

Degradation of Carotenoids from *Dunaliella salina* During Storage

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Abstract

Carotenoids, the most widespread pigments in nature, are involved in several functions in human, such as vitamin A biosynthesis, and, because of their antioxidant activity, protection of cells and tissues from free radicals and oxygen ions. However, due to their highly unsaturated structure, carotenoids are prone to degradation during processing and storage because of exposure to high temperature, light or pro-oxidant molecules. Carotenoids were extracted from *Dunaliella salina* by using methanol as a solvent at atmospheric pressure and the packaged supernatant in lined aluminium glass vials was stored under 1) air packing and environmental conditions (25 °C), 2) controlled atmosphere with N₂ and environmental conditions and 3) controlled atmosphere with N₂ and dark at 4 °C. The concentration of total carotenoids was monitored by UV-Vis every three days for 90 days. The total carotenoid content in *D. salina* was 42.18 mg carotenoids/g dry microalga and the major carotenoid found was β-carotene, which represented almost 70 % of the total carotenoids extracted. Samples stored under a modified and refrigerated atmosphere were slightly degraded, while the other two kinds of storage had a slight difference from each other, which could

indicate a synergistic effect of using an oxygen-free atmosphere and low temperature.

Keywords: Pharmaceutical product; antioxidant; β -carotene; storage study; microalgae

1. Introduction

The search for natural sources of antioxidants keeps increasing over time. These products are used to reduce the oxidative deterioration of both food and human body [1]. Microalgae represent one of the sources that have been studied due to the high amounts of bioactive components within their structure [2]. Some of these components include polyunsaturated fatty acids, antioxidants, sulphated polysaccharides (antivirals), sterols (antimicrobials), among others [3]. Nowadays there is an increasing interest in the use of antioxidants in the pharmaceutical and food industry since they have been proved to possess beneficial effects on human health [4], [5]. Carotenoids, for instance, are a class of organic pigments from the group of isoprenoids, with the main chain of 40 carbon atoms. These compounds represent highly important pigments thanks to their numerous biological functions. They are fat-soluble pigments and therefore, they present high solubility in non-polar solvents [6]. Carotenes are a specific type of carotenoids which are completely composed of hydrocarbons and with no oxygen within their structure. Carotenoids with oxygen within their structure are known as xanthophylls. β -carotene is one of the main types of carotenoids, and it is mainly known for being a natural precursor of vitamin A [7]. It is a high-molecular-weight compound that is formed by a hydrocarbon chain ($C_{40}H_{56}$); it is easily degradable by light, heat and air, and its colour can vary from yellow to dark red, depending on its pureness, source and location. Some of the main natural sources of β -carotene include plants, algae and fungi, but most part of its production comes from synthetic methods [8]. β -carotene can be used in important applications as a drug, colourant and food ingredient for human health, thanks to its antioxidant and pro-vitamin A properties [9]. It has also been used because of its anticancer activity [10].

Since carotenoids extraction involves expensive and difficult procedures, it is reasonable to evaluate the storage stability of carotenoids to monitor and regulate the quality of final products. In most cases, the contents of carotenoids vary markedly during industrial processing and storage conditions due to its significant number of double bonds in the structures [11]. Therefore, understanding how carotenoids degrade during storage is critical because it affects the nutritional value of final products. In addition, knowledge of the best packaging mechanisms is necessary. Song *et al.*, [11] studied the degradation of carotenoids in dehydrated pumpkins affected by different storage conditions. These authors found carotenoids degradation during the storage of einkorn and bread wheat flours was

influenced by temperature and time. Similar results have been demonstrated by others author [12]–[15].

On the other hand, *Dunaliella salina*, a halophilic microalga, is a unicellular biflagellate, which grows in salinities, it is very fragile due to lack of rigid cell wall and can be easily ruptured when an excessive shear stress is applied. This microalga is produced industrially due to its high content in carotenoids, which market estimation is around \$1428.12 million in 2019 [16]. In addition, 14 % of its dry weight could represent β -carotene. Taking into account the aforementioned, this study aimed at evaluating the degradation of carotenoids obtained from *Dunaliella salina* stored under normal refrigeration conditions.

2. Methodology

2.1 Chemicals and materials

Methanol (for HPLC, ≥ 99.9 %), acetonitrile (HPLC, 99.8 %), 1-butanol (for HPLC), β -carotene (for HPLC, $\geq 95\%$) and butylated hydroxytoluene (BHT) were all purchased from Sigma-Aldrich (United States). All materials were used as received.

