Journal of Current Science and Technology, September-December 2021 Vol. 11 No. 3, 375-391

ISSN 2630-0656 (Online)

Cite this article: Kusonwiriyawong, C. (2021, September). Development of spray-dried corn and tapioca starch microparticles for protein delivery. *Journal of Current Science and Technology, 11*(3), 375-391. DOI:

Journal of Current Science and Technology

Journal homepage: https://jcst.rsu.ac.th

Development of spray-dried corn and tapioca starch microparticles for protein delivery

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Received 25 August 2020; Revised 6 June 2021; Accepted 23 June 2021;
Published online 28 September 2021

Abstract

Protein drugs are susceptible to physical and chemical instabilities at every step of their product life cycle. Encapsulating the drugs within biocompatible and biodegradable microparticles made of starch is likely an efficient approach to overcome such limitations. This study aimed to develop corn and tapioca starch microparticles for the delivery of protein drugs using the Mini Spray Dryer B-290 (Büchi Labortechnik AG). Effects of processing conditions, including inlet drying air temperature (100 to 170ºC), liquid feed rate (1.9 to 7.0 g/min), atomizing air volumetric flow rate (240 to 740 L/h), aspirator vacuum (-30 to -48 mbar), and formulation parameters, namely type (corn and tapioca starch) and concentration of starch paste (2 to 8% w/w), on their physicochemical properties were characterized. Particle size and morphology were examined by light diffraction and scanning electron microscopy, respectively. The microparticle size ranged from 10.5 to 30.9 µm, depending mainly on the atomizing air volumetric flow rate and the liquid feed concentration. All microparticles showed a distorted surface. Product collection efficiency was as low as 6.0 to 34.5%. Bovine serum albumin (BSA), as a model protein drug, was incorporated into the microparticles at levels of 1.0 to 5.0%. The encapsulated protein content was determined by bicinchoninic acid assay. Actual protein loading and entrapment efficiency ranged from 1.0 to 5.2% and 94.3 to 124.9%, respectively. The protein-loaded microparticles were smaller in size than their corresponding blank microparticles, possibly due to the ease of feed atomization. The integrity of the encapsulated protein was studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It was evident that the integrity of entrapped protein was completely retained. Starch microparticles for protein delivery were efficiently developed by the spray-drying process.

Keywords**:** *biodegradable; corn starch; microparticles; protein; protein delivery; spray-drying; tapioca starch.*

1. Introduction

The broadened understanding of molecular mechanisms underlying both health and disease conditions has revealed the existence of many proteins involved in the regulation of physiological and biochemical functions of the body. These proteins possess significant medical potential, specifically for individuals who are unable to produce relevant ones sufficiently. Simultaneously, the rapid advance of modern biotechnology, especially recombinant DNA and hybridoma technology, has facilitated the large-scale production of those proteins of medical interest (Walsh, 2003). The number of protein drugs has increased dramatically, and many have been approved for clinical use. It is known that proteins usually suffer from both physical and chemical instabilities. Due to these instabilities, the manufacturing process of proteins is generally more complex than that of small molecules. Besides, the final products need specific management and storage, as controlled temperature and/or drying steps to extend their shelf-life (Emami, Vatanara, Park, & Na, 2018; Geraldes et al., 2020). Upon

administration, protein drugs also face immense challenges, such as a short half-life when administered parenterally and low bioavailability when given orally, among others (Yang et al., 2019). Thus, considerable efforts have been made to overcome these limitations and to achieve effective delivery systems for protein drugs.

Microparticulate drug carriers offer many therapeutic and technological advantages due to their structural and functional abilities. When they are formulated with appropriate polymers and excipients, these microparticles are able to protect the protein drugs from premature degradation, enhance their stability, and eventually deliver them to and/or control their release in the targeted tissues and organs. In recent years, a wide variety of biodegradable materials, including natural and synthetic polymers, has been extensively investigated for the preparation of microparticles for delivering protein drugs (Yang et al., 2019). Such studies have been well described (for reviews, see George, Shah, and Shrivastav, 2019; Lengyel, Kállai-Szabó, Antal, Laki, & Antal, 2019; Wong, Al-Salami, & Dass, 2018; Yang et al., 2019).

Starch is an edible polysaccharide found abundantly in nature. It is present in the form of granules, composed of amylose and amylopectin. Amylose is a linear helical polymer consisting of α-1,4 linked D-glucose monomers, whereas amylopectin, which is one of the highest molecular-weight natural polymers, is a highly branched polymer consisting of both α-1,4 and α-1,6 linked D-glucose monomers (Kaushik, Sharma, & Agarwal, 2016; Song et al., 2018). Among biodegradable materials, starch has long been investigated for its potential application as a drug (Bajpai & Bhanu, 2007; Desai, 2005; Janaswamy, 2014; Liu, Desai, Meng, & Chen, 2007; Mundargi, Shelke, Rokhade, Patil, & Aminabhavi, 2008), protein (Elfstrand, Eliasson, Jönsson, Reslow, & Wahlgren, 2006; Elfstrand, Eliasson, & Wahlgren, 2009; Witschi & Mrsny, 1999), and vaccine delivery carrier (Moreno-Mendieta et al., 2014) due to its biodegradability and biocompatibility (Song et al., 2018). Several approaches for the preparation of starch microparticulate drug and protein carriers have been established, including aqueous two-phase emulsification technique (Elfstrand et al., 2006; Elfstrand et al., 2009; Li, Wang, Li, Adhikari, & Mao, 2012), w/o emulsion-crosslinking technique (Bajpai & Bhanu, 2007; Fang et al., 2008; Li et al., 2009b; Mundargi et al., 2008; Phromsopha, Srihanam, & Baimark, 2012), w/w emulsion-crosslinking technique (Li et al., 2009a; Li et al., 2012), and spray-drying (Desai, 2005; Liu et al., 2007; Witschi & Mrsny, 1999).

Of those preparation methods, spray-drying is a very efficient particle engineering technique, with relatively straightforward scale-up capability for industrial production. It comprises the atomization of drug-containing liquid feed into droplets, which are subsequently dried upon contact with heated drying air. The drying droplets are usually maintained at a much lower temperature than the drying air due to the evaporative cooling effect. Additionally, the dried particles are rapidly removed from the drying chamber within seconds. Therefore, both the drying droplets and the dried particles are well protected from overheating. This technique offers relatively accurate control of particle characteristics, such as particle size and size distribution, particle density, etc., through controlling the process parameters as well as the liquid feed variables (Emami et al., 2018; Focaroli et al., 2019; Kusonwiriyawong, Pichayakorn, Lipipun, & Ritthidej, 2009). As a consequence, spray-drying is likely a promising technique for the production of microparticles intended for the delivery of proteins.

