Spray-Drying of the Microalga *Dunaliella salina*: Effects on β-Carotene Content and Isomer Composition

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The effects of spray-drying of the unicellular microalga *Dunaliella salina* on its β-carotene content and geometric isomer composition have been studied. The efficacy of a range of synthetic and natural antioxidants in preventing degradation of β-carotene has been determined. Losses of β-carotene and isomerization were minimal during processing for both the control (no exogenous antioxidants) and the samples containing butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (TBHQ). However, the use of tocopherol-based antioxidants resulted in degradation of 52−72% of β-carotene during the drying process. All dried powders of *Dunaliella* proved to be unstable during storage in the presence of light and air, with β-carotene degraded according to a first-order kinetic model. Of the antioxidants studied, only TBHQ was successful in significantly minimizing degradation (degradation constants of 0.03 and 0.04 $days^{-1}$, compared to 0.53 $days^{-1}$ for the respective control). For control powders and those with BHT added to the feed, the degradation constants were reduced to values between 0.27 and 0.37 $days^{-1}$ by restricting light and flushing with nitrogen; however, storage in the dark alone had no effect. For more slowly degrading powders having TBHQ added to the feed, it was clear that degradation of β-carotene was influenced by both light and oxygen. During storage the 9-*cis* isomer of β-carotene was significantly more unstable than the *all-trans* form. TBHQ was, however, successful in reducing relative losses of this isomer for samples stored in the dark. The results suggest a dominant photodegradative mechanism for the loss of the 9-*cis* isomer of β-carotene.

Keywords: *Antioxidants; β-carotene; Dunaliella salina; spray-drying*

INTRODUCTION

The stabilization of carotenoids in foods and foodstuffs against oxidation has for a long time been of importance in that their degradation (by either isomerization or oxidation) effectively lowers the final product quality in terms of nutritional properties as well as coloration characteristics. The stability of natural β-carotene from fruits and vegetables during thermal processing (including freezing, canning, cooking, oven-dehydration, etc.) has been extensively studied. Carotenoid degradation during these processes has usually been observed and the extent of loss found to be dependent on the type of treatment, the temperature, and the duration (Chandler and Schwartz, 1987, 1988; Craft et al., 1993; Minguez and Gandul, 1994; Ooi et al., 1994; Chen et al., 1995). Significant changes in the isomeric composition of β-carotene were also reported. *cis*-Isomer concentration, notably 9-*cis* and 13-*cis*, increased significantly while *all-trans*-β-carotene decreased. This decreased the provitamin A activity of the processed product compared to that of the fresh product (Panalaks and Murray, 1970; Sweeney and Marsh, 1971; Chandler and Schwartz, 1987, 1988; Chen et al., 1995).

The stability of carotenoids under different storage conditions has been investigated in different systems including vegetable juice (Pesek and Warthesen, 1987), freeze-dried whole egg powder (Rao et al., 1992), spinach and carrots (Kopaslane and Warthesen, 1995), spray-dried encapsulated carrot carotenes (Wagner and Warthesen, 1995), and model systems of simulated aqueous or dehydrated food (Goldman et al., 1983; Pesek and Warthesen, 1988). Although carotenoid degradation was observed in all of these, the rate and kinetics of degradation were found to be dependent on both the storage conditions (especially light, temperature, water activity, and oxygen) and the matrix characteristics (liquid or solid state and microenvironment).

The unicellular microalga *Dunaliella salina* naturally occurs in many marine habitats but is also industrially cultivated in large open raceway ponds. Mature cultures of microalgae are harvested, and the concentrated biomass is subsequently processed to provide β-carotene, notably as a supplement for the health food market. Many of the commercial algal carotenoid products are oil-based; algal carotenoids are extracted into hot vegetable oil (Nonomura, 1987) and subsequently encapsulated into gelatine capsules. Such an extraction protocol may result in large losses of carotenoids due to incomplete extraction as well as oxidative destruction during processing. An interesting alternative to carotenoid extraction is the preparation of dried algal powders by spray-drying, thus allowing the use of whole algal cells. This technique is frequently used to dry heat-sensitive products due to the short drying time. Unlike freeze-drying, which is a time-consuming process that

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Table 1. Recovery (Percentage of Original Level) of Carotenoid ($n = 3 \pm$ SE) and of β-Carotene *cis*-Isomers in Powders of Spray-Dried *D. salina*

