Impacts of microencapsulation on carotenoids in microalgae *Tetraselmis chuii.*

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Abstract

Microalgae are the basis of nutrition of the aquatic food chain. *Tetraselmis* is a unicellular flagellated marine green algae and have considerable nutritional value. This microalgae is rich in proteins, lipids and carbohydrates, contains α-tocopherol (vitamin E), carotenoids (eg. fucoxanthin and β-carotene) and as every plant also contains chlorophyll. It has a cellular composition 31% protein, 12% carbohydrates and 17% lipids. It has been reported as the most easily grown marine flagellate species on a large scale and is used as food for larvae of mollusks, crustaceans and fish. But usually in aquaculture seaweed or frozen concentrated algae pastes are used. On the question of storage and transportation is not an appropriate or easy method to handle. Besides it loose nutritional values during the storage time period and transport processes. So in this work microalgae microencapsulated products of *Tetraselmis chuii* were developed with different types of wall materials (maltodextrin:gum arabic, chitosan and gelatin) to preserve nutritional values and products evaluated for beta-carotene, antioxidant property and total carotenoid contents. Evaluations regarding impact of microencapsulation before and after the process shown different results depending on different types of encapsulation materials.

Introduction

Due to high nutritional value microalgae has importance in aquaculture. The most common genera used for aquaculture food are: *Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema, Thalassiosira,* among others [1]. Tetraselmis is a unicellular green, flagellate marine microalgae, with considerable nutritional value, being rich in proteins, lipids and carbohydrates, containing α-tocopherol (vitamin E), carotenoids (eg. fucoxanthin and β-carotene) that act as potent antioxidants. The use of microencapsulation technique has been reported as very efficient, because it is safe, feasible and simple implementation for the immobilization of living micro-organisms with the characteristic that they are released in a gradual manner. During microencapsulation process one substance surrounds or covers another substance, so as to form a polymer layer on a very small scale, obtaining capsules from one micron up to hundreds of microns in size. This work tried to microencapsulate *Tetraselmis* biomass with different kinds of wall materials to preserve the nutritional value and estimated the carotenoid and beta-carotene content, those are susceptible for degradation in normal environmental conditions.

Methodology

Algal culture: *Tetraselmis chuii* strain was collected from Centro de Investigación Biológica del Noreste (CIBNOR), La Paz, México. The microalgae cultivated in 2X Erdschreiber's Medium Recipe at room temperature 25-27 °C, with 2500 lux illumination in a photo-bioreactor and harvested at 18 days of culture.

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Encapsulation and Spray-drying: The microencapsulation of algal biomass was carried out using Spray Dryer Buchi-191 model in a matrix comprised of maltodextrin: gum arabic from acacia tree (60:40), chitosan 3% and gelatin 2%. 1mg of fresh algal biomass was added to 100mL solutions containing different proportion of wall materials. The inlet temperature was 150°C, 130°C and 110°C. The spray gas pressure, sample feed rate, and atomization pressure were kept constant at 40 bar, 1.40 mL/min, and −65 mbar respectively.

Scanning electron microscopy (SEM): The microencapsulated powders were examined by scanning electron microscopy to study morphology. The samples were deposited onto specimen stubs and a sputter coater was used to cover the samples with gold. The specimen stubs were placed into the electron microscope JEOL JSM-5400LV, using the following operational conditions: objective aperture 10 lm, accelerating voltage 5 kV–15 kV and different magnification like 500x-5000x.

Antioxidant activities assays: The antioxidant activity of the microencapsulated algae was determined by measuring the radical scavenging activity of DPPH [2]. DPPH is reduced with an antioxidant compound and the change in color due to reduction from deep violet to light violet/yellow was measured at 517 nm on a UV/visible light spectrophotometer. Inhibition of the free radical DPPH• was calculated by the following formula.

$$\% \text{ of Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Total carotenoid estimation: Total carotenoids from microencapsulated algal biomass were estimated by methods of Wellburn 1983 [3].

Beta carotene estimation: β-carotene from microencapsulated algal biomass were estimated by methods of M. A Heejazi-2002 [4] using tetrahydrofuran (THF). The extracted colored solution having pigments quantified by spectrophotometer at 455nm and compared with standard curve of β-carotene. By thin layer chromatography, quantitative analysis of β-carotene were done with concentrated extract and separated on silica gel 60 F254 TLC plates (10 x 10 cm, Merck) and developed using acetone: n-hexane (30:70) solvent .

Results

Algal culture: The microalgae cultivated in 2X Erdschreiber's Medium at room temperature 25-27 °C, with 2500 lux illumination in a photo-bioreactor with aeration and harvested at 18 days of culture. The fresh biomass obtained was 3-4g/l and collected by centrifugation for further use for microencapsulation.

Encapsulation and Spray-drying: The encapsulation efficiency of the spray drying depends critically on the selection of an appropriate wall material. Gum arabic is an effective agent as it is compatible with most gums, starches, carbohydrates, and proteins [5]. The maltodextrin is often used as a wall material because of its low cost, good flavor, high solubility in water (75%), and the low viscosity of the solutions it forms [6]. Therefore we used the mixtures of maltodextrin and gum arabic (60:40) for our algal biomass encapsulation.

