

Encapsulation of β -carotene with poly-(ϵ -caprolactones) by PGSS process

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Abstract

β -carotene is one of the most common pigments in nature. β -carotene formulations provide protection against oxidation and degradation processes. They are very attractive as natural colorants because they add value to the product due to their antioxidant and pro-vitamin activities. This work presents a study of the formulation of β -carotene with poly-(ϵ -caprolactone) by Particles from Gas Saturated Solutions (PGSS) process. Particle sizes in the range of 270 - 650 μm with a β -carotene content of up to 340 ppm were obtained using polycaprolactone with a molecular weight of 10 000 $\text{g}\cdot\text{mol}^{-1}$, while using a polycaprolactone with a molecular weight of 4 000 $\text{g}\cdot\text{mol}^{-1}$ the particle size was reduced to 110 - 130 μm . The influence of several process parameters on particle size and β -carotene content was studied, including pressure, temperature, time of contact between CO_2 and polymer melt for mixture homogenization, and molar ratio β -carotene : polymer.

1. Introduction

Carotenoids are some of the most common pigments in nature, the most abundant being β -carotene, lycopene, lutein and zeaxanthin. The main roles of carotenoids in human diet are as precursors of Vitamin A and as antioxidants. For many industrial applications, a mixture of the carotenoid with a polymer is used. Covering carotenoids with polymers provides protection against oxidation and degradation processes [1]. Nowadays the food market demands functional foods and healthy products, using natural additives which provide the final product with a healthy added value [2]. For the use of carotenoids as natural colorants, a formulation of the active compound is required with a restricted particle size. It is important to obtain an appropriate colour intensity of the formulation which depends on the properties of particles [3]. The application of supercritical fluids as an alternative to the conventional precipitation processes has been an active field of research and innovation during the past two decades [4-6]. It has been studied the applications of supercritical fluid technology to the precipitation of β -carotene [7-12]. Particles from gas saturated solutions (PGSS) process consists of saturating the solute with the carbon dioxide and upon the expansion, the carbon dioxide dissolved in the solute rapidly evaporates and produces an intense cooling effect which is the driving force for the precipitation [6]. This technique has been widely used for the encapsulation of different active compounds [13-17].

Poly-(ϵ -caprolactone) is a synthetic biocompatible semicrystalline polymer. In a previous work of the authors [18], it was determined the melting point of three different polycaprolactones under carbon dioxide pressure with different molecular weights. The maximum reduction of melting temperature ranges from 12.5 to 16.0 K, depending on the

molecular weight of the polycaprolactone. This study is necessary to develop formulations with polycaprolactones by supercritical fluid technologies. Because of this, the aim of this work has been the encapsulation of β -carotene with two different polycaprolactones (CAPA 2403D and CAPA 6100) as carrier materials by PGSS process.

2. Materials and Methods

2.1. Materials

Crystalline β -carotene with a minimum purity of 99% was manufactured by Vitatene (León, Spain). Poly-(ϵ -caprolactones) were kindly supplied by Solvay Caprolactones (Solvay Interlox Ltd., United Kingdom). Two different commercial poly-(ϵ -caprolactones) were used: CAPA 2403D and CAPA 6100. Carbon dioxide (purity: 99.5%) was provided by Air Liquide (Portugal). All products were used as received.

2.2. Equipment

Figure 1 presents the schematic flow diagram of the experimental apparatus. CO₂ is fed by a piston pump (Haskel model MCPV-71) to the high pressure mixing chamber where it is mixed with crystalline β -carotene and polycaprolactone by a mechanical stirrer. The internal temperature of this stirred vessel is controlled by a PID temperature controller (WatlowSTB3J2J1) acting over an electrical jacket and measuring the temperature with a J type thermocouple (accuracy 0.1 K). The system is depressurized by opening a valve (V-3) and atomized through a 600 μ m nozzle (Teejet TGSS, Spraying Systems Co.) into a collector where particles obtained are recovered.

