PRODUCTION OF WATER-SOLUBLE B-CAROTENE FORMULATIONS BY HIGH PRESSURE PROCESSES

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Abstract. β-carotene formulations are very attractive as natural colorants as they provide additional value to the product due to their antioxidant and pro-vitamin activities. Application of β-carotene as colorants in beverages requires an appropriate formulation in order to stabilize the particles of β-carotene in suspension and provide the desired colour. This work presents a study of the formulation of β-carotene by precipitation from a pressurized organic solvent-in-water emulsion using modified OSA-starch obtaining high encapsulation efficiencies (over 65% in most cases and as 90% with specific conditions), high antioxidant activity and a micellar particle size in the range of 300-600 nm. The process parameters which had the most influence on product properties were the concentration of different modified starches and the organic/water flow ratio. Also it was study the effect of using two different organic solvents.

Keywords: β-carotene, colorant, antioxidant, suspension, emulsion

1. Introduction

Carotenoids are some of the most common pigments in nature, the most abundant being β-carotene, lycopene, lutein and zeaxanthin. The mail roles of carotenoids in human diet are as precursors of Vitamin A and as antioxidants. Since they are authorized food ingredients, carotenoids are widely used in the food, cosmetic and pharmaceutical industries as natural colorants. For many industrial applications, a mixture of the carotenoid with a biopolymer is used. Covering carotenoids using polymers provides protection against oxidation and degradation processes [1]. Moreover, the high hydrophobicity of carotenoids makes them insoluble in aqueous systems, and therefore they have a poor intake in the body. To improve their dispersibility in water, coloring strength potential and also to increase their bioavailability during gastro-intestinal passage, carotenoid crystals must be formulated [2].

Food colorants have always been target of complains of the food industry consumers. Nowadays the food market demands functional foods and healthy products, using natural additives which provide the final product with a healthy added value [3]. For the use of carotenoids as natural colorants, a formulation of the active compound is required with a determined particle size. It is important to obtain an appropriate colour intensity of the formulation which depends on the properties of particles [4].

Recently, there has been great interest in utilizing nanoemulsions to encapsulate bioactive components for applications in food and beverage products [5]. Oil-in-water nanoemulsions consists of small lipid droplets ($r < 100$ nm) dispersed within an aqueous continuous phase. Nanoemulsions are thermodynamically unstable systems that tend to breakdown over the time [6]. Nanoemulsions containing β-carotene have been studied by several authors. Qian et al. (2012) [6] showed that β-carotene can be effectively encapsulated within food-grade nanoemulsions stabilized by globular proteins or non-ionic surfactants using a high pressure microfluidiser. The same authors [7] carried out the same study containing different kinds of carrier lipids coated by non-ionic surfactants. Mao et al. (2009; 2010) [8, 9], investigated the characteristics of β-carotene nanoemulsions prepared by high pressure homogenization using two large molecule emulsifiers (octenyl succinate starch and whey protein isolate) and two small molecule emulsifiers (tween 20 and decaglycerol monolaurate) obtaining a fine size distribution. The same technique was used by Yuan et al. (2008) [10] to

study the production of oil-in-water nanoemulsions of β-carotene. However, Silva et al. (2010) [11] presented the use of high-energy emulsification-evaporation technique to produce oil-in-water nanoemulsions of β-carotene. On the other hand, Tan and Nakajima (2005) [12] investigated the preparation of β-carotene nanodispersions by a process based on an emuylsification-evaporation technique. Ribeiro et al. (2008) [2] studied the production of β-carotene-loaded nanodispersions containing poly(D,L-lactic acid) and poly(D,L-lactic coglycolic acid) by solvent displacement method. The same technique was used by Yin et al. (2009) [13] to study the characteristics of β-carotene nanodispersions prepared with different emulsifiers.

In this research, it has been studied the formulation of β-carotene using different modified n-octenyl succinate (OSA) starches as carrier materials for application as natural colorants. Also it was studied the effect of using two different organic solvents, concretely, ethyl acetate and ethanol. Formulations have been prepared with a process based in the formation of an organic-in-water emulsion with pressurized fluids. The aim of the conception of this process is to improve over the conventional emulsion evaporation processes, accelerating the mass transfer kinetics to the time scales of the precipitation processes [4].