treatment	antioxidant level (ppm)	β-carotene content (10^{-2} g/g of solids)		recovery of β-carotene (%)		
		feed	powder	total	9-*cis*	other-*cis*
control (1)		1.39 ± 0.10	1.29 ± 0.08	92.81	96.74	123.37
BHT	500	1.44 ± 0.10	1.28 ± 0.18	88.89	82.90	116.02
BHT	1000	1.14 ± 0.06	1.08 ± 0.14	94.74	82.90	116.02
BHT	5000	0.98 ± 0.26	0.88 ± 0.08	89.80	82.90	116.02
control (2)		6.41 ± 0.19	6.38 ± 0.03	99.53	89.33	118.82
TBHQ	3000	6.41 ± 0.19	6.13 ± 0.49	95.63	97.34	99.83
TBHQ	9000	6.41 ± 0.19	5.84 ± 0.03	91.11	93.24	112.74
α-tocopherol	750	5.79 ± 0.09	1.68 ± 0.11	29.02	90.49	108.52
α-tocopherol	2000	5.79 ± 0.09	1.56 ± 0.03	26.94	84.11	79.74
tocopherol acetate	750	5.79 ± 0.09	2.39 ± 0.09	41.28	106.8	106.2
tocopherol acetate	2000	5.79 ± 0.09	1.93 ± 0.14	33.33	94.71	62.27

results in a highly hygroscopic powder and is poorly applicable on a large scale, spray-drying results in a dry powder in a relatively short time.

Antioxidants are commonly added to oils, fats, and fatty food to protect them against oxidative degradation. Such degradation is generally catalyzed by light and heat, and thus antioxidants reduce losses during processing and improve stability during storage (Kaitaranta, 1992; Dorko, 1994). However, there are few studies concerning the effect of antioxidants during spray-drying of carotenoid-rich products. The addition of lipid soluble synthetic antioxidants such as butylated hydroxytoluene (BHT) or the natural antioxidant α-tocopherol has been shown to reduce the rate of degradation of synthetic β-carotene in an aqueous emulsified model system (Barimalaa and Gordon, 1988). The protective effect of BHT on the degradation of β-carotene has also been observed in fish food preparation (Spencer, 1989), in a simulated dehydrated food model system (Goldman et al., 1983), and during extrusion cooking with starch and subsequent storage of extrudates (Berset et al., 1989). In contrast, little work has been published concerning the effectiveness of *tert*-butylhydroquinone (TBHQ) on carotenoid stabilization, even though TBHQ is probably the most effective antioxidant for most fats and oils (Dorko, 1994). Moreover, the protective effect of antioxidants on natural β-carotene preparations from algal biomass and its isomeric composition have not yet been reported. In this study we have examined the effectiveness of spray-drying of β-carotene-rich cells of *Dunaliella* and evaluated the potential use of exogenous antioxidants on preserving the stability of β-carotene and its isomeric composition.

MATERIALS AND METHODS

Preparation of Algal Concentrate. A carotenoid-rich culture of *D. salina* was cultivated by Necton (Companhia Portuguesa de Culturas Marinhas, Lda) at their facility at Belamandil, Algarve (southern Portugal). The culture was grown in raceway ponds in natural seawater enriched with selected nutrients (equivalent to 2.5 M NaCl De Walne medium; Orset and Young, 1999) outdoors under natural sunlight. The resulting harvested biomass from these ponds typically had a cellular concentration of $(0.3-0.5) \times 10^6$ cells mL^{-1} and a salinity of 18% NaCl (w/v). The culture was concentrated 10× by low-shear, cross-flow filtration (membrane pore size = 0.6 mm, shear rate = 14000 s^{-1}; Millipore Prostak system, Millipore Corp., Bedford, MA) and subsequently by centrifugation for 10 min at 2600*g* to obtain a final cell density of 4.1×10^7 cells mL^{-1}. The concentrate was then rapidly frozen at −25 °C until processed. Prior to drying, the culture was carefully defrosted and desalted in three stages by dilution with distilled water and centrifugation (2600*g* for 5 min) to obtain a cell concentrate with a final salinity of <3.5% NaCl (w/v) and a solids content of 4.5%. NaCl was considered to be an inert bulking solid. Two separate batches of algal concentrate were used in this study and, as a result, two separate control experiments were undertaken (see Table 1).

Use of Antioxidants. Tocopherol, tocopherol acetate, and BHT were obtained from Sigma (Poole, Dorset, U.K.). TBHQ was obtained from Fluka (Gillingham, Dorset, U.K.). Due to the potential of synthetic antioxidants for evaporation and thermal decomposition (Hamama and Nawar, 1991), BHT and TBHQ were added to the feed at a range of concentrations from 500 to 5000 ppm and from 3000 to 10000 ppm, respectively. α-Tocopherol and tocopherol acetate were added at 750 and 2000 ppm by dispersal with Tween 80 surfactant (added at 0.75% w/v of the concentration of the antioxidant). The final concentration of these two antioxidants was limited by their poor dispersal characteristics in the aqueous feed.

Spray-Drying. For each treatment, 25 mL of continuously agitated algal concentrate (containing antioxidant, when appropriate) was dried using a Buchi Mini spray-dryer (B-191). The temperatures at the inlet and outlet were set to 130 and 85 °C, respectively. These values were predetermined on the basis of previous optimization experiments that maximized recovery of β-carotene with minimal isomerization (data not shown). The receiving chamber was protected from exposure to light throughout the drying process. The algal material was sprayed at a rate of 200 mL h^{-1}. The resulting powder was immediately removed from the receiving vessel following drying and subjected to stability studies as described below.

