EFFECT OF EMULSION STABILITY AND SPRAY DRYING CONDITIONS ON PHYSICOCHEMICAL CHARACTERISTICS OF ENCAPSULATED POWDERS

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***Abstract*— In this investigation, four biopolymers were mixed with Maltodextrin (MDX) and used as emulsion stabilizers and wall materials to encapsulate lycopene natural pigment. These biopolymers were whey protein concentrate (WPC), soy protein concentrate (SPC), Gelatin (GEL) and Arabic Gum (AG). Some parameters such as droplet size, viscosity and creaming index of emulsions were investigated and then, to produce lycopene microcapsules, emulsions were transferred to spray dryer to be dried. Inlet air temperature (150 and 200 °C) and pressure of nozzle (1 and 2 bar) were considered as drying variables. Results showed that WPC emulsions had the smallest droplet size and lowest viscosity among other samples. The highest Encapsulation Efficiency (EE) belonged to WPC emulsions which were dried at 150 °C and 2 bar, also these samples had the highest bulk density. Results showed that samples which were atomized by using 2bar pressure at nozzle during spray drying, needed a significant longer time to be rehydrated (P<0.05).**

***Keywords*— Emulsions, microencapsulation, Spray Drying.**

I. INTRODUCTION

Lycopene is a carotenoid pigment which can be found in some red fruits and vegetables such as ripe tomato, red pepper, watermelon and red grapefruit. Investigations have proved that this natural pigment can reduce risk of certain types of cancer, such as prostate cancer, digestive-tract cancers and lung cancer (Bramley, 2000 and Southon, 2000).

However, because it has unsaturated bonds in molecular structure, is susceptible to environmental conditions such as oxidants, light and heat during storage and processing and can be easily deteriorated when exposed to such factors (Lee and Chen, 2002; Pesek and Warthesen, 1987). Therefore, the free lycopene must be protected in some forms from chemical damage before its industrial application.

Encapsulation is the technique by which one material or a mixture of materials is coated with or entrapped within another material or system. The coated material is called active or core material, and the coating material is called shell, wall material or carrier (Madene *et al*., 2006).

The wall materials must be soluble in water (Gouin, 2004) and for microencapsulation by spray-drying should have good properties of emulsification, film forming and low viscosity (Reineccius, 1988).

One of the common techniques to produce encapsulated products is spray drying, which involves conversion of liquid substances into dry powders (Reineccius, 2004).

In this research, four emulsifier biopolymers, AG, GEL, WPC and SPC were mixed with MDX coating agent to stabilize and coat lycopene and then emulsions were dried by spray dryer in order to produce microencapsulated lycopene powders. During process, effect of spray drying variables such as inlet air temperature and air pressure at atomizing nozzle on physic-chemical properties of final microcapsules were investigated.

II. METHODS

Lycopene was purchased from Anhui minmetals development company (Anhuai,China).MDX (DE20), WPC 80%, GEL, SPC and AG were produced from Qinhuangdao Lihua starch Co. Ltd (Qinhuangdao, China), Sapoto Cheese, (USA), Farmand Company (Iran), Wachsen Company (Shandung, China) and Olin Company (Shanksi, China) respectively. Soy oil was purchased from Salimi industrials (Tabriz, Iran). Sodium dodecyl sulfate (SDS) was purchased from Merck (Germany).

Solid materials (WPC, AG and SPC 37% and GEL 35.3%) were hydrated at deionized and distilled water.

WPC solution was heated in a water bath (WB 14, Memmert, Germany) in 75°C for 30 min (Chen and Subirade, 2006) and then was mixed with MDX in ratio of 1:4 (for 100cc emulsion, 7.4 g of WPC+ 29.6g of MDX).

SPC solution was heated in water bath in 90°C for 30 min (Gan *et al.*, 2008) and then was mixed with MDX in ratio of 1:9 (for 100cc emulsion, 3.7g SPC+ 33.3g MDX).

AG and GEL were mixed with MDX in ratio of 1:3 (for 100cc emulsion, 9.25g AG+27.75g MDX) and 1:19 (for 100cc emulsion, 1.76g GEL+33.53g MDX) respectively.

Lycopene (5%w/w) was dissolved in soy oil and was mixed gradually with wall material solutions using a rotor-stator homogenizer (D 91126, Heidolf industries, Germany). To prepare coarse emulsions, 10000 rpm for 5 min was used, then emulsions were homogenized at 18000 rpm with same homogenizer for 10 min.