2. Materials and methods

2.1 Materials

Crystalline β-carotene with a minimum purity of 99% was manufactured by DSM-León (Spain), using a fermentation process. Ethyl Acetate with a purity of 99.5% and ethanol with a purity of 96% were purchased from Panreac Química (Barcelona, Spain). Different modified n-octenyl succinic anhydride OSA-starches were kindly provided by National Starch Group (Hamburg, Germany).

2.2 Equipment

Figure 1 presents the schematic flow diagram of the experimental apparatus. It consists in three small storages at ambient pressure, corresponding to the feed of pure organic solvent, β-carotene suspension in the same organic solvent and the aqueous solution of the modified OSA-starch. The installation also counts with two piston pumps GILSON 305 used to feed the aqueous dissolution of modified starch and the β-carotene suspension and a piston pump JASCO PU-2080 plus used to feed the pure organic solvent. The stream of the organic solvent is preheated in a chromatographic oven to a temperature of about 165ºC in order to reach the specific operation temperature after the mixing with the β-carotene suspension (typically 145ºC). All streams are pressurized with the pumps in order to keep them in the liquid phase at this temperature (typical operating pressure: 6.0 – 6.5 MPa).

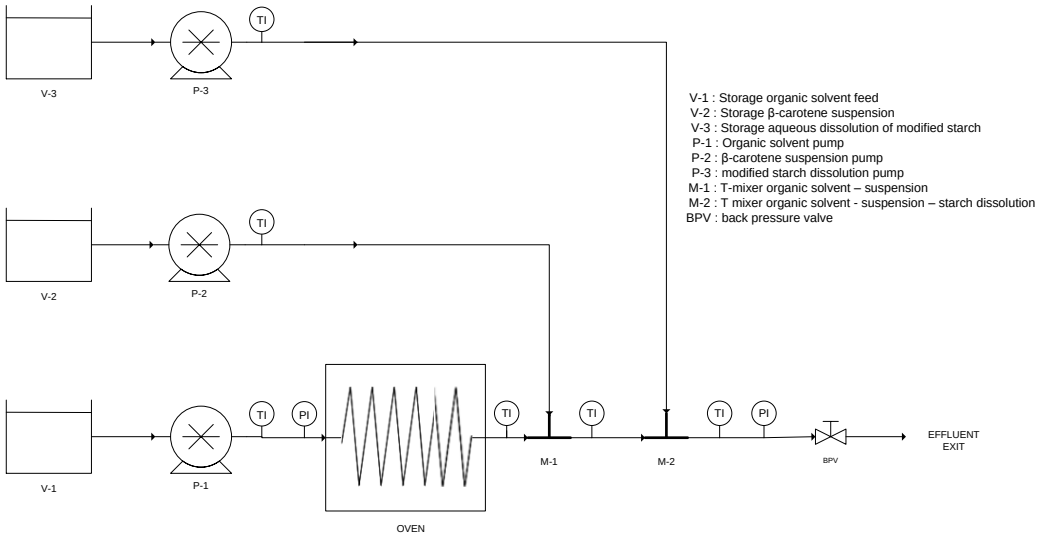

Figure 1. Schematic flow diagram of the experimental apparatus used for β-carotene formulation.

2.3 Precipitation process

The precipitation process developed in this work consists in mixing a stream of hot organic solvent with another stream of a suspension of β-carotene in the same organic solvent in a T-mixer. In this mixer takes place the complete dissolution of the β-carotene in the organic solvent due to the fact that the solubility of β-carotene increases when temperature is increased. All streams are pressurized in order to keep them in the liquid phase at the selected temperature (145ºC). Shortly afterwards, the β-carotene dissolution is mixed with a cold aqueous solution of surfactant using a second T-mixer, in order to reduce the contact time of β-carotene particles with the hot organic solvent and avoid the isomerization and degradation of the process. This second mixing causes the emulsification of the organic solvent and the precipitation of β-carotene by a combined antisolvent and cooling effect. Then, the effluent (emulsion) is collected. The organic solvent is removed from the sample using a rotary evaporator, producing a suspension of β-carotene nanoparticles in water stabilized with the surfactant. The last step in this process is to evaporate the water. For doing this, the sample is introduced in a spray-dryer. The air temperature in the spray-drying system is 160ºC, the exit temperature varies between 85 and 91 ºC and the feed flow velocity is approximately 1 L h^{-1}. The powder finally obtained is introduced in a closer container covered with aluminium paper in order to protect the sample from the effect of light and oxygen, and it is stored in a cold camera (T=6ºC) to avoid the effect of temperature.