Stability Studies. Equal weights (∼1 g) of each powder were transferred to 20 mL clear glass vials and exposed to the following conditions: (1) light (provided by 30 W white fluorescent tubes) plus air; (2) dark plus air; and (3) dark plus oxygen-free nitrogen. In each case, the vial headspace was thoroughly flushed with the appropriate gas and sealed. All powders were stored at 28 °C for up to 102 days. Aliquots (2−5 mg) of powder were removed at regular intervals for determination of total carotenoid levels and to allow HPLC analysis of β-carotene isomers (see below). At regular intervals throughout the study, the vial contents were mixed and the headspace gas was replaced.

Solids Determination. Total solids of the algal feed and the respective powders were determined in triplicate by drying to constant weight at 80 °C.

Carotenoid Analysis. Carotenoids were extracted from 50 mL aliquots of the original algal feed or 2−5 mg of powder with 1 mL of redistilled ethanol and 4 mL of redistilled, peroxide-free, diethyl ether. The extract was filtered, evaporated to dryness in the dark under a steady stream of oxygen-free nitrogen, and stored at −20 °C.

Total carotenoid content was determined by dissolving the algal extracts in redistilled acetone and measuring the absorbance at 450 nm using a double-beam spectrophotometer (Cecil Instruments Ltd., Cambridge, U.K.) and applying published extinction coefficients (Britton, 1995). Reversed-phase HPLC analysis (see below) revealed that >98% of total carotenoid in *D. salina* was present as β-carotene. The carotenoid content

of the algal powder was expressed as a percentage (w/w) of total recovered solids.

HPLC analysis of the geometric isomers of β-carotene was carried out using a diode array detector (Hewlett-Packard 1040) linked to a computer workstation. Solvents were delivered at 1.0 mL min−1 by a tertiary pump system (CM 4000, LDC Analytical). The sample was dissolved in a small volume of hexane and 20 μL injected via a Model 7125 Rheodyne injection unit. A normal phase HPLC system using a $Ca(OH)_2$ (Nakalai Chem. Ltd.) column (0.4 × 30.0 cm; packed by Professor Y. Koyama, Kwansei Gakuin University, Nishinomiya, Japan) was used for the separation of β-carotene isomers. The column was provided with a water jacket (Alltech) maintaining column temperature at 24 °C. A solvent gradient of hexane/acetone (99.5:0.5 v/v) for 20 min followed by hexane/acetone (99.0:1.0 v/v) for 70 min was employed. All solvents were of HPLC grade and dried on molecular sieves (3 Å, Fluka) prior to use. Before injection, all algal extracts underwent a final purification step on a 5 cm alumina column (aluminum oxide 90, Brockman grade I; Merck) to completely remove glycerol and chlorophylls present in the sample. The alumina column was made immediately prior to use using HPLC grade petroleum ether (40−60 °C). Diethyl ether was used as the eluting solvent. On HPLC, β-carotene isomers were identified by their retention times and absorption spectra in comparison to those of authentic standards supplied by F. Hoffman-La Roche Ltd. (Basel, Switzerland).

Reversed-phase HPLC was performed using a Spherisorb ODS2 column (250.0 × 4.6 mm; 5 μm packing). A solvent gradient of acetonitrile/water (9:1 v/v; solvent A) and ethyl acetate (solvent B) was employed to follow the degradation of β-carotene and the possible formation of its oxidative products (e.g., β-carotene-5,6-epoxide) during the study: at $t = 0$ min, A = 100%; $t = 16$ min, A = 60%, B = 40%; $t = 25$ min, A = 0%, B = 100%; $t = 25.1$ min, A = 100%, B = 0%.

Kinetic Analysis of Stability Data. The residual β-carotene content of spray-dried powders was expressed as a percentage of the original β-carotene content in the powder after the spray-drying at $t = 0$ min. Kinetic analysis of the data was performed using Stata 3 Statistics/data analysis software (Computer Resources Center, Santa Monica, CA).

RESULTS AND DISCUSSION

Spray-Drying. Reversed-phase HPLC revealed that β-carotene accounted for >95% of total carotenoids present in the algal cell on harvesting. Upon drying, both controls (antioxidant-free cell concentrates) yielded powders with high carotenoid recovery (Table 1). This is in marked contrast with the 45% loss of β-carotene reported by Cysewski (1994) during spray-drying of the microalga *Spirulina*. However, it must be noted that the inlet and outlet temperatures were different. High pigment recovery was also obtained from feeds containing BHT or TBHQ (Table 1). In contrast, the addition of either α-tocopherol or tocopherol acetate to the algal feed resulted in dramatic losses of β-carotene during drying (Table 1). The presence of the tocopherols may have altered the characteristics of the system (e.g., viscosity of the feed and size of droplets formed) and therefore the rate of evaporation. This may explain the lowest mean moisture levels of the α-tocopherol-based powders compared to all others. However, this was not observed for the tocopherol-acetate-based powders. Alternatively, the lower recoveries but unchanged isomeric composition of β-carotene might be explained by loss of material before the atomization head and in the atomizing chamber itself. This is suggested by the lower weight of powder collected from tocopherol-based feed. Blockage of the atomization head with algal material was observed when tocopherols were added to the feed but not for the controls or when synthetic antioxidants were utilized.