A. Properties of emulsions:

Droplet size:

The microstructure of emulsions was analyzed using a digital microscope (VIVA-BW 1008, Gaungdong, China) connected to a computer. Microscopic size measurement was carried out by taking photos of emulsions, and processing them by Image J (1.44P) software. Briefly, one droplet of emulsion was diluted in 100 mL 0.1% w/v SDS to prevent further droplet coalescence, and to reduce the droplet concentration down to about 0.01% v/v, because the original emulsions were too concentrated for drop size analysis (Ushikubo and Cunha, 2014). One droplet of this solution was placed on a slide glass, and photographed using microscope at 100x (Li *et al.*, 2012).

Viscosity:

Approximately, 15 mL of each emulsion was taken, and its viscosity was measured using a viscometer (D 220, Brookfield, USA) wih LV-1 spindle at 30°C. (Gharibzahedi *et al*., 2011).

Creaming index:

Stability test was carried out during storage at 30°C according to the reference method (Li *et al.*, 2012). Creaming was characterized by the creaming index (CI) as

$$\text{Creaming index}(\%) = \frac{\text{heigh of serum layer} + \text{height of cream layer}}{\text{totalheight}} \times 100 \text{ ,} \quad (1)$$

creaming index usually is defined as (Hs/Ht), where Hs is the height of the serum layer and Ht is the total height of the emulsions, but according to other investigations, in some cases which emulsion samples are separated into an optically opaque "cream" layer at the top and a transparent (or turbid) "serum" layer at the bottom after being transferred into glass vials and stored at the appropriate temperature, the sum of the turbid and transparent layers is defined as the serum layer.

Drying of emulsions:

A pilot spray dryer with 3m height and 1.5m diameter (Azar Makhzan, Iran) which was equipped by a nozzle atomizer whith outlet diameter of 0.5 mm. Temperature of inlet air (150 and 200 °C) and air pressure of nozzle (1 and 2 bar) were considered as variable and feed flow was 6kg/hr.

Encapsulation efficiency (EE):

The EE was calculated as the ratio between the initial mass of lycopene to be encapsulated and its mass in the final product. In order to measure it, 15ml Hexane was added to 0.5g encapsulated powder. Mixture was centrifuged in 1000 rpm for 2 min. supernatant was separated and its mass was determined at 470 nm by a UV spectrophotometer and standard curve (Klinkesorn *et al*. 2004). After removing surface lycopene, powders were dissolved in distilled water using magnet stirrer at 300 rpm for 30 min. then 40 ml of mixture of hexane and ethanol (3:4) was added and mixed in 3000 rpm for 2 min. then samples were centrifuged in 4000 rpm for 10 min and supernatant was removed and its mass was determined at 470 nm by a UV spectrophotometer and standard curve (Chiu *et al*., 2007). Finally, encapsulation efficiency was calculated

$$\text{EE} = \frac{\text{amount of lycopene inside the capsules}}{\text{total amount of lycopene which was added}} \times 100 \text{ .} \quad (2)$$

Moisture content of powders:

5g of powders were put in 105°C hot air oven and kept until no changes of weight was observed (AOAC, 2005).

Particle size:

Particle size was analyzed using a digital microscope (VIVA-BW1008, Gaungdong, China) connected to a computer. Microscopic size measurement was carried out by taking photos and processing them by Image J (1.44P) software. Briefly, 0.1g powder was immersed in 5 cc n-hexane and then was placed on a slide glass and observed and photographed by microscope at 100x (Li *et al*., 2012).

Bulk density:

2g of sample was weighted and filled in a graduated cylinder. Then volume was observed and bulk density was calculated according to (Goula and Adamopoulos, 2012)

$$\text{Bulk density} = \frac{\text{weight of sample}}{\text{Volume of sample}} \times 100 \text{ .} \quad (3)$$

Time of rehydration:

2g of sample was mixed with 50 ml of distilled water and agitated by magnet stirrer in 200 rpm until all powders were dissolved and hydrated. This time was reported as rehydration time (Goula and Adamopoulos, 2012).

Experimental design and statistical analysis:

Experimental data were analyzed by ANOVA (analysis of variable) method. The data used here were the mean value of three repeated experimental data.

In emulsion preparation step, a 4 × 3 full factorial design with a completely randomized design was applied.

In encapsulation step, because of two independent variables (inlet air temperature and pressure of nozzle) in two levels and having four different wall materials, a 2 × 2 × 4 full factorial design with a completely randomized design was applied.

