1:5 and 2:5 were used as the continuous phase, while dispersed phase consisted of the blueberry essential oil. Results showed that while on the one hand emulsion droplet size had a direct relationship with pH and surface blueberry oil content, on the other had an inverse relationship with encapsulation efficiency and total blueberry oil content. Also, the amount of blueberry essential oil and wall materials had effect on properties of the Nano-emulsions and the microencapsulated powders.

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1. Introduction

Microencapsulation is defined as a process that tiny particles or droplets are surrounded by a coating to give small capsules with many useful properties. There are many different microencapsulation processes such as: spray drying, spray cooling, spray chilling, freeze drying, extrusion, coacervation, liposome entrapment, molecular inclusion (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Although many techniques have been developed to microencapsulate food ingredients, but spray drying is the most common technology that was used in food industry because of low cost, and available instruments. In order to formation of microcapsules by spray dryer, it is necessity to have fine and stable emulsion of a core material in the wall solution. Hence, in this study was used ultrasonic wave for preparing Nano-emulsion. Advantages of using ultrasound are to lower energy consumption, use less emulsifier, and produce more uniform emulsion droplets with smaller size in compared with other mechanical methods.

Nano-emulsion droplet sizes fall typically in the range of 20–200 nm. Due to their characteristic size, Nano-emulsions appear transparent or translucent to the naked eye, and possess stability against sedimentation or creaming (Solan, Izquierdo, Nolla, Azemar, & Garcia-celma, 2005). Many emulsion properties such as stability, rheology, appearance, color, texture, and shelf-life depend on emulsion droplet size and size distributions (Jafari, Assadpoor, Bhandari, & He, 2008). In general, emulsion droplet size depends on different factors such as concentration and type of emulsifier, emulsion preparation method and environmental conditions such as pH.

Nano-emulsions cannot be formed spontaneously. Consequently, energy input, generally from mechanical devices or from the chemical potential of the components, is required. Nano-emulsion formation by the so-called dispersion or high-energy emulsification methods is generally achieved using high shear stirring, high pressure homogenizers and ultrasound generators (Solan, Izquierdo, Nolla, Azemar, & Garcia-celma, 2005).

Blueberry is a common name for the group of flowering plants belonging to the genus *Vaccinium*, section *Cyanococcus*. The high bush, low bush and rabbit eye are three types of blueberries grown in North America (Mohideen, 2011). They have been considered one of the fruits with the highest antioxidant potentials that a few studies have evaluated their anticancer activities (Yi, Akoh, Fischer, & Krewer, 2006). Also, blueberries are rich in health-promoting polyphenolic compounds including proanthocyanidins that are effective against prostate cancer cell growth (Schmidt, Erdman, & Lila, 2006). In this research, Inulin and β-cyclodextrin were used for microencapsulation of blueberry as wall material, for first time.

Inulin is a polysaccharide found in many vegetables that is composed of (2-1) - linked D-fructose molecules. A feature of Inulin is to be difficult to hydrolyze which qualifies Inulin as a matrix molecule for capsules that have to reach the colon and to survive the upper part of the gastrointestinal tract. The microorganisms responsible for degradation of Inulin are bifidobacteria that are abundantly present in the human gut (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004, de Vos, Faas, Spasojevic, & Sikkema, 2010). Inulin should receive more attention for colon specific delivery of chief food components as it is cheap, has many health benefits, and can be applied in combination with almost all encapsulation techniques.

Cyclodextrins (CDs) are inexpensive enzyme-modified starch derivatives that have been industrially produced. These starch derivatives are non-toxic ingredients, are not absorbed in the upper gastrointestinal tract, and are completely metabolized by the colon microflora (Szente, & Szejtli, 2004). The most extraordinary characteristic of a cyclodextrins is its ability to form inclusion complexes with a variety of compounds by trapping foreign molecules (guest) in its cavity (host) (Folch-Cano, Jullian, Speisky, Olea-Azar, 2010). β-cyclodextrin is one type of cyclodextrins that is composed of seven α-(1, 4)-linked glycosyle units. It is the most accessible, the lowest priced, and generally the most useful (Singh, Sharma, Banerjee, 2002).