2.2 Biomass and cultivation

The microalga used in this study was the *D. salina*. The biomass was grown in seawater enriched with a f/2 medium [35], at the temperature in the range of 20 °C – 35 °C and with atmospheric aeration. After growth was complete, the biomass was lyophilized and stored in a refrigerator in the absence of light until the extraction process was carried out. The final water content of the biomass was 5 %.

2.3 Carotenoids extraction

Methanol stabilized with 0.025 % BHT [17] was used as a solvent at atmospheric pressure for the extraction of carotenoids from *D. salina*. To measure the total content of carotenoids in the microalga, 0.5 g of dried material was suspended in 10 mL of solvent. The sample was ultrasonically agitated for 5 min (Bandelin SONOREX™, United States) and then maintained at 4 °C for 24 h. After that, the sample was centrifuged (Kubota Corporation, Japan) at 10 g for 10 min and the extract (supernatant) was separated from the pellet and collected in glass vials. The vials were maintained wrapped with aluminium foil until analysis. The extraction process was repeated until the pellet remained greenish in colour. After that, the sample was diluted and analysed. All the procedure was carried out at least two times.

2.4 Storage conditions

The packaged supernatant in lined aluminium glass vials was stored under 1) air packing and environmental conditions (25 °C), 2) controlled atmosphere with N₂ and environmental conditions and 3) controlled atmosphere with N₂ and dark at 4

°C. Approximately 10 g of sample was placed in per package. Each treatment was replicated at least two times. Carotenoids in dried microalga were determined every three days for 90 days.

2.5 Content of total carotenoids

The total concentrations of carotenoids were determined by measuring the absorbance of the samples by using a UV-Vis spectrophotometer (Spectronic™ GENESYS™ 20, United States). The equation proposed by Wellburn [18] was used for the determination of carotenoid concentration in the samples of *D. salina*. This equation has more parameters than other equations presented in the literature and allows the determination of the chlorophyll b contained in the samples [19].

The concentration of total carotenoids was calculated using the following equation:

$$C_{total\ carotenoids\ (x+c)} \left(\frac{\mu g}{mL} \right) = \frac{1000A_{470} - 1.63C_a - 104.96C_b}{221} \quad (1)$$

$$C_a \left(\frac{\mu g}{mL} \right) = 16.72A_{665.2} - 9.16A_{652.4} \quad (2)$$

$$C_b \left(\frac{\mu g}{mL} \right) = 34.09A_{652.4} - 15.28A_{665.2} \quad (3)$$

Where $C_{(x+c)}$, C_a and C_b are total carotenoid (xanthophylls and carotenes), chlorophyll a and chlorophyll b concentration respectively and A_{470} , $A_{652.4}$ and $A_{665.2}$ are the absorbances at 470 nm, 652.4 nm and 665.2 nm respectively.

2.6 HPLC analysis of carotenoids

Immediately after the extraction of carotenoids, the extract was analysed in order to quantify the amount of β -carotene contained in the extract. For this, a PrimeLine™ Gradient HPLC pump system (Analytical Scientific Instruments, United States) and an S-3210 photodiode-array detector (PDA) (Schambeck SFD GmbH, Germany) were used. A C30 column and a mobile phase of acetonitrile–butanol (7:3, v/v) (A) and methylene chloride (B) with the following gradient elution were used: 99 % A and 1 % B initially, increased to 4 % B in 20 min, 10 % B in 50 min and returned to 1 % B in 55 min. The identification of β -carotene was carried out by comparison of retention time and absorption spectra of the unknown peak with the reference standard.

3. Results

3.1 Carotenoids content

The total carotenoid content in *D. salina* was 42.18 ± 1.41 mg carotenoids/g dry microalga and the major carotenoid found was β -carotene, which represented almost 70 % of the total carotenoids extracted. Carotenoids found in the biomass evaluated in this work were higher than those present by Macías-Sánchez *et al.*, [19], who obtained 14.10 mg carotenoids/g dry microalga and 27.70 mg carotenoids/g dry microalga by using methanol and dimethylformamide respectively. Others author found β -carotene as the main compound contained in the same biomass [8], [20], [21]. The differences found in β -carotene content in different studies could be due to the different extraction methods or type of biomass obtained depending on the type of growth.