2.4 Product characterization

Percentage of encapsulated β-carotene. The sample was analyzed by a UV/Vis spectrophotometer model Agilent 8453. The wavelength selected was 456 nm. The absorbance determined with this method is proportional to the amount of β-carotene dispersed in solution. The particles of crystalline β-carotene not stabilized in the suspension not contribute to the absorbance determined with this method. The ratio of this concentration, corresponding to the amount of β-carotene stabilized in the suspension, to the total β-carotene concentration in the product, is reported in this work as percentage of encapsulated β-carotene.

Particle size. The particle size analysis was carried out by laser diffraction (model Beckman Coulter LS 230)

Microscopy. Pictures of the particles collected after spray-drying process were taken by means of a scanning electron microscope (SEM) model JEOL JSM-820.

3. Results and discussion

Four different n-octenyl succinic anhydride (OSA)-modified starches were used as surfactants being these: OSA-starch derived from waxy maize blend with dried glucose syrup (OSA1), OSA-dextrin derived from waxy maize (OSA2), OSA-dextrin derived from tapioca (OSA3) and OSA-starch derived from waxy maize (OSA4). The main process parameters were changed in order to analyze the influence of these parameters on product characteristics, being these parameters the concentration of OSA-starch which was varied from 37 to 367 g/L and the organic/water ratio which was varied from 0.6 to 0.9. On the other hand, it was selected OSA4 to study the effect of using two different organic solvents: ethyl acetate and ethanol.

3.1 Influence of the concentration of different OSA-modified starches

In figure 2, it can be seen the effect of the concentration of different OSA- starches on the percentage of encapsulated β-carotene and on the micellar particle size. In these experiments, ethyl acetate was used as organic solvent.

As it can be observed in figure 2, when the concentration of surfactant dissolution was increased, the percentage of encapsulated β-carotene was also higher, independently which OSA-starch was used. It is necessary to enhance that a minimum concentration of surfactant of 100 gL^{-1} was required in order to obtain a high encapsulation efficiency and high emulsion stability. On the other hand, results carried out with OSA-1 presented low encapsulation efficiencies, achieving maximum values of 30%. Comparing the rest of modified starches, best results were achieved with OSA-4, reaching an encapsulation efficiency of 70 %. Regarding the micellar particle size, results obtained with OSA-1 were not included in the figure due to the fact they were too high (from 0.5 until 136 μm) compared with the results obtained with the other modified starches. Analysing the results of micellar particle size, they were kept nearly constant in a range between 350-760 nm. However, the best results were obtained when OSA-4 was used as carrier material (360-550 nm).

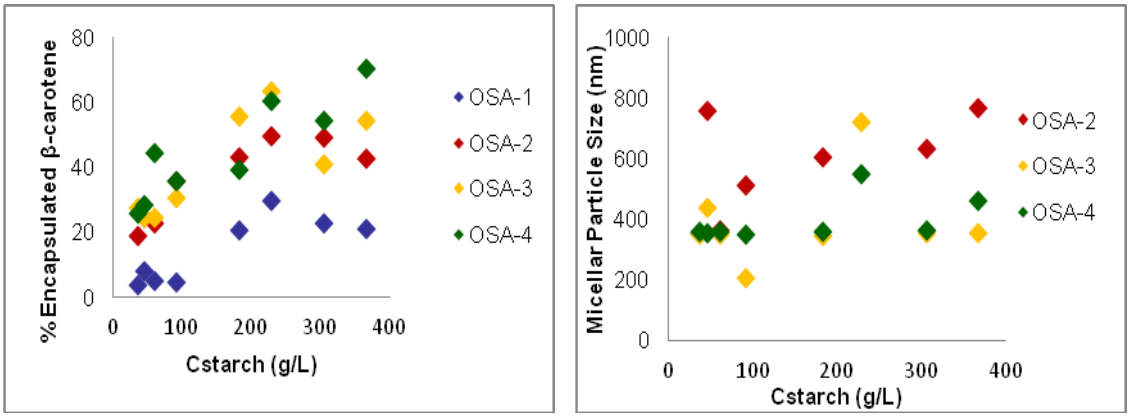

Figure 2. Effect of the concentration of different OSA-starches on the percentage of encapsulated β-carotene and on the micellar particle size.

