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Research Article

Spray-Drying Microencapsulation of β-Carotene Contents in Powdered *Dunaliella salina* Biomass

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ABSTRACT

Bioactive compounds from powdered *Dunaliella salina* biomass were encapsulated using maltodextrin (MD), gum arabic (GA) and gelatin (GE) as coating material. For encapsulation procedure, nine distinct mixtures composed of maltodextrin, gum arabic and gelatin in different ratios were studied. Microcapsules composed of MD:GA (90:10) exhibited the highest capability (93.22% efficiency) to preserve the β-carotene from thermal degradation during spray drying procedure. The obtained results also indicated that lower inlet air temperatures and flow rates could provide a better protection for β-carotene against thermal degradation. Moreover, the stored microencapsulated *D. salina* powders in 4 °C and dark conditions showed better stability during seven weeks of study. The microencapsulation protocol suggested in this study could be successfully employed for pharmaceutical and food industries handling with β-carotene-containing supplements and products.

Keywords: β-carotene, drying carrier, *Dunaliella salina*, Microencapsulation, Spray-drying.

INTRODUCTION

Carotenoids are a large family of naturally occurring lipid-soluble pigments belonging to the tetraterpenoids class of isoprenoid compounds. Among them, carotenes (α-carotene, β-carotene and lycopene) and xanthophylls (lutein, astaxanthin and zeaxanthin) are the most famous carotenoid compounds which have attained a great commercial proportion in different areas such as pharmaceutical, cosmetics, food and chemical industries. Currently, β-carotene has attained various applications as a functional food. It could play a critical role as the vitamin A precursor in humans, a coloring agent, and also as an anti-cancer agent thanks to its antioxidant properties, which have made it fascinating subject of study for scientists and different industries[1,2]. *Dunaliella salina*, a photosynthetic microalgae[3] has demonstrated to be carotenoid producer which maximally accumulates 10% of its cell dry weight[4]. A lot of attention is focused on implementation of the spray drying method to prepare the β-carotene powder from *D. salina* biomass[5,6]. β-carotene is highly sensitive to environmental factors and exposure to oxidative stress and is largely decomposed. It has been shown that the use of normal air as the dryer gas, causing oxidation of β-carotene content of 45% of the sample inlet is a spray dryer[5]. Microencapsulation[7,8] could increase the carotenoids stability in a sample. To protect the temperature-sensitive carotenoids during spray drying procedure, various coating materials, such as maltodextrin, gum arabic or modified starch[9] have been applied. The effects of operating conditions during spray drying process, such as inlet and outlet air temperature, feeding flow rate, concentration of feeding flow and feeding flow rate to carrier ratio in the β-carotene production process have not been investigated. Whilst the relative stability of the β-carotene content in *Dunaliella* biomass is of great commercial importance. The aim of this project is to investigate the effects of different operating conditions such as inlet air temperature and flow rate during the preparation of β-carotene from biomass of a naturally isolated *D. salina* using spray drying method. In addition, the effects of the encapsulation method using maltodextrin, gum arabic, modified starch and gelatin as carrier on improving the stability of β-carotene content, samples were also noted.

MATERIALS AND METHODS

Chemicals and reagents

β-carotene standard (C4582, type II synthetic, HPLC grade with ≥95% purity), maltodextrin, gum arabic and starch were obtained from Sigma-Aldrich, St. Louis, Missouri, United States. Besides, the employed solvents for liquid-liquid extraction were obtained from Merck (Darmstadt, Germany). Double distilled water was attained obtained from a Milli-Q System (Millipore, Bedford, MA, USA).

Strain, media and culture condition

D. salina (Chlorophyta, Chlorophyceae) was obtained from the Microalgal Culture Collection of Shiraz University of Medical Sciences (MCCS), Shiraz, Iran. For cultivation and preservation of the microalgal strain the Johnson medium[10] with 12% salinity was used. The

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microalgal growth was carried out for 28 days before spray-drying experiment. The study was performed in 500 mL Erlenmeyer flasks containing 200 mL of culture medium[11,12]. The Erlenmeyer flasks were then incubated at 25 °C in an orbital incubator shaker (PECO, Iran), agitated at 130 rpm for 96 h under the light intensity of 60 mol m^{-2} s^{-1}, to obtain a final cell concentration of around 30×10^4 cell mL^{-1}. The specified inoculum was employed as the standard culture for all future experiments in shake flask studies.