Although the preparation of polymeric microparticles for protein delivery has been extensively investigated, only limited information on the development of spray-dried starch microparticles for such a purpose is available. The most relevant study (Witschi & Mrsny, 1999) described the production of spray-dried soluble starch microparticles as vaccine carriers. However, the microparticles were only characterized by their bioadhesive property and the induction of cytokine release from the respiratory epithelium. Information appears to be lacking on varying the spray-drying process parameters and the feed formulation variables to produce the controlled characteristics of the starch microparticles as protein carriers. Corn and tapioca starch have long been utilized as pharmaceutical excipients in many dosage forms (Häusler, 2012) and controlled drug delivery systems (Biswas & Sahoo, 2016; Queiroz et al., 2020; Soltani, Bahri, Djerboua, & Baitiche, 2014; Wannaphatchaiyong, Heng, Suksaeree, Boonme, & Pichayakorn, 2019;). Nevertheless, the spray-drying of those starches to prepare microparticulate carriers for protein delivery still remains to be explored. Accordingly, this study

aimed to investigate the development of protein-loaded corn and tapioca starch microparticles by the spray-drying technique. The spray-drying process parameters and the feed formulation variables were manipulated to obtain the optimum microencapsulation.

2. Objectives

Corn and tapioca starch microparticles for the delivery of proteins were developed by the spray-drying process. The effects of varying the processing conditions and the formulation parameters on the physicochemical properties of the resultant microparticles were carefully explored. Bovine serum albumin (BSA), as a model protein drug, was incorporated into the microparticles. In addition to the physicochemical properties of the protein-loaded microparticles, the integrity of entrapped BSA was examined.

3. Materials and methods

3.1 Materials

Corn starch (CS) and tapioca starch (TS) were obtained from RS Foods Tech (Samut Sakorn, Thailand) and Thai Wah Food Products (Bangkok, Thailand), respectively. Bovine serum albumin Cohn Fraction V (BSA) was purchased from Sigma-Aldrich (Singapore). All other chemicals were of analytical grade and used as received.

3.2 Methods

3.2.1 Preparation of starch microparticles

Starch was first dispersed in a small amount of water at room temperature. Boiling water was then added to the required amount and stirred vigorously until a smooth paste was obtained. Subsequently, the paste was spray-dried in a bench-top spray-dryer (Mini Spray Dryer B-290, Büchi Labortechnik, Flawil, Switzerland) equipped with a standard cyclone. The liquid feed was pumped peristaltically and fed through a two-fluid nozzle (0.7 mm internal diameter), where it was atomized into fine droplets. Cooling water was circulated through the jacket around the nozzle throughout the process. The processing parameters, comprising the inlet drying air temperature, the liquid feed rate, the atomizing air volumetric flow rate, and the aspirator vacuum, were varied in the ranges of 100 to 170°C, 1.9 to 7.0 g/min, 240 to 740 L/h, and -30 to -48 mbar, respectively, to investigate their effects on the physicochemical properties of the resultant microparticles. To incorporate protein into the microparticles, BSA was first dissolved in a few mL of distilled water and subsequently mixed with the starch paste. The mixture was then adjusted with water to a pre-determined weight to obtain different protein loading levels, and eventually spray-dried under a suitable processing condition.

3.2.2 Determination of particle size

Particle size and size distribution of starch microparticles were measured by the laser light-diffraction method (Mastersizer 2000, Malvern Panalytical, Malvern, UK). A small amount of starch microparticles was dispersed in a few mL of absolute ethanol and sonicated with a 3-mm-tip diameter standard probe at an output control of 60 (Vibra Cell model VC 130 PB, Sonics and Materials, Newtown, CT, USA) for about 30 s, in order to disaggregate the microparticles. The dispersion was loaded into a stirred sample cell containing ethanol as a measuring medium. A calculation of the particle size was made from the intensity of light scattered at different angles, based on Mie's theory (Zimmerman, 1997). The particle size was presented in the volume-weighted mode and the 50% undersize diameter $d\ (v,\ 0.5)$ was referred to as the particle diameter. The result was an average of 5 runs.

3.2.3 Morphology of starch microparticles

Starch microparticles were mounted onto double-faced adhesive tape, which was attached to a sample stub. The samples were sputtered with gold and viewed under a scanning electron microscope (Jeol model JSM-5800 LV, Tokyo, Japan) at a voltage of 15.0 kV. Photomicrographs were taken at a magnification of 1500 and 5000 for the CS and TS microparticles, respectively.

3.2.4 Moisture content

The moisture content was analyzed with a moisture analyzer (Mettler PM 100 LP 16, Mettler Toledo, Columbus, OH, USA). Five hundred milligrams of starch microparticles were transferred and accurately weighed (W_0) on an aluminum pan, which was tared on the moisture analyzer. The sample was distributed as evenly as practicable by gentle sidewise shaking and subsequently heated at 105°C for 5 min. The dried sample was accurately re-weighed (W_1). The percentage of the moisture content was then calculated according to Equation (1).

$$\% \text{ Moisture content} = \frac{W_0 - W_1 \times 100}{W_0} \quad (1)$$

3.2.5 Protein content determination

Starch microparticles were dissolved in distilled water. BSA content was determined using a bicinchoninic acid kit for protein determination (Sigma-Aldrich, St. Louis, MO, USA). The reaction was run at room temperature for 2 hrs. The optical density of the sample solution was read at 562 nm on a spectrophotometer (Hitachi U-2000, Hitachi High-Technologies, Tokyo, Japan). Protein loading and entrapment efficiency were then calculated according to Equations (2) and (3), respectively. The experiment was performed in triplicate.

$$\% \text{ BSA loading} = \frac{\text{Actual weight of BSA} \times 100}{\text{Weight of microparti cles}} \quad (2)$$

$$\% \text{ Entrapment efficiency} = \frac{\text{Actual weight of BSA} \times 100}{\text{Nominal weight of BSA}} \quad (3)$$

3.2.6 Protein integrity

The integrity of the protein entrapped within the starch microparticles was investigated through sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The BSA was recovered by dissolving the microparticles in distilled water. One part of the sample was mixed with one part of reducing sample buffer (5% β-mercaptoethanol in Laemmli Sample Buffer, Bio-Rad Laboratories, Hercules, CA, USA) and heated at 95°C for 1 min. The mixture equivalent to 10 µg of BSA was loaded onto a 12% Mini-PROTEAN TGX Precast gel (Bio-Rad Laboratories, Hercules, CA, USA) and subjected to electrophoresis (Bio-Rad model Mini-PROTEAN 3, Bio-Rad Laboratories, Hercules, CA, USA) in pH 8.3 Tris/Glycine/SDS running buffer at 200 V for about 45 min. The gel was first rinsed three times for 5 min each with an excess of distilled water. It was then stained with 20-40 mL of EZBlue™ Gel Staining Reagent (Sigma-Aldrich, St. Louis, MO, USA) for 1 hr and destained several times with distilled water until the protein bands were visualized.

4. Results

4.1 Characterization of corn starch microparticles

Starch microparticles were prepared by the spray-drying technique at different processing conditions and formulation parameters. Physicochemical properties of the resultant microparticles are presented in Table 1.