3.2 Comparison between ethanol and ethyl acetate as organic solvent, using OSA-4 as carrier material

In figure 3 it can be seen the influence of the organic/water ratio on the encapsulation efficiency and on the micellar particle size. Also it is shown a comparison of results using ethanol and ethyl acetate as organic solvent.

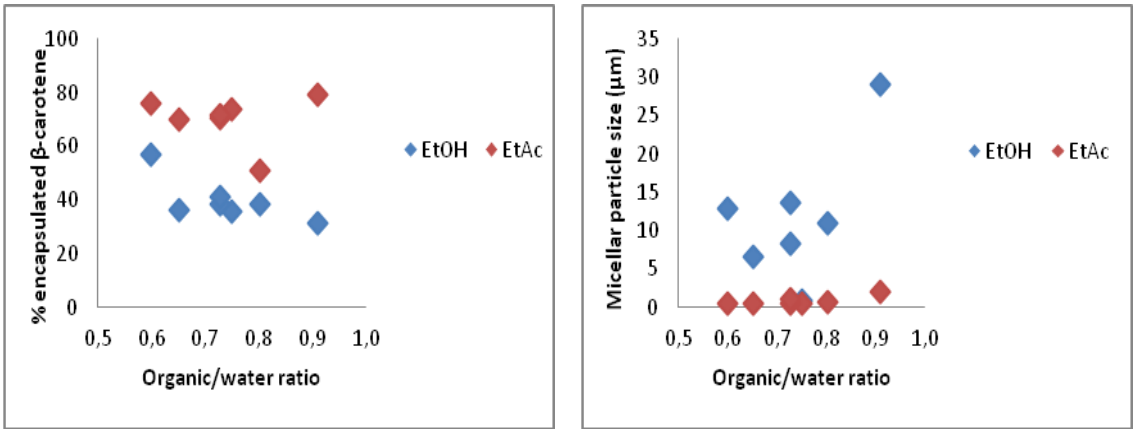

Figure 3. Effect of the organic/water ratio on the percentage of encapsulated β-carotene and on the micellar particle size. Comparison between ethanol and ethyl acetate as organic solvent.

As it can be seen in figure 3, the organic water ratio has a strong influence on the micellar particle size. When ethanol was used as organic solvent, the micellar particle size increases when the organic-water ratio increased as well, ranging from 0.8 to 29 µm for the results. However, when ethyl acetate was used, results present an increase of the micellar particle size when the organic-water ratio was increased as well but ranging from 0.417 to 2 µm. This strong difference in the range of micellar particle size using ethanol and ethyl acetate as organic solvent could be explained by the miscibility of the organic solvents in water. Regarding the encapsulation efficiency, it is not observed a clear variation with the organic-water ratio. Comparing the encapsulation efficiencies obtained with ethanol and ethyl acetate as organic solvent, higher encapsulation efficiencies are obtained using ethyl acetate, between 70 and 80 % in most experiments. Analyzing the obtained results together (micellar particle size and percentage of encapsulated β-carotene), a better aqueous dispersion and consequently, a better formulation and encapsulation of β-carotene was developed using ethyl acetate.

3.3 Characterization of product morphology and colour parameters

In figure 4, pictures of encapsulated β-carotene dispersions in water and powder collected after spray-drying process are shown, using four different concentrations: a total concentration (β-carotene + OSA-starch) of 0.01, 0.005, 0.0025 and 0.00125 g mL^{-1}, with corresponding β-carotene concentrations of 298, 149, 74.5 and 37.5 ppm, respectively.