Cell growth measurement

The microalgal growth rates were monitored every two days during 28 days of study using dry cell weight method[13]. *D. salina* cells (5 mL) were isolated aseptically and then centrifuged for 5 min at 6000 *g*. The obtained cell pellets were washed twice with an isotonic normal saline solution (0.09 g NaCl L^{-1}) and centrifuged again to remove the possible impurities. After it, the microalgal pellets were dried at 88 °C overnight for cell dry weight determination.

Spray-drying conditions

To obtain the β-carotene powder, spray-drying process was conducted through a mini spray dryer (BÜCHI, B-290, Labortechnik AG, Flawil, Switzerland). The spray-dryer was set for a concurrently drying and was equipped with a spray nozzle system, two-fluid atomizer technology with an orifice of 0.7 mm diameter. The temperature of inlet air was set at 120 °C and 140 °C and also atomizing air flow rate was set at 400 mL min^{-1} and 600 mL min^{-1} for all experiments. The outlet air temperature controlled at 95 °C.

Analytical procedure

For evaluation of the produced chlorophyll *a*, 3 mL of microalgae culture was centrifuged at 2500 rpm, for 10 min. The supernatant was replaced with 80% acetone solution and the suspension was shaked well by vortex, then after centrifugation (2500 rpm, 10 min), absorbance of the supernatant solutions in 668.2 and 664.8 nm were measured by using a spectrophotometer. After it, the amount of chlorophyll *a* was calculated as (Eq. 1): Eq. 1: Chl. *a* (μg mL^{-1}) = $12.25_{A668.2} - 2.79_{A664.8}$

All experiments were done in triplicate with three time observations. For evaluation of produced β-carotene, 1 mL of microalgal culture was centrifuged in 3000 rpm for 10 min, and then the supernatant was replaced with 3 mL hexane/ethanol solution (1:2). After vortexing the solution was centrifuged (3000 rpm, 10 min) and supernatant was separated into two isolated phases. The upper phase was hexane phase including β-carotene compounds. According to equation below (Eq. 2), the amount of β-carotene was calculated in μg mL^{-1}: Eq. 2: β-carotene (μg mL^{-1}) = $25.2 \times A_{450}$

Statistical analysis

To assess the significance of the obtained results, IBM SPSS software version 22.0 (Armonk, NY: IBM Corp.) and GraphPad prism version 6.00 (GraphPad Software, La Jolla California, USA) were employed. In ANOVA analysis, the statistical different levels at 5% were considered as significant.

RESULTS AND DISCUSSION

Cell growth, β-carotene and chlorophyll production

The studied microalgal strain was cultured for 28 days Johnson culture medium. After finishing the cultivation, *D. salina* was grown up to 4.142 g L^{-1} (Fig. 1). Findings of this study were in agreement with some previously reported growth rates for *D. salina*[3]. The obtained data exhibited a typical sigmoidal growth pattern for *D. salina* in Johnson culture medium (12%). The studied cells were reached to their maximum amounts (D_{max}) in their 19th day after commencing the experiment. Further experiments were conducted on the total cell numbers and chlorophyll *a* contents (as the two major factors to monitor the microalgal growth rates). The results indicated that the final cell number of *D. salina* after 28 days of experiment reached to 1.462×10^6 cell mL^{-1}. The total chlorophyll contents of *D. salina*, during cultivation was also scrutinized. Since the total chlorophyll contents are generally regarded as a mirror reflecting the growth pattern of microalga. After 28 days of experiments, it comprehends that the final chlorophyll *a* content in the culture medium reached to 11.587 mg L^{-1}.