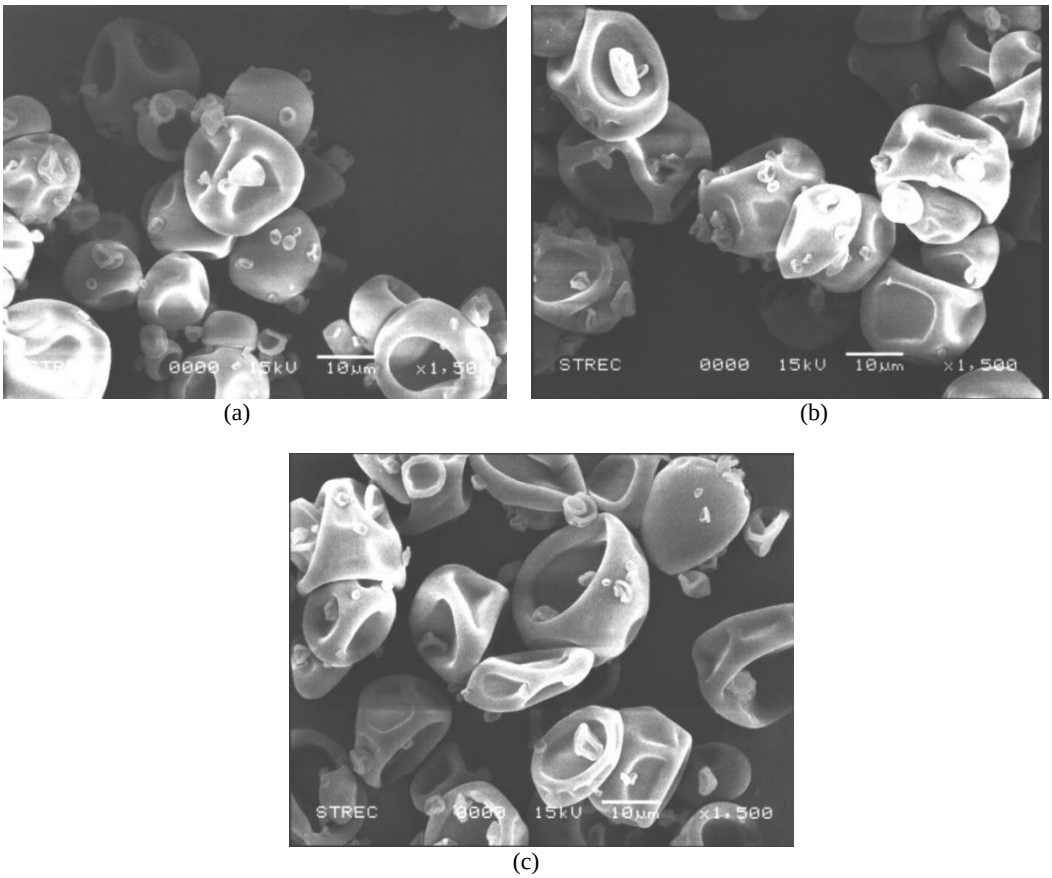
Spray-drying of 2% w/w CS paste at an inlet drying air temperature of 100°C resulted in small microparticles (15.9 µm), with the moisture content and product yield of 12.6% and 20.1%, respectively. When the inlet drying air temperature was increased to 120°C, slightly larger microparticles, lower moisture content and higher product yield were obtained. However, further increase of the inlet drying air temperature to 140°C had little or no effect on the investigated microparticle characteristics. The surface topography and morphology of the CS microparticles spray-dried at varied inlet drying air temperature are illustrated in Figure 1. Both smooth spherical and dented microparticles were obtained when they were prepared at the inlet drying air temperature of 100°C (Figure 1a). As the inlet drying air temperature was increased, more dented and/or distorted microparticles were apparent (Figures 1b and 1c).

Varying the liquid feed rate had little effect on the microparticle size (Table 1). However, as the liquid feed rate was increased from 3.4 g/min to 5.0 g/min and 7.0 g/min, the product yield was significantly reduced from 22.8% to 15.4% and 6.0%, respectively. During the spray-drying process, especially at a high liquid feed rate, it was observed that some starch paste was deposited on the wall of the drying chamber. This likely resulted in a lowered product yield, especially when the production of microparticles was carried out at a high liquid feed rate. The observation under the scanning electron microscope revealed dented and distorted CS microparticles (Figure 2). When the higher liquid feed rate was applied, some smooth spherical particles were observed (Figures 2b and 2c).

Table 1 Effect of formulation and process parameters on the physicochemical properties of the starch microparticles

Starch	Conc. (% w/w)	Temp.[1] (°C)	Aspirator (mbar)	Air flow rate (L/h)	Feed rate (g/min)	Particle size (μm)	Moisture content (%)	Yield (%)
CS	2	100/66	-32	240	3.2	15.9 (0.4)[2]	12.6	20.1
		120/79	-32	240	3.3	17.5 (0.4)	11.8	27.6
		140/91	-32	240	3.3	17.5 (0.4)	11.9	26.7
	4	120/76	-32	240	3.4	19.9 (0.3)		22.8
		120/77	-32	240	5.0	17.9 (0.4)		15.4
		120/76	-32	240	7.0	19.1 (0.4)		6.0
		120/75	-32	470	3.4	12.9 (0.5)		34.5
		120/70	-32	740	3.3	10.5 (0.6)		31.0
		120/83	-38	240	3.3	20.5 (0.4)		14.7
		120/89	-48	240	3.3	20.1 (0.4)		15.1
		140/91	-32	240	3.3	20.7 (0.4)	11.0	22.9
	6	140/89	-32	240	3.3	22.9 (0.4)	9.6	22.2
	8	140/93	-32	240	3.4	24.8 (0.4)		11.1
TS	2	161/100	-30	740	1.9	25.3 (0.4)		
		140/90	-30	740	3.0	20.0 (10.0)		
		156/100	-30	740	3.0	21.2 (1.4)		
		173/107	-30	740	3.0	22.2 (6.1)		
	3	162/102	-30	740	1.9	30.9 (1.5)		
		166/104	-30	740	3.0	30.3 (0.4)		