Again the chitosan was used as encapsulating material for its biodegradability, low toxicity and biocompatibility properties. Chitosan was prepared at 3% solution (w/v) for our algal biomass

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encapsulation. Another wall material we tried in this study that is 2% gelatin, it's a commercial protein biopolymer used as various food component. To every 100ml of the wall material solution 1g of algal biomass was added and then mixed with a homogenizer. Then all the materials were spay dried with three different temperatures and the average yield of the powder was ~20-25% in case of chitosan and gelatin but in case of maltodextrin it was 62%.

Scanning electron microscopy (SEM): Electron microscopy image analysis can play an important role in the evaluation of microcapsules. Visually was found that the encapsulated samples was extremely fine, slightly green color and water soluble powders. Moreover it was found that the drying temperatures do not affect that much for the resulted particle size and surface structure. But wall material type affects the size and surface structure greatly. With maltodextrin: gum arabic, the resulted particles found irregular shape with adherent property (fig.1). The surface was smooth and homogeneous with particle size ranges between 10-40μ. With chitosan the particle were rounded and surface was bit wrinkled, but the particles are well separated from one another and size was very small in between 5-15 μ (fig.1). With gelatin the particles presented highly irregular size and wrinkled but the particles were less crowded than those obtained through Chitosan (fig.1). The size of particles was in between 2-10μ. All of the particles were consistent by structure with very lees or no fragile particles. Also there was found no unbound algal biomass.

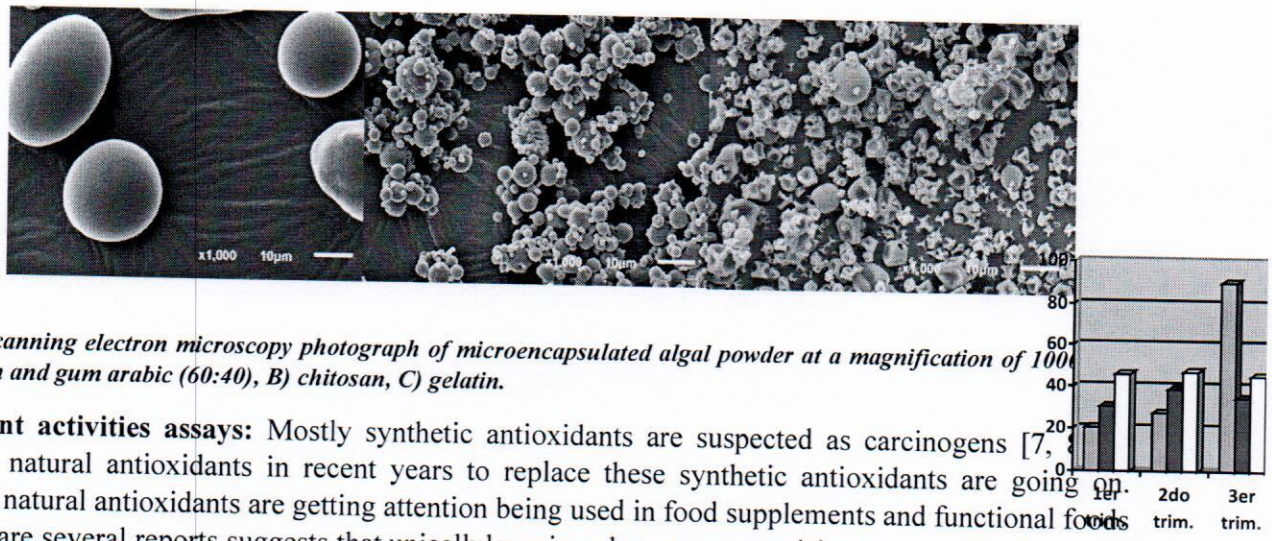

Figure 1. Scanning electron microscopy photograph of microencapsulated algal powder at a magnification of 100(maltodextrin and gum arabic (60:40), B) chitosan, C) gelatin.

Antioxidant activities assays: Mostly synthetic antioxidants are suspected as carcinogens [7, 8]. search for natural antioxidants in recent years to replace these synthetic antioxidants are going on. Therefore, natural antioxidants are getting attention being used in food supplements and functional foods [9]. There are several reports suggests that unicellular microalgae are a promising source of antioxidants [10, 11, 12]. Carotenoids contribute significantly towards the total antioxidant capacity of microalgae [13]. But there is no much reports on *Tetraselmis Chuii* antioxidant capacity and β-carotene content. So this study evaluated the antioxidant capacity of *Tetraselmis chuii* after microencapsulation which can be used as food ingredient.

In the present study, DPPH activity was expressed in terms of inhibition %(I).The microencapsulated algae shown inhibition % very similar results with maltodextrin: gum arabic (60:40) and gelatin nearly 32-41% (fig. 2, 3, 4). Whereas chitosan shown little lower inhibition capacity (33%). It may be due to acetic acid used to dissolve chitosan. But there was no significance difference among the inlet temperature effects on inhibition capacity with the same wall material used for encapsulation.