Microencapsulation and spray-drying the β-carotene

For encapsulation procedure, nine distinct mixtures composed of maltodextrin (MD), gum arabic (GA) and gelatin (GE) in different ratios (90:10 and 50:50) were studied. As the provided results in Fig. 2, microcapsules composed of MD:GA (90:10) exhibited the highest capability (93.22% efficiency) to preserve the β-carotene from thermal degradation during spray drying procedure. Furthermore, it was shown that lower inlet temperature (120 °C vs. 140 °C) could provide more protection against thermal degradation of β-carotene (11.187 mg L^{-1} vs 10.894 mg L^{-1}). Besides, the lower atomizing air flow rates (400 mL min^{-1}) brings more protection for β-carotene in comparison with higher flow rates as 600 mL min^{-1} (11.342 mg L^{-1} vs 10.569 mg L^{-1}). Continuing our analyses, regarding the stability of the β-carotene-containing *D. salina* biomass, we investigated the effects of different temperatures (4, 25 and 37 °C) and illuminations (preservation in light and dark conditions) on β-carotene stability in the obtained microalgal powder. The resulted powder in normal and microencapsulated conditions were monitored for seven weeks and after it the β-carotene composition in each powder was measured. The results are depicted in Fig. 3. It could be comprehended that preservation in dark and colder conditions cause to more stability for microencapsulated β-carotene. The best result was seen in 4 °C, at dark conditions. Generally, the pharmaceutical industries use direct chemical synthesis or some natural resources like microorganisms and vegetables for β-carotene production. Direct chemical β-carotene synthesis is faced with some critical problems such as hazardous waste products which could damage the environment. Owing to its higher growth rate, simple cultivation and scale up, *D. salina* offers a great potential for pharmaceutical β-carotene production in large scale. Although this process has also some short comings such as uncontrollable changes in production procedures due to geographic and seasonal changes and more importantly instability of the obtained β-carotene. To improve the

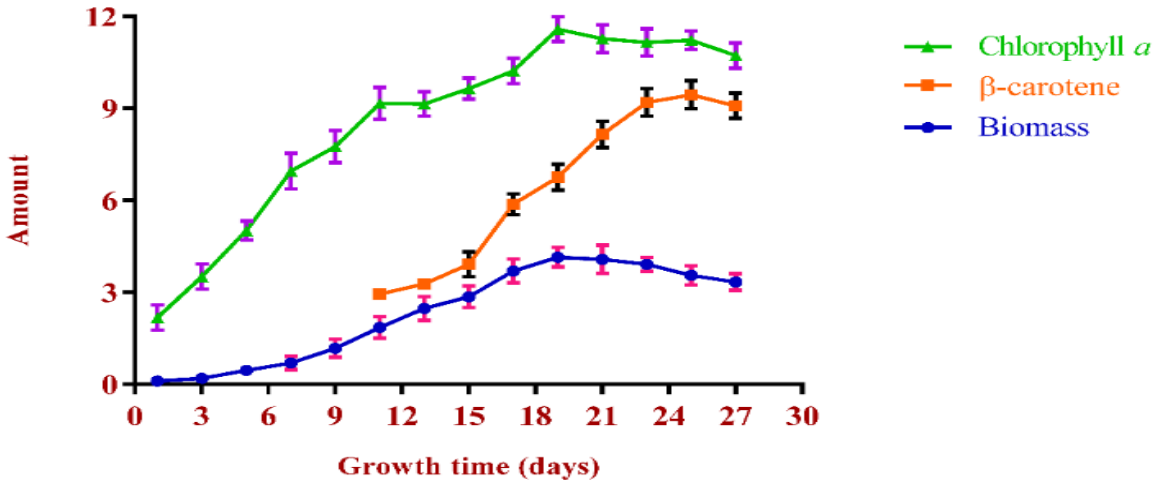

Figure 1: Growth trend (g L^{-1}), β-carotene (mg L^{-1}) and chlorophyll *a* (mg L^{-1}) production from *D. salina* in shake flask scale during 28 days of study using dry cell weight method.