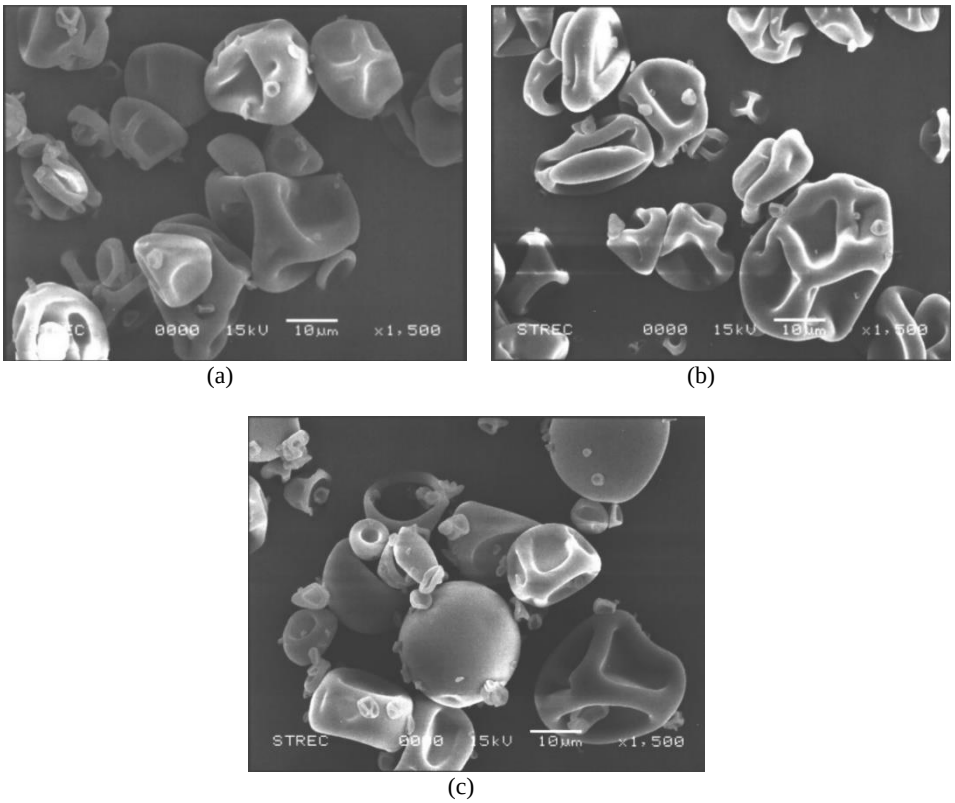
[1]Inlet air temperature/outlet air temperature
[2]Absolute deviation from the median (Uniformity)



(a) (b)

(c)

Figure 1 Scanning electron photomicrographs of the CS microparticles obtained by spray-drying of 2% w/w corn starch paste at an inlet temperature of (a) 100°C, (b) 120°C, and (c) 140°C, an aspirator of -32 mbar, an atomizing air volumetric flow rate of 240 L/h, and a spray rate of 3 g/min.



(a) (b)

(c)

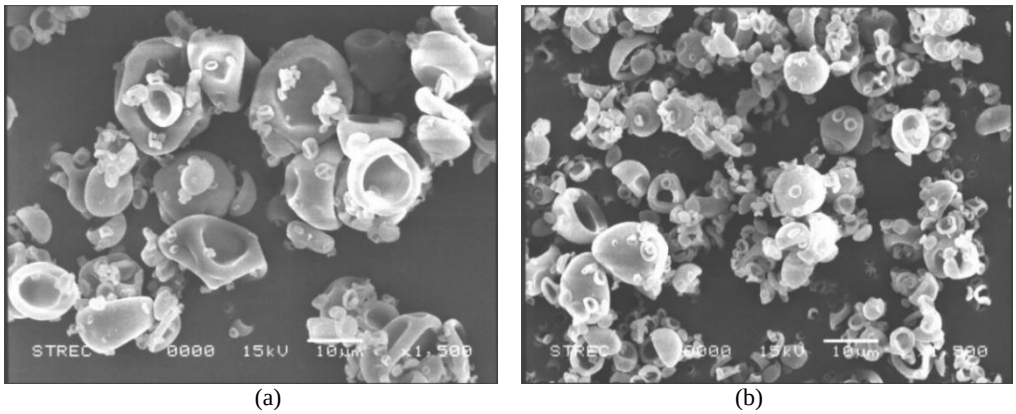
Figure 2 Scanning electron photomicrographs of the CS microparticles obtained by spray-drying of 4% w/w corn starch paste at an inlet temperature of 120ºC, an aspirator of -32 mbar, an atomizing air volumetric flow rate of 240 L/h, and a spray rate of (a) 3 g/min, (b) 5 g/min, and (c) 7 g/min.

When the atomizing air volumetric flow rate was increased from 240 L/h to 470 L/h and 740 L/h, the microparticle size was significantly reduced from 19.9 µm to 12.9 µm and 10.5 µm, respectively (Table 1). Increasing the atomizing air volumetric flow rate also caused a more broadened size distribution, according to higher Uniformity (Table 1). Additionally, the product yield was improved as the atomizing air volumetric flow rate was increased. Figures 2a and 3 depict a higher proportion of the small microparticles when the higher air volumetric flow rate was applied. This likely supported the effects found on the size and size distribution of the CS microparticles, as described above.

Although varying the aspirator vacuum seemed to have little effect on size (Table 1) and morphology of the microparticles (Figures 2a and 4), decreasing the aspirator vacuum resulted in a slight reduction of the product yield (Table 1).

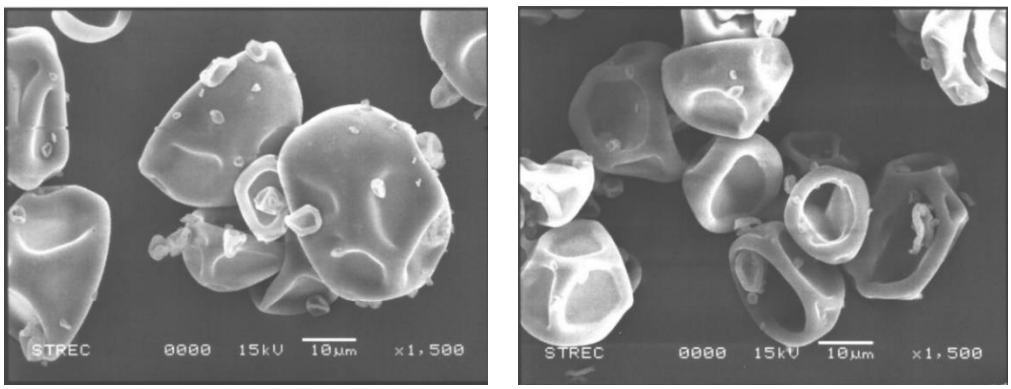
The effects of modifying the concentration of the CS paste were investigated at the inlet drying air temperature of 140ºC. Increasing the concentration of the starch paste resulted in slightly larger microparticles (Table 1). In most cases, the effect on the product yield was not obvious; however, the product yield of the microparticles prepared from 8% w/w CS paste was remarkably reduced. Spray-drying of 8% w/w CS paste resulted in a dried starch film on the wall of the drying chamber. Consequently, a lowered product yield was obtained. A moisture analysis of the microparticles showed a lowered percentage of the moisture content, as the concentration of the CS paste was increased. The scanning electron photomicrographs revealed a proportion of the large microparticles when they were prepared from a high concentration of CS paste (Figures 2a and 5).

With the exception of the atomizing air volumetric flow rate and the formulation parameters, in most cases the investigated processing conditions had little influence on the size distribution of the CS microparticles according to the consistency of Uniformity (Table 1).