The isomeric composition of β-carotene in the algal biomass was largely unaltered by processing. In all dried powders, a high recovery (90−95% of the original level) of 9-*cis*-β-carotene was obtained (Table 1). Using algal concentrates with different ratios of 9-*cis*-:*all-trans*-β-carotene resulted in similar high yields, suggesting good stability of the 9-*cis* isomer during the drying process. The compartively low losses (4−10%) suggest the degradation process is independent of the initial isomeric composition of the feed.

Except for the high concentration of tocopherols, a significant increase in the relative levels of other mono- and di-*cis*-isomers of β-carotene compared with the original feed composition was noted in all powders (accounting for up to 23% of total β-carotene). This suggests that the 9-*cis*- and *all-trans*-isomers of β-carotene may have been isomerized into other-*cis* forms or preferentially degraded during drying. The formation of *cis*-isomers during a range of heat treatments of fruits and vegetables has been reported (Panalaks and Murray, 1970; Sweeney and Marsh, 1971; Chandler and Schwartz, 1987, 1988; Pesek and Warthesen, 1988; Chen et al., 1995). An increase in the levels of *cis*-isomers (notably 13-*cis*-) was reported by Chandler and Schwartz (1988) during the processing of sweet potatoes. Similarly, an increase of *cis*-isomers of β-carotene was observed during the processing of carrot juice by Chen et al. (1995). The 13-*cis*-, 15-*cis*-, 13,15-*cis*-, and 9-*cis*-isomers were formed, although the rate of formation of the 13-*cis*- and 15-*cis*-isomers was much greater than that of the 9-*cis*- and 13,15-*cis*-isomers. In this study, the observation that 9-*cis*-β-carotene was destroyed as a result of spray-drying rather than being formed by *trans* → *cis* isomerization (as commonly seen during the processing of fruits and vegetables) may be related to the fact that the 9-*cis*-isomer was present in very high proportions in the algal feed before processing. In comparison, levels of 9-*cis*-β-carotene in unprocessed fruits or vegetables are generally very low. In cases when *cis*-isomers were not present in the unprocessed material, the formation of *cis*-isomers as a result of different processing methods was reported (Chandler and Schwartz, 1987). This may suggest that during spray-drying, isomerization of the *all-trans* (and possibly 9-*cis*) into other-*cis* forms occurred rather than a preferential degradation of the *all-trans* form per se.

In most cases, heat treatments have been reported to lead to high levels of degradation of β-carotene in fruits and vegetables or in a model system (Chandler and Schwartz, 1988; Minguez and Gandul, 1994; Chen et al., 1995). In contrast, spray-drying of *D. salina* biomass yielded powders with high recoveries of β-carotene and its isomers. Spray-drying thus appears to be suitable for the preparation of *cis*-isomer-rich β-carotene preparations. The presence of antioxidants failed to improve the recovery of total carotenoid and *cis*-isomers of β-carotene during the drying process itself.

Storage: Light plus Air. Carotenoid degradation in all powders including those with exogenous antioxidants (with the exception of the TBHQ-based powders) was extremely rapid with <10% of the original β-carotene remaining in the powder after 5 days of storage (Figure 1). Analysis by SEM of spray-dried *D. salina* cells revealed that the cell structure was damaged and the cell surface would be likely be porous (Figure 2). Such

