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Optimization of Solid Lipid Nanoparticles for the Encapsulation of Carotenoids from *Cucurbita moschata* Pulp†

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Abstract: This work aimed to optimize the production of Solid Lipid Nanoparticles (SLN) for the future encapsulation of carotenoid-rich extracts obtained from pumpkin (*Cucurbita moschata*) pulp by ultrasound-assisted extraction. The extracts were characterized by *in vitro* spectrophotometric assays and by high-performance liquid chromatography coupled with diode array detector. Hot high-pressure homogenization was the method selected for SLN production and β-carotene was used as a model molecule for the optimization. This choice was supported by the chemical-analytical characterization, which identified β-carotene as the main carotenoid of the pumpkin extracts. SLN loaded with 1% β-carotene showed dimensions compatible with increased intestinal absorption. Furthermore, antioxidant assay results showed that the technological process did not alter the antioxidant capacity of β-carotene.

Keywords: nanoencapsulation; binary mixture; unconventional extraction; pumpkin; nanoparticles; antioxidant properties; β-carotene; pharmaceutical technology

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1. Introduction

The use of nanotechnology is a growing trend in several sectors, such as agriculture, biochemistry, medicine, and recently in food field. Nanocarrier small dimensions and large surface area can contribute to improve the solubility, gastrointestinal protection and bioavailability of lipophilic bioactive compounds, such as carotenoids [1]

Pumpkins are one of the vegetables with the highest content of carotenoids in nature, with β-carotene, a vitamin-A precursor, as the most representative compound. Even though carotenoids exhibit interesting antioxidant and health properties, they have numerous drawbacks that severely restrict their use as food components. These limitations are: very poor bioavailability from natural sources; limited absorption *in vivo*; high instability to light and oxygen [2]

In this paper, Solid Lipid Nanoparticles (SLN), produced using the hot high-pressure homogenization method, have been selected as nanocarriers for the encapsulation of carotenoids, using β-carotene as model compound.

2. Methods

2.1. Plant Materials and Reagents

Pumpkins (*Cucurbita moschata*) were purchased in October 2022 in Perugia (Umbria, central Italy). Hydrogenated Sunflower Oil (HSO) (VGB 5 ST, free fatty acids 0.07%) was obtained from ADM-SIO (Saint-Laurent-Blangy, France). β-carotene (>97.0%; m.p. 184 °C) was purchased from Tokyo Chemical Industry (Toshima, Tokyo, Japan). Soy lecithin and dibasic sodium phosphate were purchased from VWR (Milan, Italy). 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were from Sigma-Aldrich (Milan, Italy).

2.2. Pumpkin Pulp Preparation and Carotenoid Extraction

The pulp of pumpkins, cut into cubes, was dehydrated at 40 °C in a ventilated oven (Binder, Series ED, Tuttlingen, Germany). The dried pumpkin was grinded, sieved and immediately subjected to extraction. The carotenoids were isolated by a sonication bath (mod. AU-65, ArgoLab, Carpi, Italy) with hexane:isopropanol (60:40, *v/v*) for 30 min at 45 °C [3] The extracts were filtered, collected in amber glass vials, and kept at −20 °C until further analysis.

2.3. Total Carotenoid Content (TCC)

The determination of TCC was carried out by the assay reported in a previous paper [4].

2.4. Analysis by HPLC-DAD of Carotenoids

Chromatographic equipment and conditions for the determination of the carotenoid profile of the extracts were reported in a previous work [4]

2.5. ABTS Assay of Extracts and β-Carotene Loaded SLN

ABTS assay was performed according to the procedure described in a previous paper [5]; results expressed as μg of Trolox equivalents/gram of dry pumpkin pulp (μg TE/g). The antioxidant properties of SLN β-carotene-loaded were determined following the Durmaz' method [6]; results expressed as μg TE/mg SLN.

2.6. Production and Characterization of Solid Lipid Nanoparticles

Hot high-pressure homogenization (HPH) was employed to prepare SLN. HSO, melted in a water bath (~75 °C) and soy lecithin (0.8% *w/v)* were added to the lipid phase under magnetic stirring. After adding the lipid phase to the aqueous buffer (sodium phosphate buffer solution, 4 mM, pH = 7) containing sodium cholate (0.3% *w/v*), the pre-emulsion was obtained by using an Ultraturrax homogenizer (8,000 rpm, 1 min), maintaining emulsion in a hot bath. Then, the pre-emulsion was homogenized (5 cycles at 1,000 bar) using an Avestin EmulsiFlex-C5 high-pressure homogenizer (ATA Scientific, Taren Point, Australia), at 75 °C. For the recovery of the SLN, the final emulsion was put in an ice bath. Different amounts of β-carotene (i.e., 0.5, 1, 5, and 10% *w/w*), added in the lipid phase, were used to load SLN.