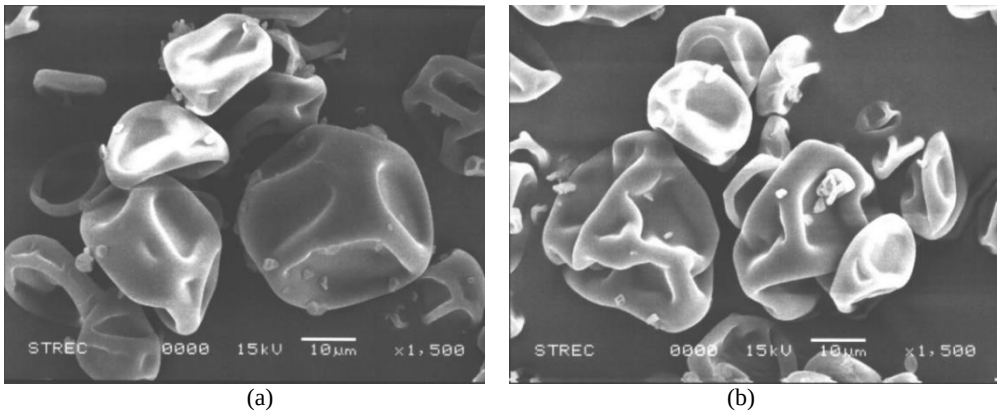
(a) (b)

Figure 3 Scanning electron photomicrographs of the CS microparticles obtained by spray-drying of 4% w/w corn starch paste at an inlet temperature of 120ºC, an aspirator of -32 mbar, an atomizing air volumetric flow rate of (a) 470 L/h and (b) 740 L/h, and a spray rate of 3 g/min.



(a) (b)

Figure 4 Scanning electron photomicrographs of the CS microparticles obtained by spray-drying of 4% w/w corn starch paste at an inlet temperature of 120ºC, an aspirator of (a) -38 mbar and (b) -48 mbar, an atomizing air volumetric flow rate of 240 L/h, and a spray rate of 3 g/min.



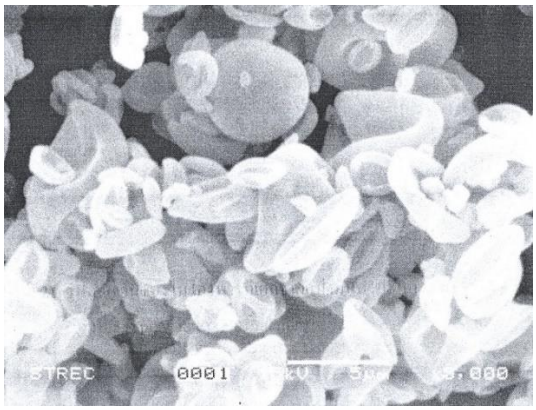
(a) (b)

Figure 5 Scanning electron photomicrographs of the CS microparticles obtained by spray-drying of (a) 4% w/w and (b) 6% w/w CS paste, at an inlet temperature of 140ºC, an aspirator of -32 mbar, an atomizing air volumetric flow rate of 240 L/h, and a spray rate of 3 g/min.

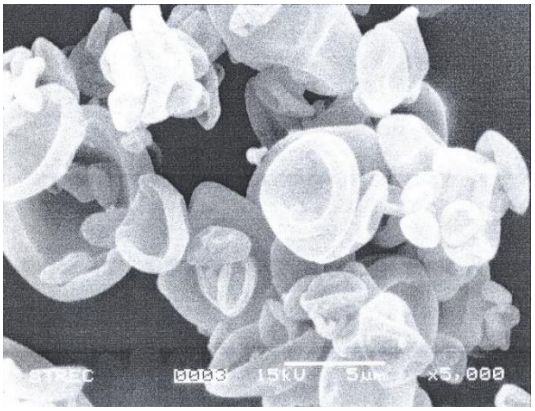
4.2 Characterization of tapioca starch microparticles

Tapioca starch microparticles were prepared by spray-drying at selected processing conditions. Increasing the inlet drying air temperature resulted in slightly larger microparticles than those prepared at a lower temperature (Table 1). The effect was similar to that on the CS microparticles. However, the effect of altering temperature on the size distribution varied. The scanning electron photomicrographs showed smooth distorted microparticles of various sizes (Figures 6a-6c), corresponding well with the Uniformity (Table 1). Similarly, spray-drying at different liquid feed rates had little effect on the TS microparticle size. Increasing the feed rate from 1.9 g/min to 3.0 g/min resulted in the microparticles with the sizes of 25.3 µm vs. 21.2 µm and 30.9 µm vs. 30.3 µm for spray-drying of 2% w/w and 3% w/w TS paste, respectively. Taking their surface characteristics and morphology in Figure 6 into consideration, the microparticles spray-dried at different feed rates resembled one another (Figures 6d vs. 6b and 6f vs. 6e). The concentration of the TS paste clearly affected the size of microparticles. When the concentration was increased, the size of the microparticles increased, i.e., 25.3 µm vs. 30.9 µm and 21.2 µm vs. 30.3 µm for spray-drying of 2% w/w vs. 3% w/w TS paste, respectively. The scanning electron photomicrographs confirmed the effect (Figures 6d vs. 6f and 6b vs. 6e). The microparticles were larger when they were prepared from the higher concentration of the TS paste. Additionally, some small particles were scattered throughout the photos.