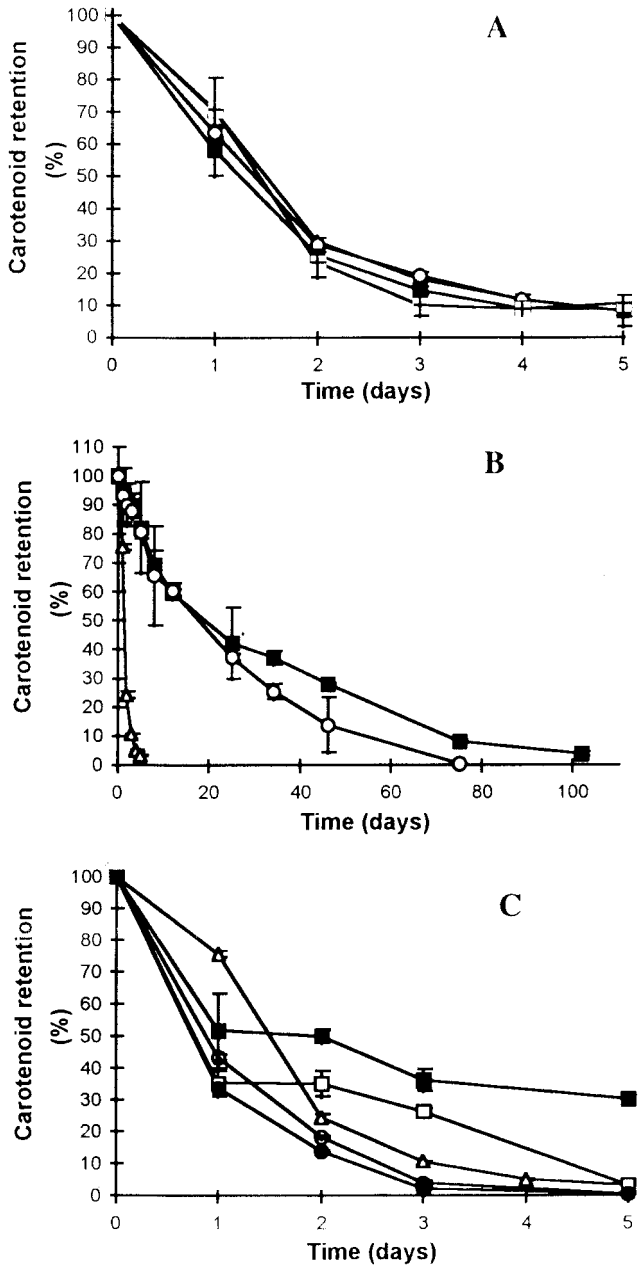

Figure 1. Carotenoid retention (percent of original) in powders of *D. salina*, stored in light plus air, with antioxidants (added to the algal biomass before spray-drying): (A) BHT concentrations were (△) control (no antioxidant), (○) 500 ppm, (■) 1000 ppm, and (+) 5000 ppm; (B) TBHQ concentrations were (△) control (no antioxidant), (○) 3000 ppm, and (■) 9000 ppm; (C) tocopherol concentrations were (△) control (no antioxidant), (○) 750 and (●) 2000 ppm of tocopherol acetate, and (□) 750 and (■) 2000 ppm of α-tocopherol ($n = 3 \pm$ SE).

damage could be due to an increase of vapor pressure inside the cell during the process, resulting in cell bursting. Consequently, β-carotene would not be protected by a continuous outer membrane but highly exposed to environmental degradative factors such as oxygen and light. As β-carotene degraded, the powder decolorized from orange to pale green, suggesting that chlorophylls were still present in the sample. Chlorophyll is a well-known photosensitizer and may be expected to accelerate the degradation of β-carotene in these powders by stimulating the formation of reactive oxygen species, especially singlet oxygen. However, comparison of the rates of degradation of β-carotene in powders maintained in the dark and the light suggest

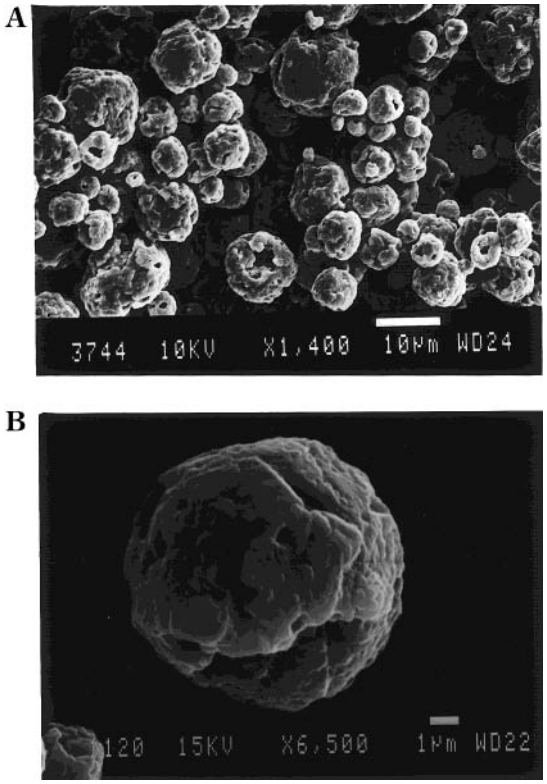

Figure 2. Scanning electron micrographs of spray-dried cells of β-carotene-rich *D. salina* (control powder with no exogenous antioxidant added): (A) magnification ×1400 (scale bar = 10 μm); (B) magnification ×6500 (scale bar = 1.0 μm). (Figure is reproduced here at 75% of the original.) The SEMs of powders prepared in the presence of antioxidants revealed no discernible difference in cell morphology from that seen in the control powders.

that this effect is negligible and the presence of chlorophyll does not accelerate the degradative process under the conditions tested in this study.