In both steps, results were analyzed by SAS software and Duncan's Comparisons to determine significant differences between means. Treatments means were considered significantly different at $P < 0.05$.

Results showed that emulsions had different stability and properties. This was because of different kinds of solid materials which were used as wall material and emulsifier. Details about droplet size, creaming index and viscosity of emulsions during storage and in compare with each other exactly after production, are given in Table 1 and Table 2, respectively.

As it is shown in table 2, viscosity and droplet size of emulsions were significantly different ($P<0.05$). SPC and WPC emulsions had the highest and lowest viscosity respectively. Also the largest droplet size which were observed belonged to GEL emulsions. WPC and AG had the lowest ones.

Table 1: Changes of emulsions specifications during storage

Sample	Measured parameter	Day 1	Day 2	Day 3	Day4	Day 5	Day 6
AG+MDX	Viscosity (cp)	89.75^{a}	81.23^{b}	76.81^{c}	74.22^{d}	73.91^{e}	71.76^{f}
	Droplet size(μm)^2	18.12^{e}	19.26^{d}	20.34^{cd}	20.76b^{c}	21.15^{b}	22.43^{a}
	Creaming index (%)	16.89^{e}	17.53^{d}	18.24^{c}	19.32^{b}	19.98^{ab}	20.71^{a}
SPC+MDX	Viscosity (cp)	121.32^{a}	115.21^{b}	112.45^{c}	109.79^{d}	108.92^{d}	107.22^{e}
	Droplet size(μm)^2	22.85^{d}	23.05^{d}	24.03^{c}	24.87^{b}	25.12^{ab}	25.83^{a}
	Creaming index (%)	43.45^{f}	44.21^{e}	45.62^{d}	47.12^{c}	48.31^{b}	49.05^{a}
WPC+MDX	Viscosity (cp)	45.12^{a}	44.03^{b}	43.12^{c}	42.54^{d}	41.11^{e}	40.78^{e}
	Droplet size(μm)^2	17.67^{c}	18.05^{bc}	18.45^{b}	18.92^{b}	19.23^{ab}	19.74^{a}
	Creaming index (%)	18.34^{b}	18.56^{b}	18.98^{b}	19.45^{ab}	19.72^{a}	19.93^{a}
GEL+MDX	Viscosity (cp)	113.46^{a}	110.82^{b}	105.24^{c}	102.19^{d}	100.12^{e}	98.22^{f}
	Droplet size(μm)^2	27.92^{f}	30.45^{e}	33.25^{d}	37.19^{c}	40.15^{b}	42.78^{a}
	Creaming index (%)	54.38^{f}	59.14^{e}	65.13^{d}	68.45^{c}	71.98	75.28^{a}

Different letters in each row show significant difference in 5% level

Table2: Emulsions specifications

specification / Emulsion	Viscosity (cp)	Droplet size (μm)^2	Creaming index (%)
AG+MDX	89.75^{c}	18.12^{c}	16.89^{c}
SPC+MDX	121.32^{a}	22.85^{b}	43.45^{b}
WPC+MDX	45.12^{d}	17.67^{c}	18.34^{c}
GEL+MDX	113.46^{b}	27.92^{a}	54.38^{a}

Different letters in each column show significant difference in 5% level

Table 3: Encapsulation efficiency of microcapsules

Emulsion type	Inlet air temperature(centigrade)	Pressure of nozzle (bar)	Encapsulation efficiency (%)
AG+MDX	150	1	85.70^{b}
AG+MDX	150	2	76.80^{d}
AG+MDX	200	1	61.77^{g}
AG+MDX	200	2	52.14^{i}
WPC+MDX	150	1	89.59^{a}
WPC+MDX	150	2	82.84^{bc}
WPC+MDX	200	1	65.08^{f}
WPC+MDX	200	2	58.86^{g}
SPC+MDX	150	1	72.08^{e}
SPC+MDX	150	2	64.98^{f}
SPC+MDX	200	1	48.24^{j}
SPC+MDX	200	2	42.49^{k}
GEL+MDX	150	1	80.61^{c}
GEL+MDX	150	2	75.87^{d}
GEL+MDX	200	1	55.66^{h}
GEL+MDX	200	2	46.91^{j}

Different letters in each column show significant difference in 5% level

Because emulsions were dried under different situations, encapsulated powders had different characteristics. Encapsulation efficiency of microcapsules is given in Table 3.