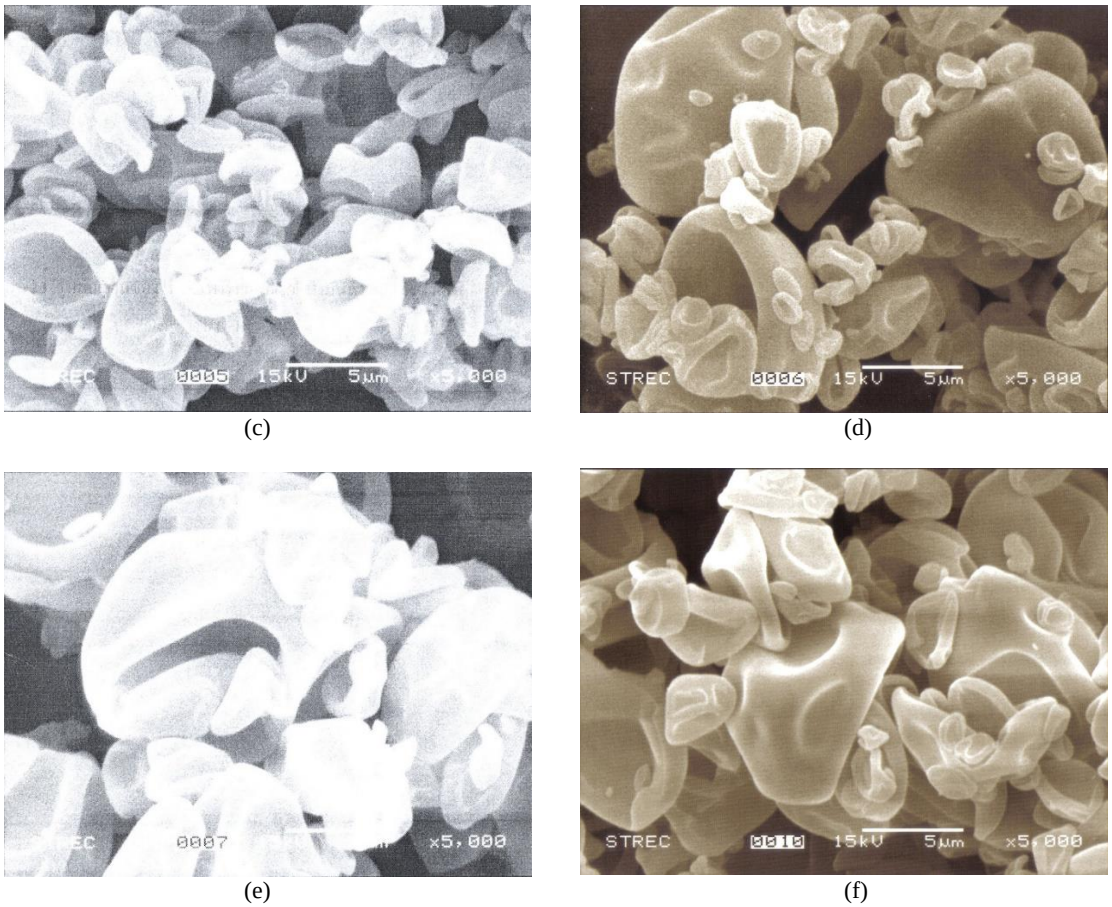
Generally, the processing conditions and the formulation parameters affected the size and size distribution of the resultant CS and TS microparticles in the same way. When the microparticles were prepared by the same processing and formulation parameters, i.e., spray-drying of 2% w/w starch paste at the inlet drying air temperature of 140°C and the feed rate of about 3 g/min, the TS microparticles (20.0 µm) were slightly larger than the CS microparticles (17.5 µm). Moreover, the size distribution of most TS microparticles was larger than that of the CS microparticles (Table 1). The morphology and topography of the CS and TS microparticles spray-dried at the same processing and formulation variables were then compared. The CS microparticles (Figure 1c) were observed to be generally less distorted than the TS microparticles (Figure 6a). Additionally, the CS microparticles seemed to be more uniform in size than the TS microparticles. This effect corresponded with the higher Uniformity of the TS microparticles than the CS microparticles (Table 1).



(a)



(b)



(c) (d)

(e) (f)

Figure 6 Scanning electron photomicrographs of the TS microparticles obtained by spray-drying of 2% w/w tapioca starch paste at a spray rate of 3 g/min and an inlet temperature of (a) 140ºC, (b) 156ºC, (c) 173ºC, (d) a spray rate of 1.9 g/min and an inlet temperature of 161ºC, (e) 3% w/w tapioca starch paste at an inlet temperature of about160ºC and a spray rate of 3 g/min, and (f) 1.9 g/min.

4.3 Characterization of BSA-loaded CS microparticles

Suitable processing conditions and formulation parameters were selected to prepare the BSA-loaded starch microparticles. CS was selected instead of TS because it was less difficult to handle and process due to lower viscosity at the same concentration (7.0 ± 0.3 cps vs. 35.9 ± 0.2 cps for CS paste and TS paste, respectively, at the concentration of 2% w/w, determined by using the Brookfield Viscometer DV-II+ Pro equipped with the spindle SC4-18 rotating at the set speed of 30.0 rpm). Consequently, the liquid feed rate was more controllable when the CS paste was spray-dried. The physicochemical properties of the CS microparticles at different levels of BSA loading are presented in Table 2.

BSA was successfully encapsulated in the CS microparticles with very high entrapment efficiency, indicating that most of the protein was incorporated into the microparticles. In some cases, the entrapment efficiency was found to be larger than 100%. This likely resulted from inhomogeneous mixing the BSA solution with the slightly viscous starch paste prior to spray-drying and unintentional sampling only the BSA-enriched microparticles for the protein content determination. Incorporation of BSA into the CS microparticles resulted in smaller microparticles (11.6 to 12.8 µm, Table 2) than the corresponding blank CS microparticles (19.9 µm, Table 1). Further increasing the loading of BSA did not significantly affect the size and size distribution of the protein-loaded microparticles (Table 2). The product yield was about doubly increased relative to

the blank CS microparticles (22.8%, Table 1). Figure 7 depicts the surface topography and morphology of the protein-loaded CS microparticles. Some tiny particles were observed to be deposited on the surface of large distorted microparticles, which resembled the blank CS microparticles. The appearance of tiny particles was more pronounced as the BSA loading was increased.

Table 2 Effects of protein loading on physicochemical properties of BSA-loaded CS microparticles prepared by spray-drying of 4% w/w starch paste at a spray rate of 3.2 to 3.3 g/min and an inlet temperature of 120ºC

Nominal protein loading (%)	Actual protein loading (%)	Entrapment efficiency (%)	Particle size (μm)	Yield (%)
1.0	1.0 ± 0.1[1]	94.3 ± 5.9[1]	11.6 (0.5) [2]	40.6
2.5	3.1 ± 0.2	124.9 ± 8.1	12.8 (0.4)	42.2
5.0	5.2 ± 0.5	104.0 ± 10.2	12.4 (0.5)	45.6

[1]Standard deviation
[2]Absolute deviation from the median (Uniformity)

The integrity of the protein entrapped within the CS microparticles was investigated in comparison with that of the unprocessed BSA. The SDS-PAGE showed that the spray-drying process used in this study did not deteriorate the BSA integrity. As illustrated in Figure 8, all bands of BSA, including the unprocessed BSA (Lane 2) and BSA recovered from CS microparticles with 1%, 2.5%, and 5% protein loadings (Lanes 3-5), moved through the electrophoretic gel similarly to one another. No band was observed in the lower molecular weight region. The bands appearing in the higher molecular weight region were believed to result from the aggregation of the proteins that occurred during the recovery and/or SDS-PAGE sample preparation process.