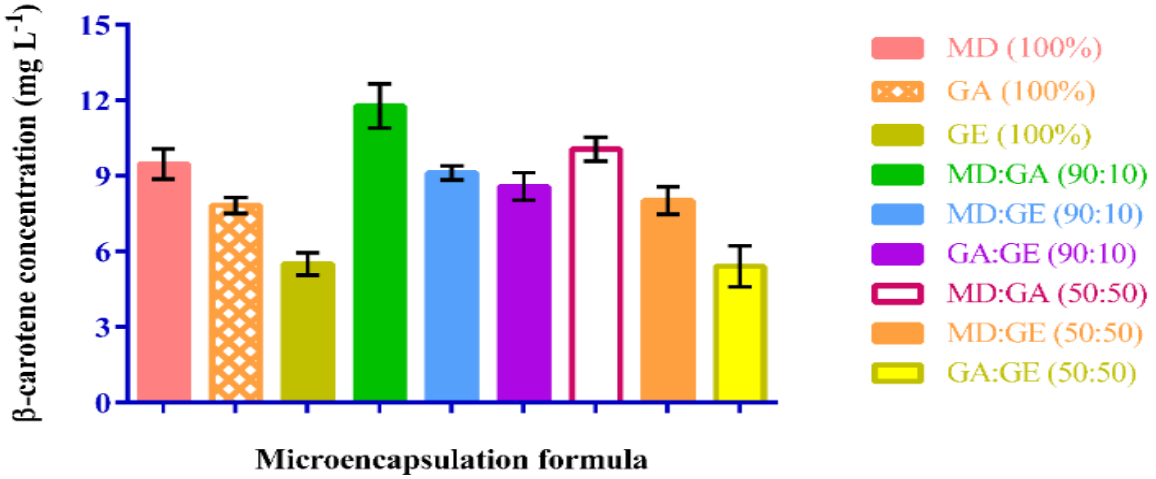

Figure 2: Capability of each studied microencapsulation formula for β-carotene preservation (*n*=3) in *D. salina* powder (MD: maltodextrin; GA: gum arabic and GE: Gelatin).

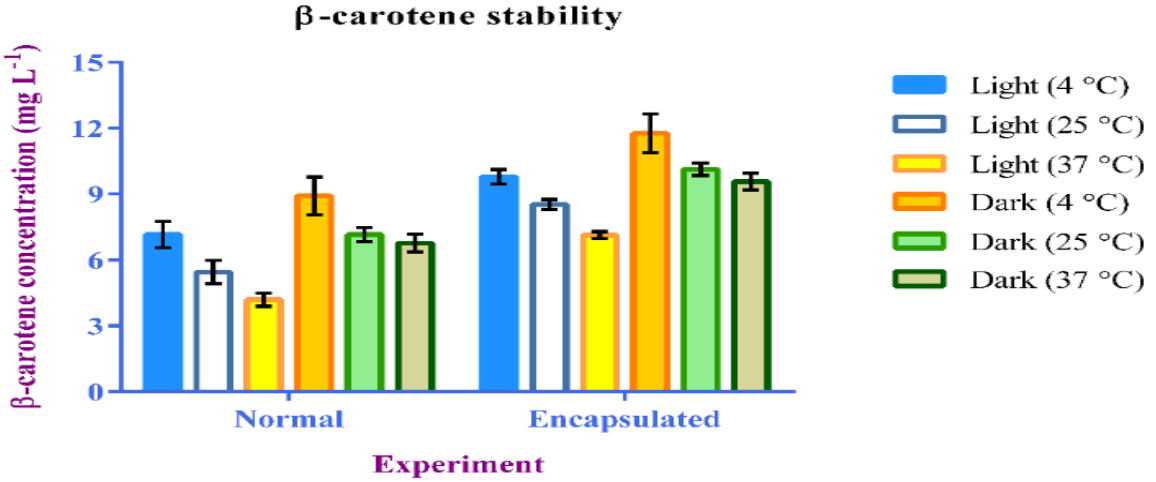

Figure 3: Stability of β-carotene under different temperature (4, 25 and 37 °C) and illumination (preservation in light and dark conditions) in normal and microencapsulated *D. salina* powder (*n*=3).

thermal sensitivity microencapsulation strategy could be considered as a robust strategy. Some previous studies have indicated the capability of encapsulation method, to increase the stability of lycopene[14] pigment or other carotenoid content in oleoresins of green cardamom[15], caraway[16], black pepper[17], and carrot powder[18]. In the current study, we also indicated the effectiveness of using microencapsulation method for protection of β-carotene from thermal degradation during spray-drying process. The quality of the resulting product and its efficacy

depends on various operating conditions such as inlet and outlet air temperature, feeding flow rate, concentration of feeding flow and feeding flow rate to carrier ratio. Using the optimal operating conditions can trap active ingredients within a carrier material, protect a high level of natural pigments such as β-carotene from destruction[19].

CONCLUSION

Maltodextrin, gum arabic and gelatin could be successfully employed as carrier to protect β-carotene from thermal degradation during spray-drying process. The mixture of MD:GA (90:10) was considered as the best formulation for microencapsulation. Our findings also indicated that lower inlet temperatures and flow rates could bring higher yields of β-carotene. The highest stability of β-carotene was reported in dark conditions at 4 °C. The obtained β-carotene containing biomass could be used for pharmaceutical or nutritional purposes. Further optimization studies are needed to improve the suggested spray-drying conditions. Moreover, the developed formula might be used for spray-drying of other valuable pigments obtained from microalgal biotechnology.

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