This study repots an emulsification method for producing blueberry Nano-emulsions by ultrasound, and a microencapsulation process by spray dryer. This research investigated effects of various proportions of blueberry essential oil and wall materials on different factors.

2. Materials and methods

2.1. Materials

In this study, blueberry essential oil (minimum 97%) was purchased from Fermotec Company (Holland). Inulin and β-cyclodextrin were supplied by Aldrich Chemical Company (USA). Isopropanol, N-hexane and Tween 80 were purchased from Merck Company (Germany).

2.2. Nano-emulsion preparation

Inulin and β-cyclodextrin (0, 25, 50, 75 and 100% concentration) dissolved in distilled water at 60 ˚C by magnetic stirrer to obtain 20% total solids concentration. The emulsions were kept overnight in room temperature. Blueberry essential oil was added into the emulsion to obtain ratios of 1:5 and 2:5 (Blueberry: Total solid), and 1 wt% Tween 80 was added to emulsions as emulsifier. They were mixed by magnetic stirrer for 15 min. pH (pH Lab, Model 827, Swiss) was measured after adding blueberry essential oil in all emulsions. Emulsions were emulsified using an ultrasound (Ultrasonic Liquid Processor, Model S-4000-010, USA) with 24KHz intensity for 130s to produce fine oil-in-water emulsions.. Then, the size distribution of emulsion droplets was investigated by a Stabisizer (Model PMX 200C, Germany).

2.3. Spray drying

Nano-emulsions were dried by spray dryer (Model B-191, Buchi, Switzerland). The operational conditions of spray drying were: air inlet temperature of 120 ˚C, air outlet temperature of 65 ˚C, and nozzle air pressure of 6 bar.

2.4. Microcapsule powder analysis

2.4.1. Encapsulation efficiency

There are two factors that are important in Encapsulation efficiency: surface oil and total oil. Surface oil has been named as free or extractable oil, as it is measured mostly through solvent extraction. Total oil is oil retention inside encapsulate powders (Jafari, Assadpoor, Bhandari, & He, 2008).

To determine blueberry on surface (surface oil); first, 1 wt% encapsulated powder was mixed with 8 ml N-hexane, and shaken by magnetic stirrer. Next, it was centrifuged at 8000 rpm for 20 min, and filtered with Whatman filter paper. After evaporating solvent, amount of blueberry was weighed.

To survey total blueberry (total oil); first, 0.5 wt% encapsulated powder was mixed with 10 ml distilled water and stirred by magnetic stirrer for 2 min. Next, Isopropanol and N-hexane were added at the ratio of 1:3, 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 total blueberry was weighed.

Encapsulation efficiency was calculated as:

$$EE = \frac{A-B}{A} \times 100$$

Where A was the total blueberry content and B was the free blueberry content on surface.

2.4.2. Scanning electron microscopy (SEM)

The morphology and size of encapsulated powders were investigated by scanning electron microscopy at a voltage of the 15 kV (Model 7538, England). Before testing, the samples were mounted on the SEM stubs with double-sided adhesive tape, and coated with gold using Vacuum Coater (Model E5200, England) to make the sample conductive. The images were obtained with instrument's software installed on a PC connected to the system.

2.5. Experimental design

An analysis of variance (ANOVA) was used for determination of differences between treatments with SPSS 19 program. Pearson correlation was used for investigation of relationship between factors. All the experiments were done in triplicate, and average values reported.

3. Results and discussion

3.1. pH

Our results revealed that amount of blueberry essential oil had a very significant influence ($p<0.01$)

on pH. Increasing amount of blueberry essential oil (1:5 to 2:5) led to decrease pH. On the other hand, amount of wall materials (Inulin and β-cyclodextrin) had influence on pH. Using β-cyclodextrin as the wall, to illustrate, was reducing pH to compared with sample containing Inulin.

pH in emulsions with the ratio of 1:5 varied from 2.81 ± 0.01 to 4.94 ± 0.03 (Table1). β-cyclodextrin samples had the lowest pH (2.81 ± 0.01), and samples containing Inulin/ β-cyclodextrin had the highest pH (4.94 ± 0.03). pH in emulsions with the ratio of 2:5 varied from 2.84 ± 0.07 to 4.36 ± 0.06 (Table2). pH was at minimum (2.84 ± 0.07) in β-cyclodextrin samples, and at maximum (4.36 ± 0.06) in Inulin samples.