As it is shown in Table 3, the highest EE belonged to WPC+MDX emulsions which were dried at 150 °C and 2 bar. Also SPC+MDX which were dried at 200 °C and 1 bar, had the lowest EE. Also, in one block with same materials, changing inlet air temperature from 150°C to 200°C, resulted to a significant decrease of EE ($P<0.05$).

Effect of droplet size and viscosity of emulsions:

Small oil droplets (as dispersed phase) have more tendency to be enclosed and embedded efficiently within the wall matrix (as continuous phase) of the microcapsules. Also, the resulted emulsion will be more stable during the spray drying which is one of the critical parameters to have the optimum efficiency of encapsulation (Jafari *et al*., 2008). It is reported that high viscosity can complicate the droplet formation process and in lower viscosities, less energy or pressure is required to form a particular spray pattern (Patel *et al*., 2009). On the other hand, higher viscosity, can decrease the spray angle in spray dryer. Spray angle can be between 50º to 90° according to shape and size of nozzle. Usually a decrease in spray angle, result to increase of outlet droplets size which is an improper for encapsulation because bigger droplets might reach faster to the end of chamber which can cause non-uniform drying and permeable shell formation (Montgomery and Bovey, 1956).

Therefore, overall, it was expected that by using WPC+MDX, produced emulsions could result to encapsulated powders with the minimum amount of unencapsulated lycopene (surface lycopene) because of smaller droplet size and lower viscosity. Danviriyakul *et al*. (2002), Carmona *et al*. (2011) Shu *et al*. (2006) and Montgomery and Bovey (1956) reported similar results by spray drying different emulsions.

Effect of inlet air temperature:

Results showed that higher inlet air temperature (200°C), resuled to lower encapsulation efficiency in all emulsions. It can be described that increasing inlet temperature from 150 to 200 °C while keeping other parameters unchanged, may break the balance between rate of water evaporation and film-formation, which finally would result to breaking down of wall system of microcapsules and decrease of EE. Shu *et al*. (2006) and Liu *et al*. (2000) reported similar results by spray drying of emulsions.

Effect of pressure of nozzle:

As it is shown in Table 3, increasing nozzle pressure from 1 to 2 bar, while keeping other parameters unchanged result to a significant increase of EE ($P<0.05$). Increasing of pressure of nozzle, result to a decrease in droplets diameter. These smaller droplets, have bigger surface area and higher rate of heat and mass transfer, so semi-permeable layer can be formed faster. These phenomena finally can result to a higher encapsulation efficiency. These results were similar to what Chang *et al*. (1988) and Silva and Rey (1996) reported. Other specifications of powders such as moisture content, bulk density, rehydration time are shown in Table 4.

Table 4: Physicochemical properties of encapsulated powders

Emulsion type	Pressure of nozzle (bar)	Inlet air temperature (°C)	Moisture Content wet basis (%)	Rehydration Time (sec)	Diameter of Encapsulated Powders (µm)	Bulk Density (g/cm^3)
AG+MDX	2	150	3.10^{d}	361^{l}	37.060^{defg}	0.36^{c}
AG+MDX	1	150	3.74^{c}	254^{p}	42.510^{ab}	0.30^{d}
AG+MDX	2	200	2.04^{f}	440^{i}	34.980^{fg}	0.24^{ef}
AG+MDX	1	200	2.53^{e}	400^{k}	40.100^{bcde}	0.20^{gh}
WPC+MDX	2	150	4.12^{b}	300^{n}	33.930^{g}	0.48^{a}
WPC+MDX	1	150	4.80^{a}	275^{o}	39.240^{bcde}	0.36^{c}
WPC+MDX	2	200	2.13^{f}	383^{g}	33.720^{g}	0.29^{d}
WPC+MDX	1	200	2.89^{de}	347^{m}	38.190^{cdef}	0.24^{ef}
SPC+MDX	2	150	4.01^{bc}	460^{h}	40.150^{bcde}	0.40^{b}
SPC+MDX	1	150	4.76^{a}	420^{j}	45.380^{a}	0.33^{c}
SPC+MDX	2	200	2.10^{f}	710^{c}	38.120^{cdef}	0.27^{de}
SPC+MDX	1	200	2.83^{de}	602^{e}	41.555^{abc}	0.23^{fg}
GEL+MDX	2	150	3.14^{d}	690^{d}	38.200^{cdef}	0.34^{c}
GEL+MDX	1	150	3.75^{c}	492^{f}	43.230^{ab}	0.29^{d}
GEL+MDX	2	200	2.01^{f}	765^{a}	36.030^{efg}	0.22^{fg}
GEL+MDX	1	200	2.68^{e}	719^{b}	40.600^{bcd}	0.18^{h}