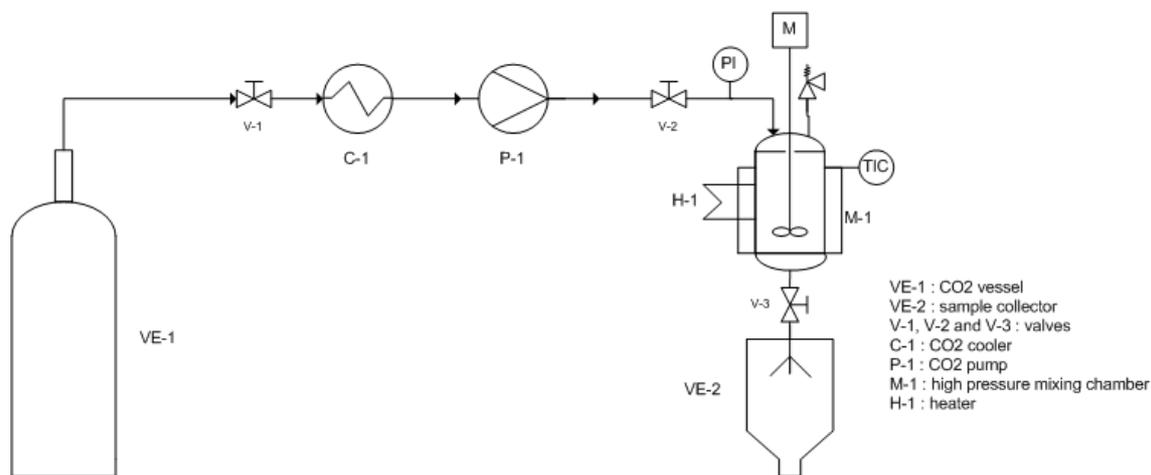


Fig.1. Schematic flow diagram of the experimental apparatus employed for the formulation of β -carotene with poly-(ϵ -caprolactones) by PGSS process.

2.3. Experimental procedure

A typical experiment starts with the preparation of the mixture of the active compound (crystalline β -carotene) and the carrier (polycaprolactone) and put it in the high pressure mixing chamber. This mixture was prepared with three different molar ratios (moles of β -carotene: moles of polycaprolactone), being specifically 1:4, 1:6 and 1:8. For this formulation, CAPA 6100 (molecular weight: 10000 g/mol) was used. However, few experiments were carried out with CAPA 2403D (less molecular weight than CAPA 6100, 4000 g/mol). CO₂ was pumped to the high pressure mixing chamber until the desired pressure was achieved and then, when the desired temperature inside the chamber is reached, the mechanical mixing is

switched on during a specific time of mixture homogenization. Two pressures and two temperatures were used, being 11 and 15 MPa and 50 and 70 °C, respectively. Different times of the mixture homogenization were used to carry out the formulation, concretely 60, 120 and 240 minutes. After stirring the selected time, the system is depressurized by opening a valve and the particles obtained are recovered in a collector.

2.4. Product characterization

2.4.1. Yield of collected particles

The yield of the collected particles by PGSS process was determined by difference of mass, being the amount of the obtained particles in the sample collector divided by the amount of mass introduced in the mixing chamber.

2.4.2. β -carotene content

The sample was analysed by UV/VIS spectrophotometer model HITACHI U-2000. The wavelength selected was 456 nm. The absorbance determined with this method is proportional to the amount of β -carotene dissolved in the solution.

2.4.3. Particle size

The particle size analysis was carried out by laser diffraction model Malvern Mastersizer 2000.

3. Results and discussion

Table 2 shows a summary of the process conditions in all experiments performed. The main process parameters were changed in order to analyse the influence of these parameters on product characteristics, being these parameters the molar ratio (moles of β -carotene: moles of polycaprolactone), time of mixture homogenization, temperature and pressure inside the mixing chamber. Table 3 presents the main experimental results obtained in each experiment. The experimental results are the yield of the collected particles, the percentage of β -carotene content and the particle size.