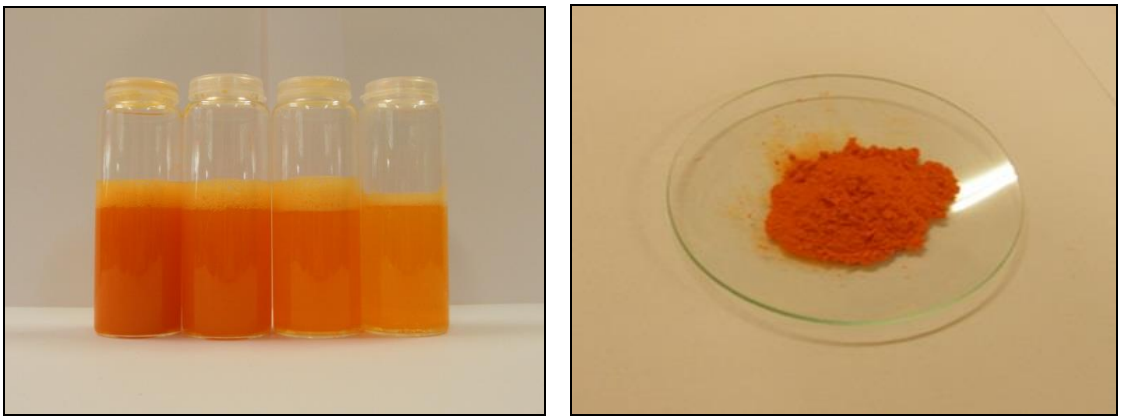

Figure 4. a) Encapsulated β-carotene dispersions in water with β-carotene concentrations of 298, 149, 47.5 and 37.5 ppm (left to right). b) Powder collected after spray-drying process.

In figure 5 it can be observed SEM micrographs of the particles collected after spray-drying process. After spray-drying process, a powder is obtained which is formed by particles of modified starch containing the nanoparticles of β-carotene produced with the emulsion precipitation process. Because of this, the size of these particles is higher than micellar particle size.

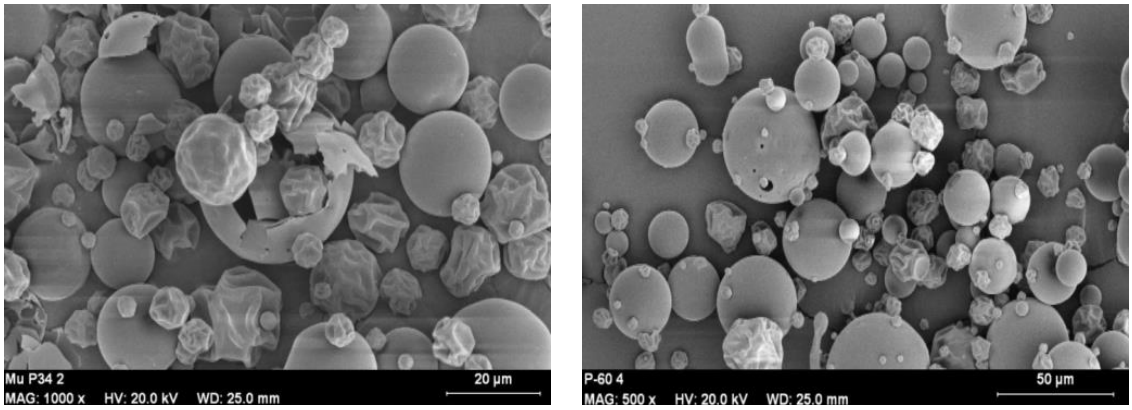

Figure 5. SEM micrographs of particles obtained after spray-drying.

4. Conclusions

The formulation of β-carotene using four different n-octenyl succinic anhydride (OSA)-modified starches using pressurized ethyl acetate emulsions was investigated in this work. Also, it was studied the effect of using ethanol as organic solvent. The results showed that it is possible to obtain a formulation of β-carotene with a micellar particle size in the range of 300-600 nm and with a high percentage of encapsulated β-carotene (70%) when ethyl acetate was used as organic solvent and OSA-4 as surfactant. It was demonstrated that high concentrations of modified starch (over 100 gL^{-1}) were required to obtain a high percentage of encapsulated β-carotene and high emulsion stability. When ethanol was used as organic solvent, it is possible to obtain a formulation of β-carotene with maximum encapsulation efficiency of 60% and with a micellar

particle size in the range of 1-23 μm. It was investigated the influence of the organic-water ratio in the encapsulation efficiency and in the micellar particle size. Resuls showed that an increase in the micellar particle size when the organic-water ratio was increased as well. The same effect was observed with ethyl acetate as organic solvent, obtaining the best results with low ratios.

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