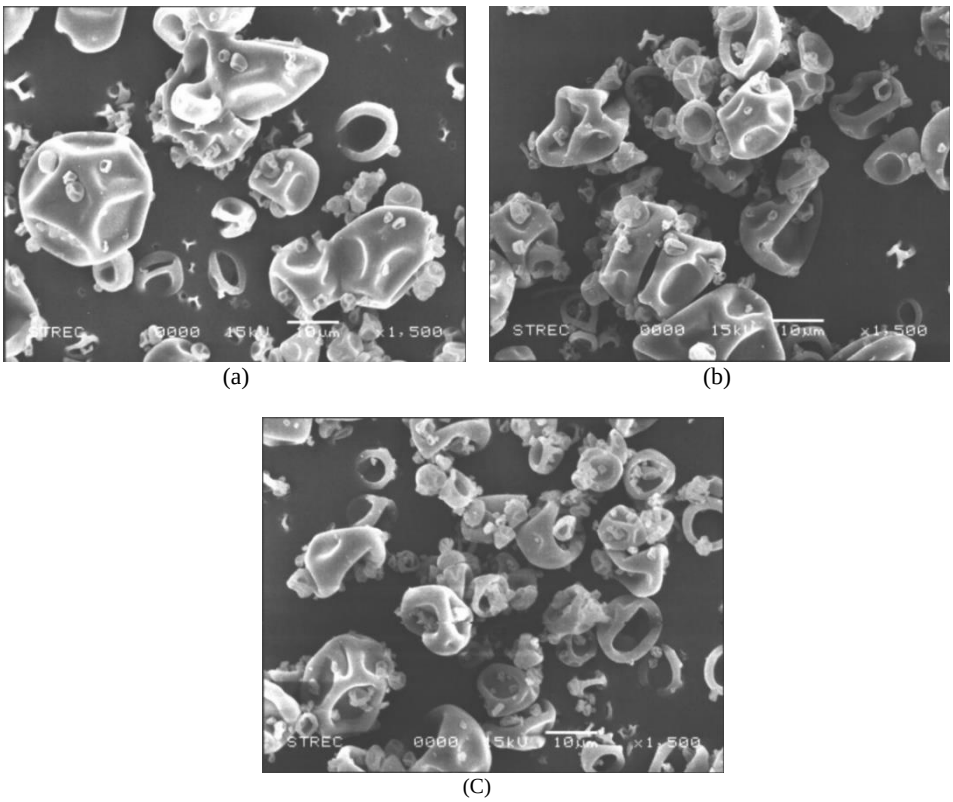


Figure 7 Scanning electron photomicrographs of the BSA-loaded CS microparticles obtained by spray-drying of (a) 1%, (b) 2.5%, and (c) 5% w/w BSA loaded in 4% w/w CS paste at a spray rate of 3.2 to 3.3 g/min and an inlet temperature of 120ºC.

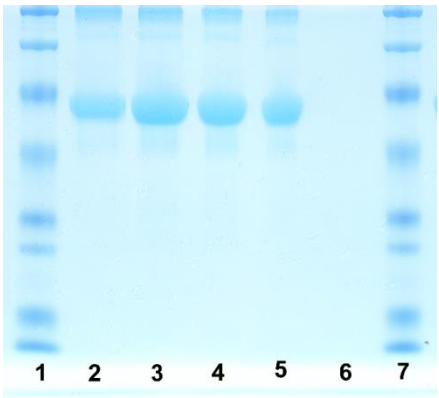


Figure 8 SDS-PAGE. Lanes 1 and 7: Prestained SDS-PAGE molecular weight standards, broad range (Bio-Rad Laboratories, Hercules, CA, USA); Lane 2: Unprocessed BSA; Lanes 3-5: BSA recovered from 5%, 2.5% and 1% w/w BSA-loaded CS microparticles, respectively; Lane 6: Sample buffer.

5. Discussion

In this study, the size of the spray-dried starch microparticles was affected by both processing and formulation parameters, mainly the atomizing air volumetric flow rate and the composition and/or concentration of the liquid feed. Increasing the atomizing air volumetric flow rate resulted in increased energy for breaking up the liquid feed by the nozzle (Focaroli et al., 2019; Ståhl, Claesson, Lilliehorn, Lindén, & Bäckström, 2002). Consequently, smaller droplets of liquid feed and subsequent smaller microparticles were obtained upon atomization and drying, respectively. Nevertheless, some large droplets and subsequent large microparticles were also formed, as illustrated in Figure 3. Thus, increasing the atomizing air volumetric flow rate produced a broader size distribution of the microparticles. In contrast, increasing the solid content and/or the viscosity of the liquid feed—either by increasing the concentration of starch paste or by spray-drying the TS paste, which possessed higher viscosity than the CS paste—created some difficulty for atomizing the liquid feed, resulting in larger droplet size and larger microparticles when dried (Goula & Adamopoulos, 2004; He, Davis, & Illum, 1999; Kusonwiriyawong et al., 2009). Furthermore, increasing the concentration of starch paste was also attributed to the presence of a higher amount of starch in the droplets, leading to the formation of larger microparticles (Liu et al., 2007).

In addition, spray-drying both CS and TS pastes at higher temperatures resulted in slightly larger microparticles. This was possibly due to the rapid development of particle structure at a high inlet drying air temperature, preventing the subsequent deflation and shrinkage upon drying (Wang, Dufour, & Zhou, 2015; Wei, Huang, Cheng, & Song, 2020). Thus, slightly larger microparticles were obtained.

Proteins are known to be surface-active molecules. Incorporation of BSA into the CS paste likely reduced the surface tension of the liquid feed, delivering ease of atomization to some extent. Consequently, smaller microparticles were produced compared with the corresponding blank CS microparticles.

Ståhl et al. (2002) described that the collection efficiency of the bench-top spray-dryer was relatively low, ca. 20 to 60%, which has been a common drawback of this system design. It was estimated that, depending on the processing conditions, approximately 15% and 24 to 77% of the spray-dried material adhered to the walls of the equipment and was carried through the cyclone with the outgoing air, respectively. These figures are in line with earlier results (O'Riordan, Andrews, Buckle, & Conway, 2001). In this study, all investigated processing and formulation parameters seemed to affect the percentage of product yield. Generally, the droplets produced from the two-fluid nozzle tended to project toward the wall of the drying chamber. If the drying had been proceeding inadequately, the droplets and/or the forming microparticles impacted with and subsequently adhered to the wall of the drying chamber, leading to the formation of wet deposits and/or dried starch film on the wall. As a result, the percentage of product yield was reduced (Maury, Murphy, Kumar, Shi, & Lee 2005). This was the case when

a high feed rate and a high feed concentration were used (Chang, Tan, & Pui, 2020). On the other hand, increasing the inlet drying air temperature likely allowed the droplets to dry before hitting the wall of the drying chamber. The extent of wall adhesion was thus reduced, resulting in the progressive improvement of the yield percentage (Bazaria & Kumar, 2018; Focaroli et al., 2019).

Atomizing air volumetric flow rate has been reported to positively affect the product yield (Focaroli et al., 2019). Increasing the volumetric flow rate resulted in the atomization of liquid feed into smaller droplets, contributed to the greater specific surface area and consequently a better contact between the drying air and the surface of the forming microparticles. This led to the improved drying and the properly dried microparticles, which had a lower tendency to deposit on the wall of the drying chamber or the cyclone separator before reaching the collection vessel. As a result, a higher yield percentage was obtained. It was likely the case when BSA was incorporated into the CS microparticles. As mentioned above, the addition of protein resulted in the production of smaller microparticles, leading to more complete drying and hence a higher product yield. Additionally, the aspirator vacuum was observed to be another important parameter that could display a positive effect on the product yield (Telang & Thorat, 2010; Thirugnanasambandham & Sivakumar, 2017). Increasing the aspirator vacuum provided a higher amount of drying air and hence higher energy for solvent evaporation. This condition contributed to sufficient drying of the microparticles in the chamber and accordingly enhanced product yield.