3.2. Emulsion droplet size

When comparing the emulsion droplet size for two ratios (1:5 and 2:5), it was found that the emulsion droplet size increased very significantly ($p<0.01$) with increasing amount of blueberry essential oil. The reason could be low stability of emulsions, and low ability of β-cyclodextrin for entrapping blueberry droplets due to increasing amount of blueberry essential oil. Not only amount of blueberry essential oil, but also amount of wall materials had very significant influence on emulsion droplets size. The emulsion droplet size in Nano-emulsion with the ratio of 1:5 (core: wall) varied from 8.28 ± 0.11 to 206.12 ± 5.08 nm. It was at minimum (8.28 ± 0.11 nm) in the Nano-emulsion containing β-cyclodextrin, and at maximum (206.12 ± 5.08 nm) in Nano-emulsion consisting of Inulin/ β-cyclodextrin. The emulsion droplet size in Nano-emulsion with the ratio of 2:5(core: wall) was ranged from 42.10 ± 6.38 to 127.54 ± 10.89 nm. It was at minimum (42.10 ± 6.38 nm) in the Nano-emulsion containing β-cyclodextrin, and at maximum (127.54 ± 10.89 nm) in Nano-emulsion consisting of Inulin/ β-cyclodextrin. The Nano-emulsions consisting of β-cyclodextrin had smallest size of emulsion droplets. It could be due to Nano-structure of β-cyclodextrin. While emulsion droplet size increased in emulsions consisting of Inulin and β-cyclodextrin. It might be due to some structural changes in wall materials.

The results of the present study fit well the conclusion of Klaypradit and Huang (2008) who have also investigated amount of tuna oil influence on micro-emulsions consisting of chitosan, maltodextrin and whey protein isolate. They found that increasing tuna oil, increased emulsion droplet size.

3.3. Effect of pH on emulsion droplet size

The overall results were that there was a direct relationship between pH and emulsion droplet size. Higher pH increases the emulsion droplet size. For example, the emulsions containing β-cyclodextrin at two ratios of 1:5 and 2:5 had the lowest pH that it led to produce the lowest emulsion droplet size, among all samples. Khanmohamadi, Razavizadeh, and Azizi (2010) reported similar results by producing Nano-emulsions containing rice oil, Arabic gum and whey protein with Ultraturrax. They found that pH decreased with decreasing emulsion droplet size due to increasing Arabic gum.

3.4. Effect of emulsion droplet size on encapsulation efficiency

In general, there was a direct relationship between emulsion size droplet and encapsulation efficiency: smaller the emulsion droplet size, better the encapsulation efficiency.

Jafari, Assadpor, Bhandari, and He (2008) showed that emulsion droplet size of encapsulated fish oil by maltodextrin and surface-active biopolymer had a direct relationship with encapsulation efficiency. Such results are in agreement with the results obtained here.

3.5. Effect of emulsion droplet size on surface blueberry oil

There was a direct relationship between emulsion droplet size and surface blueberry oil. Decreasing emulsion droplet size, decreased surface blueberry oil. It is obvious that small blueberry oil droplet will be embedded more efficiently within the wall matrix of the microcapsules. Therefore, the resulted emulsion will be more stable during the spray drying. Consequently, it will lead to encapsulated powder with the minimum amount of surface blueberry oil. Danviriyakul, Mclements, Decker, Nawar, and chinachoti (2002) reported similar results by spray drying milk fat emulsions with sodium caseinate, corn syrup solids and lecithin. They explained that increasing amount of surface oil, increased emulsion droplets size due to instability of emulsions with bigger droplets.