BHT had no protective effect at any concentration (Figure 1A). This is contrast with the results obtained by Goldman et al. (1983) and Berset et al. (1989), who both reported a protective effect of BHT toward *all-trans*-β-carotene in dehydrated systems at antioxidant levels of 1400 and 50 ppm, respectively. Other workers have also observed an efficient protection of BHT toward synthetic β-carotene in fats, in solution, or in solid systems (Barimalaa and Gordon, 1988; Katusin-Razem and Razem, 1994). This result suggests possible degradation of BHT during the drying process. However, using a higher temperature (170 °C) during the extrusion process, Berset et al. (1989) were able to observe a protective effect of BHT on β-carotene in the final product. It is important to note that BHT also failed to protect β-carotene from degradation following the freeze-drying of cells of *Dunaliella* (Orset and Young, unpublished data). BHT at a concentration of 10000 ppm was found to efficiently slow astaxanthin degradation in a fish feed formulation obtained by cryogrinding of *Haematococcus* cells (Spencer, 1989).

Unlike BHT, the phenolic antioxidant TBHQ showed a significant protective effect (Figure 1B). After 102 days of storage, 30−40% of carotenoids remained in the powders and there was little difference in the effectiveness of TBHQ at the two concentrations utilized. It is possible that the greater water solubility of TBHQ may be an important factor in the case of aqueous dryer feed. The solubility of TBHQ in water is ∼1% at 25 °C,

Table 2. Kinetic Parameters of Carotenoid Degradation in Powder of Spray-Dried *D. salina* Stored under Light plus Air

treatment	antioxidant level (ppm)	kinetic order	k (days^{-1})	SD	R^2
control (1)		first	0.52	0.04	0.98
BHT	500	first	0.55	0.03	0.99
BHT	1000	first	0.62	0.04	0.99
BHT	5000	first	0.80	0.11	0.97
control (2)		first	0.74	0.05	0.98
TBHQ	3000	first	0.07	0.01	0.91
TBHQ	9000	first	0.03	0.00	0.99
α-tocopherol	750	first	0.22	0.05	0.84
α-tocopherol	2000	first	0.62	0.12	0.90
tocopherol acetate	750	first	1.06	0.12	0.97
tocopherol acetate	2000	first	1.24	0.15	0.97

whereas BHT is completely insoluble between 0 and 60 °C (Kirlies and Stine, 1978; Sherwin, 1989). The effectiveness of TBHQ in oil-based systems against lipid oxidation has been noted (Warner et al., 1986; Onyeneho and Hettiarachchy, 1991; Kaitaranta, 1992; Wanasundara and Shadidi, 1994). A greater efficiency of TBHQ compared to BHT in an aqueous system has also been reported by Lee and Klein (1989).

The two natural antioxidants assessed, namely, α-tocopherol and tocopherol acetate, showed different responses (Figure 1C). α-Tocopherol had a small protective effect on β-carotene degradation, which was greater at lower concentrations of antioxidant. This is consistent with the results obtained by Barimalaa and Gordon (1988), Berset et al. (1989), and Katusin-Razem and Razem (1994), who reported a protective effect of α-tocopherol toward *all-trans*-β-carotene in a range of conditions, although greater effectiveness was noted at the highest antioxidant concentrations (140−7000 ppm; Katusin-Razem and Razem, 1994). In contrast, in the presence of tocopherol acetate, β-carotene degraded more rapidly than the control. Tocopherol acetate thus appeared to act as a pro-oxidant. However, the pro-oxidant effect might result from degradation products of tocopherol acetate formed during the process rather than from the intact molecule.

For all powders the degradation of the pigment was found to follow a first-order kinetic. A linear regression of ln[carotenoid retention (as a percentage of original levels)] against time was performed to obtain the degradation constant k, together with relevant statistical parameters (Table 2). The high value of correlation coefficient obtained confirmed the applicability of the first-order kinetic model. A first-order degradation pattern was also reported for natural carotenoids from stored, photoexposed spinach and carrot (Kopaslane and Warthesen, 1995), spray-dried encapsulated carrot carotenes (Wagner and Warthesen, 1995), and β-carotene in aqueous or solid model systems and in vegetable juice (Pesek and Warthesen, 1987, 1988). However, the rate of degradation of β-carotene was found to be highly dependent on the matrix (Pesek and Warthesen, 1987, 1988). Conversely, Goldman et al. (1983) described a three-phase sigmoidal degradation pattern for a synthetic *all-trans*-β-carotene model system (synthetic *all-trans*-β-carotene mixed with microcrystalline cellulose). This indicates a dependence of the degradation pattern on the source of β-carotene as well as the biological matrix in which they are present.

Storage: Dark plus Air. As in the powders exposed to a combination of light plus air, the degradation of β-carotene was found to follow a first-order kinetic. The degradation constants k, together with relevant statistical parameters, are presented in Table 3. Results similar to those observed earlier (see above) were obtained under dark plus air storage conditions (Figure 3). The constants of degradation of the controls and powders prepared with BHT were identical to that observed in light plus air conditions. In the presence of TBHQ, the degradation of β-carotene was found to be slower in the dark (Figure 3B). This result suggested that oxygen may be a major factor of β-carotene degradation and that oxidative degradation of the pigment could be catalyzed by light. More rapid oxidation of β-carotene in the light, compared with the dark, was also reported by Gloria et al. (1993).