3.2 Storage study

The changes of the total carotenoids content from *D. salina* under different storage conditions are presented in Figure 1. As can be seen in Figure 1, the total content of carotenoids decreased rapidly under air packaging conditions as compared to other storage conditions, which indicated that the degradation degree of the original pigments was influenced by storage conditions [11]. At the end of the study, the higher carotenoid content was found in samples stored under modified atmosphere with N_2 and dark at 4 °C, which showed that the degradation of carotenoids depended on the temperature, storage duration and the presence of oxygen, as it has been found by other authors who studied carotenoid degradation in different matrices such as dehydrated pumpkin [11], orange juice [22], spinach chloroplasts [14], oil [15], einkorn and bread wheat [12]. Song *et al.*, [11], claimed that during the storage, the predominating mechanism of carotenoid degradation should be isomerization and oxidation (autoxidation).

Figure 1 shows that after 90 days of storage, approximately 48 %, 35 % and 10 % of total carotenoids were lost respectively under air packing and environmental conditions, controlled atmosphere with N_2 and environmental conditions and controlled atmosphere with N_2 and dark at 4 °C. According to Figure 1, only samples stored under a modified and refrigerated atmosphere were slightly degraded, while the other two kinds of storage had a slight difference from each other, which could indicate a synergistic effect of using an oxygen-free atmosphere and low temperature. Similar degradative behaviour of carotenoids during storage were reported previously [12], [14], [15], [22], [23]. As known, carotenoids, including β -carotene, typically contain 40 carbons and multiple conjugated double bonds are highly susceptible to degradation due to oxygen and high temperatures [11]. However, dried biomass could represent a complex system, because of the numerous other compounds present. Compounds such as

proteins, carbohydrates, and lipids may act as co-oxidants and promote oxidation or may have a protective effect [13].

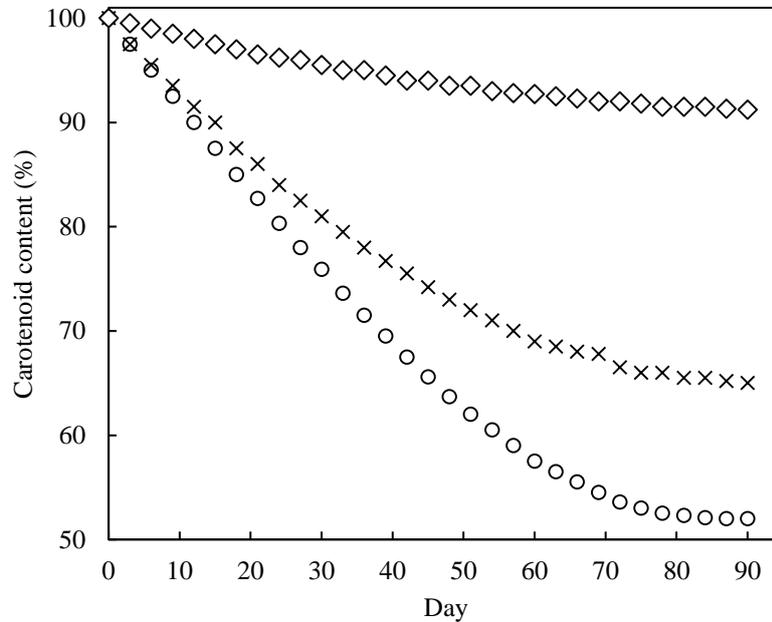


Figure 1. Total carotenoids content from *Dunaliella salina* during storage under air packing and environmental conditions (○), controlled atmosphere with N₂ and environmental conditions (◇) and controlled atmosphere with N₂ and dark at 4 °C (×).

4. Conclusions

Carotenoids, the most widespread pigments in nature, are liposoluble antioxidants produced by plants, where they contribute to the photosynthetic pathway as both light collectors and photo protectors. In humans, they are involved in several functions, such as vitamin A biosynthesis, and, because of their antioxidant activity, protection of cells and tissues from free radicals and oxygen ions. However, due to their highly unsaturated structure, carotenoids are prone to degradation during processing and storage as a result of exposure to high temperature, light or pro-oxidant molecules. B-carotene was the main carotenoid found in extract. On the other hand, samples stored under a modified and refrigerated atmosphere were slightly degraded, while the other two kinds of storage had a slight difference from each other, which could indicate a synergistic effect of using an oxygen-free atmosphere and low temperature. On the other hand, the numerous other compounds present in the biomass could act as co-oxidants and promote oxidation. Finally, package atmosphere should be paid more attention during long-term storage and a synergistic effect of using an oxygen-free atmosphere and low temperature could exist.

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