Nanoencapsulation of carotenoids *via* temperature-induced phase transition of triblock polymer in Supercritical Carbon dioxide (scCO$_2$)

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1. Introduction

In the food industry, β-carotene is primarily applied as a food colourant but there is a growing demand for β-carotene-based food, drinks, and beverages with the rising interest in nutraceuticals[1]. However, β-carotene-based formulations are uncommon because of their low bioavailability, poor solubility, and stability concerns during processing and storage[(2, 3,4)]. Hence, solubility and/or dissolution rate improvement of carotenoids is required to enhance their bio-efficiency and expand their applications. This study aims to develop an effective, solvent-free, and novel approach to enhance the aqueous solubility and stability of carotenoids by their nanoencapsulation in triblock polymers known as Pluronics via temperature-induced phase transition (TIPT) in scCO$_2$.

2. Materials and Methods

Carotenoid-loaded pluronic nanoparticles with a 1:4 (PEG: Pluronic) ratio were prepared by TIPT. 10 wt% of the carotenoid extract was first dissolved in PEG and then Pluronic F 68 was added before heating the mixture to a specified temperature in either scCO$_2$ or at atmospheric pressure for 90 min. The mixture was then cooled either by placing it in an ice bath or via depressurisation during scCO$_2$ processing. The processing conditions included; 60 °C/100 bar[4] (Car-PNP-1), 60 °C/1 bar (Car-PNP-2), 120 °C/1 bar (Car-PNP-3) and 60 °C/100 bar without PEG (Car-PNP-4). Thereafter, 50 mg of the mixture was sonicated in 20 mL of distilled water to obtain nanoparticle dispersion. Samples were stored in fridge at 5 ºC in amber coloured bottles until needed for analysis. Formulations were analysed via dynamic light scattering (DLS), high performance liquid chromatography (HPLC) and scanning electron microscopy (SEM). The stability tests were performed by determining the weekly recovery of carotenoids from samples stored at 5 ºC for 5 weeks.

3. Results and discussion

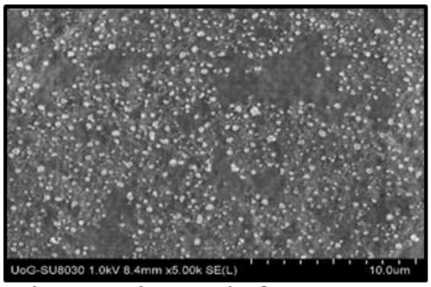

Figure 1: Micrograph of Car-PNP-1

Table 1. Percent recovery of carotenoids in Car-PNP formulations.

Carotenoids	Car-PNP-1	Car-PNP-2	Car-PNP-3	Car-PNP-4
9′ -*cis* β-carotene	95.9 ±1.5	63.3 ±0.3	44.0 ±0.5	100.9 ±0.3
All-trans β-carotene	98.5 ±0.8	68.3 ±0.2	43.2 ± 0.6	100.0 ±0.7
All-trans α-carotene	99.1 ±0.2	73.8 ±0.3	55.5 ±0.7	101.1 ±0.4
Lutein	92.2 ±1.5	67.6 ±0.8	29.2 ±0.2	89.3 ±2.7
Zeaxanthin	97.5 ±1.2	64.2 ±2.3	28.0 ±0.9	87.9 ±3.7

Nanoparticles obtained after resuspension were spheroidal (Figure 1) with a size of ~200 (±5) nm as determined by DLS. The percent recovery of various carotenoids as determined by HPLC (Table 1) confirmed the superiority of scCO$_2$ processing and its ability to avoid detrimental effect of temperature on carotenoid stability during TIPT. Almost all carotenoids could be recovered from scCO$_2$-processed formulations (Car-PNP-1 and Car-PNP-4) in comparison to 40-70% loss from the samples prepared at atmospheric pressure (Car-PNP-2 and Car-PNP-3).

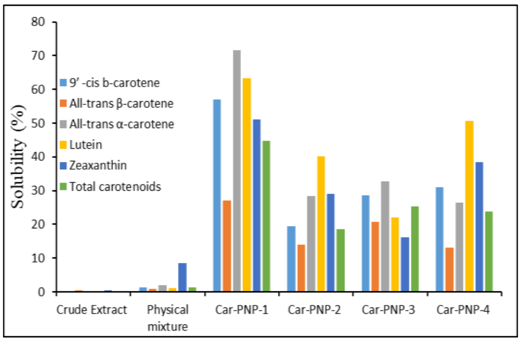

Figure 2: Solubility of carotenoids

The aqueous solubility of carotenoids in Car-PNP formulations (Figure 2) was significantly higher than both the physical mixture and crude extract. The total recovery of identified carotenoids in water at room temperature was found to be: *Car-PNP-1 > Car-PNP-3 > Car-PNP-4 > Car-PNP-2*. In general, the 5-weeks storage study suggested improvement in carotenes' stability except for the xanthophylls, that had either no or slightly negative impact on the stability after nanoencapsulation in PEG/Pluronic mixture which could be attributed to their hydrophilic nature. Although it is difficult to exactly postulate why this happened, but we believe that storage of these formulations in a moisture-controlled environment may help in improving the stability of all carotenoids further. Nonetheless, this study provides a novel solvent-free method to develop formulations containing carotenoids with improved stability and solubility which could potentially to be used in nutraceutical and food industries.

4. Conclusions

Polymeric nanoparticles containing carotenoids were prepared via solvent-free temperature induced phase transition method in scCO_2. The stability of carotenoids during processing and storage was found to better than the samples prepared at atmospheric pressure. The solubility of carotenoids from scCO_2-processed formulations was also found to be up to 30-70% higher, amongst various carotenoid tested in this study.

References:

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