Dynamic Light Scattering (DLS) (Particle sizer NICOMP 380 ZLS, Santa Barbara, CA, USA) was employed for the dimensional evaluation of SLN. All measurements were carried out at 23 °C for 12 min. The diameter was determined using the NICOMP, based on the variation in the intensity of scattered light (INTENSITY-WT). The β-carotene content in the suspension was determined after drying 200 μL of SLN and solubilizing the residue in hexane. The solutions were analyzed using a UV-Vis spectrophotometer set at λ=450 nm and β-carotene encapsulation efficiency (*EE*) was determined as reported in a previous paper [4]

2.7. Statistical Analysis

The data were performed in triplicate. The results were expressed as mean ± standard deviation (SD) on dried weight (DW).

3. Results and discussion

3.1. Characterization of Pumpkin Carotenoid Extracts

Pumpkin carotenoid extracts were characterized by: extraction yield (%), TCC, and ABTS assay results. Extract from pumpkin pulp showed an average extraction yield, evaluated as reported in a previous work [4], of 2.32 % ± 0.05, a TCC of 147.53 μg/g ± 5.68, and antioxidant activity of 1023 μg TE/g ± 12.36, determined by ABTS assay. These data are comparable to those obtained for the same pumpkin variety (*C. moschata*), collected in the 2021 in the same geographical area [4] To evaluate the qualitative composition of the extract, an HPLC-DAD procedure was performed, showing the presence of two main peaks (α-carotene and β-carotene). Regarding the presence of other compounds, only a few traces of lutein were found in our extract. These findings were in line with other works since both Provesi et al. [7] and Azevedo-Meleiro et al. [8] reported a similar trend in *C. moschata*, where concentrations of α- and β-carotene were higher than lutein, and β-carotene was one of the main carotenoids. Starting from this consideration, β-carotene was chosen as a model molecule to optimize the parameters for the subsequent encapsulation of the extract. Therefore, the first step was to develop and optimize a methodology for the encapsulation of β-carotene within SLN.

3.2. Development and Characterization of β-Carotene Loaded SLN

For β-carotene encapsulation, SLN were selected since they are safe, present good tolerability, and can guarantee an enhancement of carotenoid solubility, stability during storage, and absorption in the gastrointestinal tract [9]. The optimized blank SLN were composed of 2% *w*/*v* HSO, 0.8% *w*/*v* soy lecithin and 0.3% *w*/*v* sodium cholate as the emulsifier and co-emulsifier, respectively.For blank SLN, DLS analysis showed the presence of a main population (93.5%) with a mean diameter of 230 nm (2.29 ± 32.6 nm). β-carotene loading affected the original dimensions of SLN that progressively increased encapsulating growing amounts of the carotenoid. SLN achieved the largest particle size with 10% *w*/*w* β-carotene loading, while minor cargo had a smaller impact, maintaining the SLN mean diameter under 500 nm. Furthermore, suspensions containing 5% and 10% β-carotene showed the presence of red agglomerates, as a result of non-encapsulated β-carotene. Consequently, only formulations with 0.5% and 1% β-carotene were further characterized for the determination of β-carotene content and antioxidant capacity. Regarding the EE, nearly 95% of the β-carotene was encapsulated in the 0.5% loaded SLN, while around 80% was encapsulated for the 1% loaded SLN, suggesting that some β-carotene was lost during the manufacturing process. ABTS was successively performed to investigate the influence of the HPH method on the antioxidant activity of β-carotene in the freshly prepared formulations. ABTS assay was also carried out on soy lecithin and β-carotene. Antioxidant capacity was determined on three different batches of SLN (0.5% and 1% β-carotene loaded) prepared on different days. A very low RSD% was achieved for both the 0.5% and 1% formulations, suggesting that the production process and the assay were reproducible. Overall, the average antioxidant capacity was of 0.060 ± 0.002 mg of TE/mg SLN for the 0.5% loaded SLN and 0.071 ± 0.007 mg of TE/mg SLN for the 1% loaded SLN. Regarding soy lecithin, a very low value (0.005 ± 0.001 mg of TE/mg of lecithin) was observed, suggesting that the antioxidant activity of the formulations was ascribable to β-carotene, that, tested alone, showed 7.875 ± 0.032 mg of TE/mg of β-carotene.

4. Conclusion

Based on the results, we can confirm that pumpkin pulp can be considered a valuable source for the recovery of carotenoids, by using an unconventional method. These bioactives can be used for food and nutraceutical applications. β-carotene was successfully encapsulated in SLN, with suitable dimensions maintained by adding 0.5% and 1% *w*/*w* β-carotene to the lipid phase. Furthermore, we were also able to demonstrate that the technological process guarantees the preservation of the antioxidant activity of β-carotene. All the obtained results (chemical and technological data) are important because they allow us to confirm the reproducibility of the extraction method, the chemical composition of the fruit (same variety collected after one year), and the reproducibility of the encapsulation method. Further studies are currently underway to enhance the loading capacity of β-carotene and translate the encapsulation process on the whole carotenoid extract, taking into consideration also other pumpkin varieties (*i.e.*, *C. maxima*).

Supplementary Materials: Not available.

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