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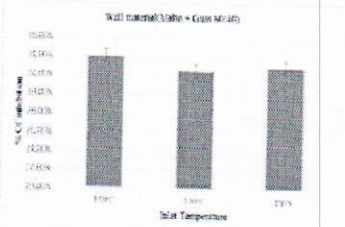

Figure 2. Antioxidant activity with maltodextrin and gum arabic.

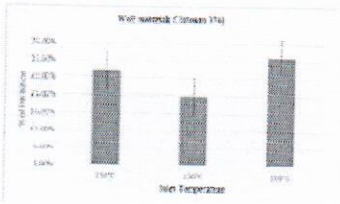

Figure 3.Antioxidant activity with chitosan 3%.

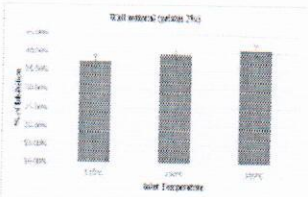

Figure 4. Antioxidant with gelatin 2%.

Total carotenoid and β-carotene estimation: In plants, carotenoids contribute to the photosynthetic machinery and protect them against photo-damage. Carotenoids and phenolic acids is proven beneficial effects on human Health [14]. About to 90% of the carotenoids in the diet and human body is represented by β-carotene, α-carotene, lycopene, lutein and cryptoxanthin [15]. There is a great interest on caroteneoids as antioxidants [16]. Again vitamin A deficiency presents a serious public health problem in many parts of the world. Now days carotenoids used as food additives as a natural colorant and also due to its antioxidant properties. Marine microalgae offer great potential as a source for compounds those are receiving attention in many industries, like food, pharmaceutical and cosmetic industries. So this study evaluated total carotenoid and β-carotene in microencapsulated microalgae *Tetraselmis chuii.* There was found carotenoid content like 95mg/g in case of Maltodextrin and 75mg/g in case of chitosan encapsulated dried biomass. In case of gelatin without application of heat we cannot separate the algal biomass for proper extraction of pigment, so it was not compared.

Presence of β-carotene in the microalgae *Tetraselmis chuii* has shown a clear band in thin layer analysis (fig.5). Then was quantified the β-carotene content and found it is nearly 0.5- 1% of dry weight. Usually algal biomass dried to use as food ingredient. Also was compared β-carotene content in the microencapsulated algal biomass and it was found nearly similar or higher in case of maltodextrin: gum arabic (60:40) encapsulated with non-encapsulated dried biomass like 5-7mg/g dry wt (fig.6). But wall material affects about the preservation of β-carotene content, it was found more preserved with gelatin as we could not succeed to extract properly as biomass was not fully recovered after washing (fig.8). With maltodextrin: gum arabic (60:40) and chitosan encapsulated material, the β-carotene content was found similar within 5-7mg/g dry wt. (fig.6, 7). There was no significant impact with difference of inlet temperature on β-carotene content when same wall material is used. The results shown that maltodextrin: gum arabic (60:40) is little better than chitosan for preserving β-carotene.

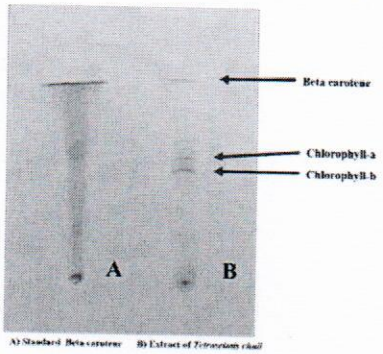

Figure 5. TLC profile of Tetraselmis chuii, showing β-carotene band.

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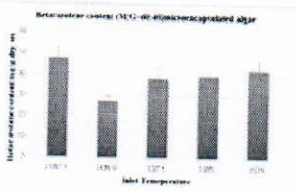

Figure 6. β-carotene content with maltodextrin and gum arabic.

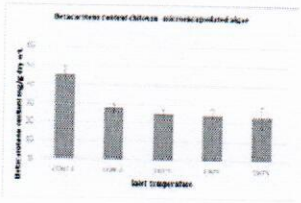

Figure 7. β-carotene content with chitosan 3%.

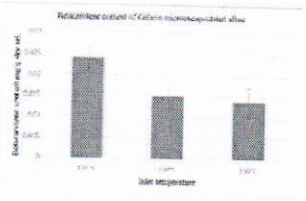

Figure 8. β-carotene content with gelatin 2%.

Conclusion: In this study whole algal biomass was used to preserve the entire nutritional value rather than the purely extracted compounds. The microalgae with maltodextrin and gum arabic wall material shown better preservation of β-carotene and the better antioxidant activity was shown by the gelatin. Both can be used for encapsulation material as they are edible and cheaper. It will be easier to preserve and transport the microalgae biomass as microencapsulated powder for the use in aquaculture and can be added to other food ingredients for increment of nutritional value.

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