Table 3. Kinetic Parameters of Carotenoid Degradation in Powder of Spray-Dried *D. salina* Stored under Dark plus Air

treatment	antioxidant level (ppm)	kinetic order	k (days^{-1})	SD	R^2
control (1)		first	0.51	0.04	0.98
BHT	500	first	0.52	0.04	0.98
BHT	1000	first	0.57	0.05	0.98
BHT	5000	first	0.57	0.08	0.94
control (2)		first	0.80	0.07	0.97
TBHQ	3000	first	0.01	0.00	0.96
TBHQ	9000	first	0.01	0.00	0.96

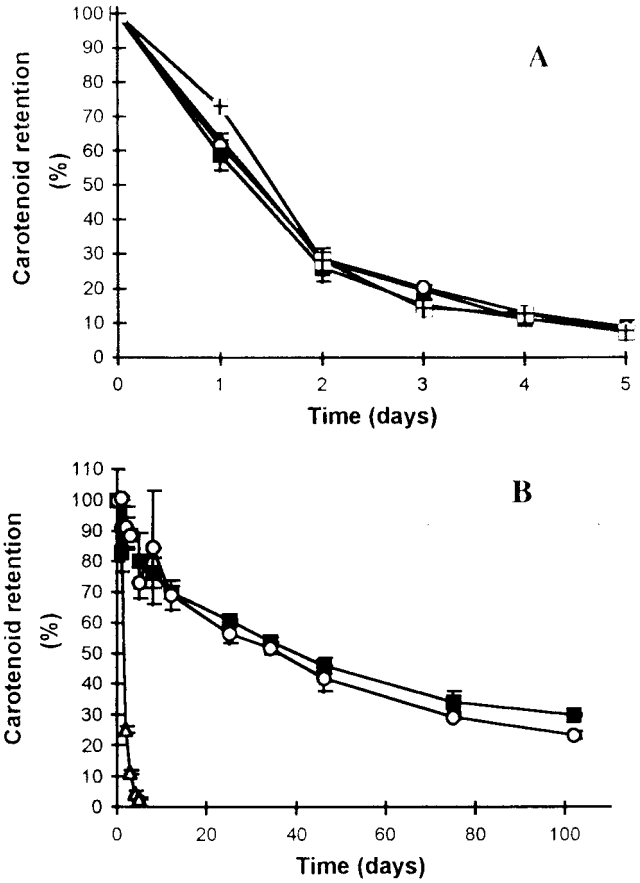

Figure 3. Carotenoid retention (percent of original) in powders of *D. salina*, stored in dark plus air, with antioxidants (added to the algal biomass before spray-drying): (A) BHT concentrations were (△) control (no antioxidant), (○) 500, (■) 1000, and (+) 5000 ppm; (B) TBHQ concentrations were (△) control (no antioxidant), (○) 3000, and (■) 9000 ppm; ($n = 3 \pm$ SE).

Storage: Dark plus Nitrogen. The degradation constants k, together with relevant statistical parameters for β-carotene degradation in dried powders under

Table 4. Kinetic Parameters of Carotenoid Degradation in Powder of Spray-Dried *D. salina* Stored under Dark plus N_2

treatment	antioxidant level (ppm)	kinetic order	k ($days^{-1}$)	SD	R^2
control (1)		first	0.32	0.27	0.97
BHT	500	first	0.51	0.05	0.98
BHT	1000	zero	16.25	1.75	0.96
BHT	5000	zero	15.46	2.54	0.90
control (2)		first	0.30	0.07	0.83
TBHQ	3000	first	0.01	0.00	0.95
TBHQ	9000	first	0.01	0.01	0.97

conditions of dark plus nitrogen, are presented in Table 4. In the control powders and in the powders supplemented with TBHQ β-carotene degradation followed a first-order kinetic. In the powders prepared with high concentrations of BHT (i.e., 1000 and 5000 ppm), the degradation of pigment was found to fit better a zero-order kinetic.

For both control powders, the rate of β-carotene degradation under dark plus N_2 was slower than that observed in dark plus air. This reduced rate of degradation of β-carotene in an inert atmosphere suggests that oxygen is a major factor controlling β-carotene degradation. A decrease in β-carotene degradation, in a simulated dehydrated food model system, was also observed by Goldman et al. (1983) when samples were stored under N_2. Although β-carotene degradation was largely slowed under an inert gas, β-carotene breakdown was still observed and could result from other pro-oxidant compounds present in the powder, combined with the high temperature (28 °C) under which the powders were stored. Despite the fact that powders were completely flushed with oxygen-free N_2, it cannot be excluded that traces of oxygen were still present in the headspace of the vials. Goldman et al. (1983) observed that the retention of β-carotene, in a dry model system, decreased with the percentage of oxygen present in the headspace. A slower degradation of powder prepared with TBHQ was also observed in dark plus N_2] (Figure 4) compared with dark plus air storage (Figure 3). After 34 days, retention of pigment reached 62 and 46% in an inert atmosphere and in air, respectively. However, after 102 days, the percentage of pigment retained in the powders was not significantly different between these two storage conditions.