3.6. Characterization of microencapsulated blueberry powder

3.6.1. Encapsulation efficiency

The amount of blueberry essential oil had a very significant influence ($p<0.01$) on encapsulation efficiency. Whereas increasing amount of blueberry (from 1:5 to 2:5), decreased encapsulation efficiency. It seems that by increasing amount of blueberry, decreases the ability of wall material for covering. Also, it should be mentioned that Inulin has a high covering ability in compared with β-cyclodextrin. The encapsulation efficiency of dried powder at the ratio of 1:5 varied from 97.19± 0.05% to 99.45± 0.05%, and at the ratio of 2:5 from 62.85± 0.02% to 95.37±

0.07%. The maximum and minimum of encapsulation efficiency at the ratio 1:5 were in sample containing Inulin, and sample containing Inulin/ β-cyclodextrin (50:50), respectively. Also, the maximum and minimum of encapsulation efficiency at the ratio 2:5 were in sample containing Inulin, and sample containing Inulin/ β-cyclodextrin (75/25), respectively.

3.6.2. Surface blueberry oil content

Our results indicated that amount of blueberry essential oil and wall materials had a very significant influence ($p<0.01$) on surface blueberry oil, separately. In fact, increasing amount of blueberry essential oil from the ratio of 1:5 to 2:5 increased surface blueberry oil. The reason could be low ability of wall materials for maintenance of core material at higher ratio. Also, it should be mentioned that surface blueberry oil at two ratios of 1:5 and 2:5 altered from 0.003± 0.001 to 0.02± 0.002 (gr/100gr powder) and 0.02± 0.001 to 0.127± 0.039 (gr/100gr powder), respectively. It was at minimum (0.003 ± 0.0.001) in microcapsulated powder containing Inulin / β-cyclodextrin (75/25) at the ratio 1:5, and at maximum (0.02 ± 0.002) in sample consisting of β-cyclodextrin (100%). Also, It was at maximum (0.127 ± 0.0.039) in microcapsulated powder containing Inulin / β-cyclodextrin (75/25) at the ratio 2:5, and at minimum (0.02 ± 0.001) in sample consisting of Inulin (100%).

3.6.3. Effect of encapsulation efficiency on surface blueberry oil

There was an inverse relationship between encapsulation efficiency and surface blueberry oil content. Surface blueberry oil increased in sample consisting of encapsulated blueberry by β-cyclodextrin. In fact, there was a slow crust formation in this sample that could be associated with high levels of surface blueberry oil content. In other words, there is more opportunity for the core material (blueberry essential oil) droplets to come onto the surface of particles. While in Inulin sample, crust formation is fast that less blueberry oil droplets could come onto the surface.

3.6.4. Morphology of microcapsules

Spray dried matrix consisting of blueberry essential oil (core) and Inulin/ β-cyclodextrin (wall) at the ratio of 1:5 was observed under scanning electron microscope. Because this samples had the best encapsulation efficiency. As can be seen in fig. 1A, the majority of the particles containing β-cyclodextrin were spherical with smooth surfaces, less pores and without shrinkage. Also, there was no evidence of cracks in this sample. In fig. 1B, particles consisting of Inulin were observed more surfaces dents. We observe in fig. 1C, incorporating Inulin with β-cyclodextrin produced particles with more surface shrinkages and dents. In other words, Inulin had a profound influence on the structure and surface morphology of microencapsulated powders, resulting in particles with more indentation and shrinkages.

It should be mentioned that the formation of surface indentation in spray dried powders not only depends on the composition of wall materials and drying parameters, but also depends on emulsion size (Jafari, Assadpoor, Bhandari, & He, 2008).

4. Conclusion

Inulin was found to be an efficient encapsulation matrix for blueberry essential oil encapsulation in spray drying due to high encapsulation efficiency and low surface blueberry oil content of microcapsules. Although we do not ignore influence of emulsion droplet size on the properties of microcapsuled powder such as encapsulation efficiency. This research revealed that different factors could be affected on emulsion droplet size. Amount of blueberry essential oil, pH, type and concentration of wall materials, for example, affected on emulsion droplet size.

بررسی خوصیات نانوامولسیون و نانوکپسول های بلوبری در دو سطح مختلف

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