Different letters in each column show significant difference in 5% level

Moisture content:

As it is shown in Table 4, moisture content of the samples which were dried at higher temperature, was significantly lower than other samples ($P<0.05$). Also SPC and WPC emulsions which were dried at 150 °C and 1 bar, had the highest and AG and GEL which were dried at 200 °C and 2 bar had the lowest levels of moisture content. The pressure of nozzle had significant effect on moisture content of samples ($P<0.05$) and the ones which passed through nozzle under higher pressure, had lower moisture content. According to previous investigations, increasing of inlet air temperature, results to decrease of moisture content due to higher rate of mass and heat transfer and finally faster drying (Prabhanjan, *et al*., 1995; Methakhuo *et al*., 2004).

Encapsulated powder size:

Results showed that pressure of nozzle had significant effect on final size of dried powders ($P<0.05$) but inlet air temperature did not have a significant effect on it. Also WPC powders had the lowest and SPC had the highest particle size.

Smaller particles loose moisture in a faster rate and this can accelerate the formation of semi permeable shell. So the samples which were dried under high pressure, had lower particle size. On the other hand, temperature had a minor effect on size of samples due to faster operation and less shrinkage but that was not significant. These results were similar to what Jafari *et al*. (2008) and Reineccius (2001, 2004), reported. Also some investigations showed that in high temperatures, shell forms rapidly whereas moisture is trapped inside the particle (ballooning phenomena). This phenomenon result to swelling and cracking of shell which can affect the size of particle (Walton, 2000).

Bulk Density:

Results showed that bulk densities of encapsulated powders were significantly different ($P<0.05$). WPC samples which were dried at 150 °C and 2 bar had the lowest and GEL samples which were dried in at 200 °C and 1 bar had the highest bulk density. Also, same samples which were dried under higher pressure of nozzle, had higher bulk density.

Bulk density is defined as the weight of mass per unit volume of mass. It considers both the mass and the pore spaces. So bigger and non-uniform particles, need more volumes and have lower bulk densities. Another parameter which can affect bulk density of powders, is moisture content. The higher moisture contents result to more adhesion of particles and less porosity, so bulk density decrease (Walton, 2000). Also samples which were dried in higher temperatures, because of ballooning phenomena, had bigger size and lower bulk density. Goula *et al.* (2012) and Walton (2000) reported same results.

Rehydration time:

As it is shown in Table 4, AG powders had the shortest rehydration time but GEL samples needed the longest time to be rehydrated. Also between powders with same solid material and drying temperature, the ones which were dried under higher pressures, needed a significant longer time to be rehydrated ($P<0.05$). On the other hand, higher inlet air temperature during drying of samples, resulted to significantly longer time for complete rehydration ($P<0.05$).

In a drying process, some factors can have significant influence on quality and rehydration ratio of final product such as drying conditions, size of samples and water temperature (Salimi *et al*., 2011; Drusch *et al*., 2006) during drying, capillary tubes which transfer mass to the surface, loose their efficiency due to shrinkage and sedimentation of solid materials which were dissolved in unbound water of samples. So the vessels which could intake moisture during rehydration, loose their efficiency. Other problems during drying such as coagulation of proteins, burning and some other parameters can also affect rehydration time (Nour *et al*., 2011; Kulshreshtha *et al*., 2009).

Particle size of samples is another parameter which might have significant effect on rate of rehydration. Very small and fine particles, have a large surface area and higher surface energy, so stick together and have

less contact with water to be rehydrated. As it can be seen in Table 4, samples which smaller particle size, had longer rehydration time. Gaula *et al.* (2012) and Walton (2000) reported same results.

III. CONCLUSIONS

Lycopene microcapsules were prepared by a spray-drying method using a wall system consisting of WPC, SPC, AG and GEL which were mixed with MDX. In first step, some emulsions were prepared and investigated. WPC emulsions had the proper conditions such as small droplets and low viscosity. Some characteristics of final microcapsules such as EE, bulk density, particle size and rehydration time were significantly affected by the initial emulsion stability, drying conditions and wall materials. The optimal condition for getting to higher EE was determined as follows: WPC+MDX as wall materials, inlet temperature of 150 °C, and nozzle pressure of 2 bar.

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Received: April 9, 2017.
Sent to Subject Editor: April 27, 2017.
Accepted: December 18, 2017.
Recommended by Subject Editor: Orlando Alfano