Table 2. Summary of operating conditions

	Poly- ϵ - caprolactone	R molar	Temperature (°C)	Pressure (MPa)	Time mixture homogenization (minutes)
E 1	CAPA 6100	0.25	70	15	60
E 2	CAPA 6100	0.25	70	15	120
E 3	CAPA 6100	0.16	70	15	60
E 4	CAPA 6100	0.16	70	15	120
E 5	CAPA 6100	0.13	70	15	60
E 6	CAPA 6100	0.25	50	15	60
E 7	CAPA 6100	0.25	70	11	60
E 8	CAPA 6100	0.13	70	15	120
E 9	CAPA 6100	0.16	50	15	60
E 10	CAPA 6100	0.16	70	11	60
E 11	CAPA 6100	0.13	50	15	60
E 12	CAPA 6100	0.13	70	11	60
E 13	CAPA 6100	0.25	70	15	240
E 14	CAPA 6100	0.13	70	15	240
E 15	CAPA 2403D	0.25	70	15	60
E 16	CAPA 2403D	0.16	70	15	60
E 17	CAPA 2403D	0.13	70	15	60

Table 3. Summary of experimental results.

	Yield of collected particles (%)	β -carotene content (ppm)	Particle size (μm)
E 1	28	336	578
E 2	15	227	591
E 3	15	306	501
E 4	16	35	613
E 5	21	57	467
E 6	8	183	606
E 7	23	161	467
E 8	19	27	523
E 9	19	158	652
E 10	27	10	394
E 11	5	124	510
E 12	33	17	276
E 13	19	49	525
E 14	18	37	431
E 15	32	89	111
E 16	35	94	120
E 17	44	32	132

3.1. Influence of the molar ratio

The effect of the different molar ratios has been studied, being concretely 0.13, 0.16 and 0.25. As it can be seen in Table 2, the temperature and pressure inside the mixing chamber have been kept constants, being 70 °C and 15 MPa respectively. The molar ratio has a strong influence on the particle size, and as this molar ratio is increased, the particle size increases as well independently of the time of the mixture homogenization used. With regard to the β -carotene content in the sample, it was low in all cases. However, this content increases when the molar ratio increases as well independently of the time of the mixture homogenization.

3.2. Influence of the time of mixture homogenization

The effect of the time of the mixture homogenization has been studied, being concretely 60, 120 and 240 minutes the used times. As it can be seen in Table 2, the temperature and pressure have been kept constants, being 70 °C and 15 MPa respectively.

Analysing the results presented in table 3, it can be observed that, when the time of the mixture homogenization increases (keeping constant the molar ratio), the particle size tends to decrease. It can be seen that, when it was used the maximum time of homogenization (240 minutes), the smallest particle size was obtained (431 and 525 μm). It means that the mixture homogenization was better than in other cases in which the selected time might not be enough to stabilize and homogenize the mixture correctly. However, the highest particle size was obtained using a time of homogenization of 120 minutes. Regarding the β -carotene content in the sample, a higher β -carotene content was obtained when the selected time of the mixture homogenization was the lowest, concretely 60 minutes.

3.3. Influence of the temperature

The effect of the temperature inside of the high pressure mixing chamber has been studied, being concretely 50 and 70 °C keeping constant the pressure and time of homogenization, being 15 MPa and 60 minutes respectively.