To further improve product recovery, using a high-efficiency cyclone or a specially-designed dual cyclone was recommended (Gikanga et al., 2015). The air flow system of the Büchi Spray-dryer has also been improved by detaching the bag-filter unit, relocating the aspirator to control the air input from upstream, and mounting a vacuum-filter unit to draw the exhaust air (Maa, Nguyen, Sit, & Hsu, 1998). Such an adapted system enhanced product collection efficiency in the receiving vessel due to the reduction of air flow resistance and the increment of the drying air flow rate. Additionally, increasing the batch size from laboratory to pilot scale seemed to improve product yield. Zhu et al. (2014) investigated the development of influenza vaccine formulations using the Büchi Spray-dryer B-290 and the subsequent production of selected formulations at a pilot scale on the Bend Research Spray-dryer BLD-1. A higher yield of about ≥ 20% (from ≥ 70% to ≥ 90%) was obtained.

Residual moisture content is thought to influence the physical stability of protein encapsulated within the spray-dried microparticles. Moisture content was found to depend directly on the inlet drying air temperature (Focaroli et al., 2019; Zier, Schultze, & Leopold, 2018). Increasing the inlet temperature likely supplied enhanced energy to the drying process and hence allowed more efficient solvent evaporation (Amaro, Tajber, Corrigan, & Healy, 2011; Chang et al., 2020), resulting in microparticles with lowered residual moisture content. Nevertheless, only a small effect was observed in this study. Additionally, the residual moisture content of the spray-dried microparticles was controlled by the feed solids concentration (Gong, Zhang, Mujumdar, & Sun, 2008). A lower residual moisture content could be achieved by applying a higher feed solids concentration (Goula & Adamopoulos, 2004), possibly due to the reduction of total water for removal (Chang et al., 2020).

Morphology and topography of the microparticles upon spray-drying were principally controlled by the rate of droplet drying and composition of the liquid feed (Ameri & Maa, 2006). A concept of the Péclet number, which is a correlation of convective transport and diffusive transport phenomena (Rapp, 2017), could be applied to describe the particle formation process and the subsequent morphology of the spray-dried microparticles. In cases of proteins and polymers, the Péclet number was estimated to be larger than 1, that is, the rate of droplet evaporation was faster than the diffusion of the components within the droplets (Vehring, 2008). As the atomized droplets dried, the components likely accumulated at the outer surface. A theoretical shell was then formed, once the saturation was reached. Meanwhile, the diffusional motion of the components would be too low to evenly distribute within the droplets during evaporation, due to the intrinsic viscosity of the starch paste. Both the forming shell and the viscosity possibly prevented the outward evaporation of water, resulting in the increase of vapor pressure inside the drying droplets. When the internal pressure exceeded what the shell could resist, it was then prone to disrupt before the complete drying could occur. As a result, collapsed

or deformed microparticles were produced, particularly in the fast drying process (Ameri & Maa, 2006; Liu, Wu, Selomulya, & Chen, 2011; Sukaraseranee, Watcharamaisakul, Golman, & Suwanprateeb, 2017). These might presumably be inherent characteristics of the spray-dried starch microparticles (O'Riordan et al., 2001). Either decreasing the inlet drying air temperature or increasing the liquid feed rate would result in a slower and more complete drying. Consequently, some completely spherical microparticles were observed, as illustrated in Figures 1a and 2c. CS and TS microparticles were observed to have similar morphology and topography, although the TS paste possesses higher viscosity than the CS paste.

There are several concerns in spray-drying sensitive molecules. Application of high temperature could deteriorate the sensitive proteins (Kusonwiriyawong et al., 2009). Two other possible causes of stress during spray-drying including shear stress and large air-liquid interface, generated through the atomization, could induce the adsorbed protein to unfold, expose the hydrophobic regions, and undergo aggregation by the interaction with other unfolded protein molecules until the precipitation occurs (Ameri & Maa, 2006). Several studies also supported that protein denaturation at the air-liquid interface of spray droplets played a major role in protein degradation during spray-drying (Maa & Hsu, 1997; Maa et al., 1998; Mumenthaler, Hsu & Pearlman, 1994). Furthermore, the protein denaturation could occur during the dehydration step as well (Griebenow & Klibanov, 1995; Prestrelski, Tedeschi, Arakawa, & Carpenter, 1993; Tzannis & Prestrelski, 1999). Removal of hydration water molecules that are required to form hydrogen bonds with protein molecules could alter the native structural conformation and thus compromise the biological activity of proteins.

Despite those potential stresses on protein stability, the spray-drying process appeared to be very efficient in encapsulating stable protein into the CS microparticles according to the comparable nominal and actual protein loading (Table 2). As a consequence, protein encapsulation efficiency was high, implying a reliable encapsulation of protein into the CS microparticles. Because all biological phenomena involve the process of molecular recognition, it is important for delivery systems to retain the native integrity of the protein. No cleavage of protein backbone was evident on the electrophoretic gel; therefore, it was believed that the spray-drying process used in this study did not deteriorate the integrity of encapsulated proteins, corresponding to the findings of a previous study (Kusonwiriyawong et al., 2009).

Overall, the findings of this study provide some practical information that could enable further development of starch microparticles with more specific properties. Depending on the intended route of administration or target of the protein drugs, a modification of the prospective CS microparticles, either by surface functionalization or by incorporation of pharmaceutical excipients into the microparticles, should be examined.

6. Conclusion

Starch microparticles for protein delivery were successfully developed by a conventional spray-drying process. The microparticle size was mainly controlled by the atomizing air volumetric flow rate and the concentration and/or the viscosity of the starch paste, whereas the morphology and topography of the microparticles were directly controlled by the rate of droplet drying and the composition of the liquid feed. The product collection efficiency was affected by all investigated processing and formulation parameters. Increasing the inlet drying air temperature or the feed solids concentration resulted in low residual moisture content within the microparticles. Incorporation of protein into the CS microparticles was very efficient, and the entrapment efficiency of the protein was high. Moreover, the integrity of the encapsulated protein was well retained. Thus, this study provides useful information on controlling both the process parameters and the formulation variables to achieve microparticles with desired physico-chemical properties, promoting the potential application of starch microparticles as protein drug carriers.

7. Acknowledgements

Partial funding from the Research Institute, Rangsit University, is gratefully acknowledged. The courtesy of Prof. Dr. Garnpimol C. Ritthidej, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, for the Mastersizer 2000 is appreciated. The author also would like to thank N. Charoen, A. Thanessakul, K. Artharn and P. Sakunthong for their technical assistance in spray-drying.

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