Results showed that the temperature inside the high pressure mixing chamber has a strong influence on the particle size obtaining higher particle size when the temperature used was the lowest (50°C). Consequently, 50°C is not enough temperature to carry out the formulation of β -carotene by PGSS process. On the other hand, the particle size tends to increase when the molar ratio increases as well in both trends of data, with the exception of one experiment which corresponds to the maximum molar ratio used at 50°C. In another work of the authors [18], the determination of the first melting point of different polycaprolactones under pressure was investigated. In comparison, the first melting point of CAPA 6100 at 15 MPa was 43°C. It means that 50°C is not enough operating temperature due to the fact it is close to the first melting point and probably, the mixture homogenization was not carried out correctly. However, at 70°C, the particle size was lower because the mixture was completely melted obtaining a stable and homogeneous mixture. Regarding the amount of β -carotene in the sample, it was low in all cases. However, it is necessary to enhance that higher β -carotene contents were obtained when the temperature inside the high pressure mixing chamber was 70°C. These results corroborate that a better co-precipitation was carried out at 70°C.

3.4. Influence of pressure

The effect of the pressure inside the mixing chamber has been investigated, being the selected pressures 11 and 15 MPa, keeping constant the temperature and time of homogenization (70°C and 60 minutes respectively). Analysing the results presented in table 3, it can be seen that the pressure inside the mixing chamber has a strong influence on the particle size. As it is shown, lower particle sizes were obtained operating with 11 MPa. It is necessary to enhance that the difference between both pressures with regard to the particle size was high. On the other hand, both trend of data of the particle size increase when the molar ratio increases as well. Regarding the amount of β -carotene in the sample, in all cases was very low. There is a drastic difference in this β -carotene content using 15 and 11 MPa. At the highest pressure, higher amounts of β -carotene were obtained.

3.5. Effect of the molar ratio: comparison between CAPA 6100 and CAPA 2403D

The effect of the molar ratio with two different polycaprolactones, concretely CAPA 6100 and CAPA 2403D, has been studied, keeping constant the temperature (70°C), pressure (15 MPa) and time of homogenization (60 minutes). Observing the results showed in table 3, there is a drastic difference in the particle size between both polycaprolactones. With regard to CAPA 2403D, it can be seen that the particle size was lower (111-132 μm) and the trend of data of the particle size tends to be constant when the molar ratio is increased. However, regarding CAPA 6100 results, when the molar ratio increases, the particle size increases as well. Regarding the literature [18], the first melting point of CAPA 2403D and CAPA 6100 at 15 MPa was 41°C and 43°C, respectively. Consequently, 70°C was a good operating temperature because was higher than the first melting point in both cases. On the other hand, it is necessary to enhance that the morphology of the particles was completely different. In the case of CAPA 6100, in all cases agglomerated and soft particles were obtained. However, in the case of CAPA 2403D, powder particles were obtained. It might be due to the low molecular weigh of the polycaprolactone used.

3.7. Yield of collected particles

The yield of collected particles by PGSS process is presented in Table 3. It is necessary to enhance that the yield of collected particles was low in all cases due to the fact the nozzle was obstructed during the expansion. However, in the experiments where CAPA 2403D was used,

it was observed that the yield of collected particles was high because a polycaprolactone with lower molecular weight was used.

4. Conclusions

The formulation of β -carotene with Poly-(ϵ -caprolactones) by PGSS process was investigated in this work. The obtained particles from high pressure precipitation technique have a particle size in the range of 276-652 μm when CAPA 6100 was used. In the case of CAPA 2403D, particles with a size of 111-132 μm were obtained. Because of this difference in the particle size, a better formulation was carried out with CAPA 2403D. It was demonstrated that there are several parameters that have a strong influence on the particle size. With regard to the molar ratio, when it is increased the particle size increases as well. Temperature and pressure inside the mixing chamber were other important factors. Higher particle sizes were obtained when the temperature inside the mixing chamber was 50°C and when the selected pressure was 15 MPa. Regarding the β -carotene content, it was low in all cases. However, the modified parameters have an important effect on the amount of β -carotene in the sample. When the molar ratio increases, the β -carotene content increases as well and higher amounts of β -carotene were obtained when the selected time of the mixture homogenization was 60 minutes. With regard to the temperature and pressure inside the mixing chamber, higher β -carotene contents were obtained (306-336 ppm) when the selected temperature and pressure was 70°C and 15 MPa, respectively.

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