The results from this study suggest that in spray-dried algal cells of *D. salina* both TBHQ and α-tocopherol demonstrated a protective effect against β-carotene oxidation. The protection afforded by TBHQ appeared to be much greater than that afforded by α-tocopherol. In contrast, BHT and tocopherol acetate, although chemically related to TBHQ and α-tocopherol, respectively, did not have any protective effect. Degradation of these two antioxidants during the spray-drying process may have occurred, although these compounds were also ineffective in protecting β-carotene from oxidation in freeze-dried powders of *D. salina* (data not shown). The levels of TBHQ used in this study were higher than that permitted for human use. At 200 ppm, the effectiveness of this antioxidant was greatly reduced but β-carotene retention was better than in the control powders (data not shown). In this case, β-carotene degradation followed a zero-order kinetic with a degradation constant of $k = 9.58 \pm 0.31$ $days^{-1}$ ($r^2 = 0.98$). This contrasts with first-order kinetics seen at the higher antioxidant concentrations. The retention of β-carotene in spray-dried *D. salina* cells was shown to be greater in the dark and could be improved by storing the powder under an inert gas atmosphere.

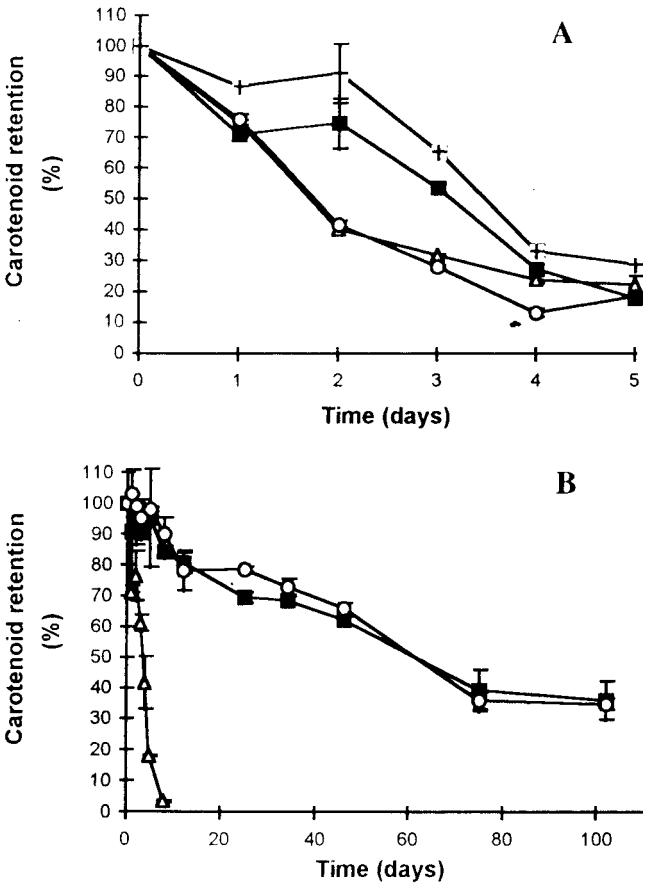

Figure 4. Carotenoid retention (percent of original) in powders of *D. salina*, stored in dark plus N_2, with antioxidants (added to the algal biomass before spray-drying): (A) BHT concentrations were (△) control (no antioxidant), (○) 500, (■) 1000, and (+) 5000 ppm; (B) TBHQ concentrations were (△) control (no antioxidant), (○) 3000, and (■) 9000 ppm ($n = 3 \pm$ SE).

Effect of Storage on the Isomeric Composition of β-Carotene. Although powder formulations and storage conditions that could delay β-carotene degradation have been established (see above), it is also important to determine whether these conditions stabilize the isomeric composition of β-carotene in dried cells of *D. salina*. After only 2 days of storage in air (in either the presence or absence of light), >90% of 9-*cis*- and >80% of other-*cis*-β-carotene isomers were degraded in the control powders. Much greater stability of the *cis*-isomers was observed in the powders stored under N_2 atmosphere in the dark, where the degradation of 9-*cis*-isomer was 15% and that of other-*cis*-isomers was 10%. However, after 5 days, under any of the storage conditions tested, the β-carotene remaining in the control powders was ~100% in the *all-trans* form. A similar trend was observed for the powders containing BHT and tocopherol acetate, although, in each of these formulations, the retention of *cis*-isomers was found to be even poorer than in the control after only 2 days. Improved protection was seen for powders containing α-tocopherol. In light plus air, >50% of the initial levels of the 9-*cis*-isomer was noted after 2 days at 2000 ppm, but this fell to only 24% at 750 ppm. As with the control powders, in all of the powders containing either BHT or tocopherol, almost no *cis*-isomers (~2%) of β-carotene could be detected following 5 days of storage.

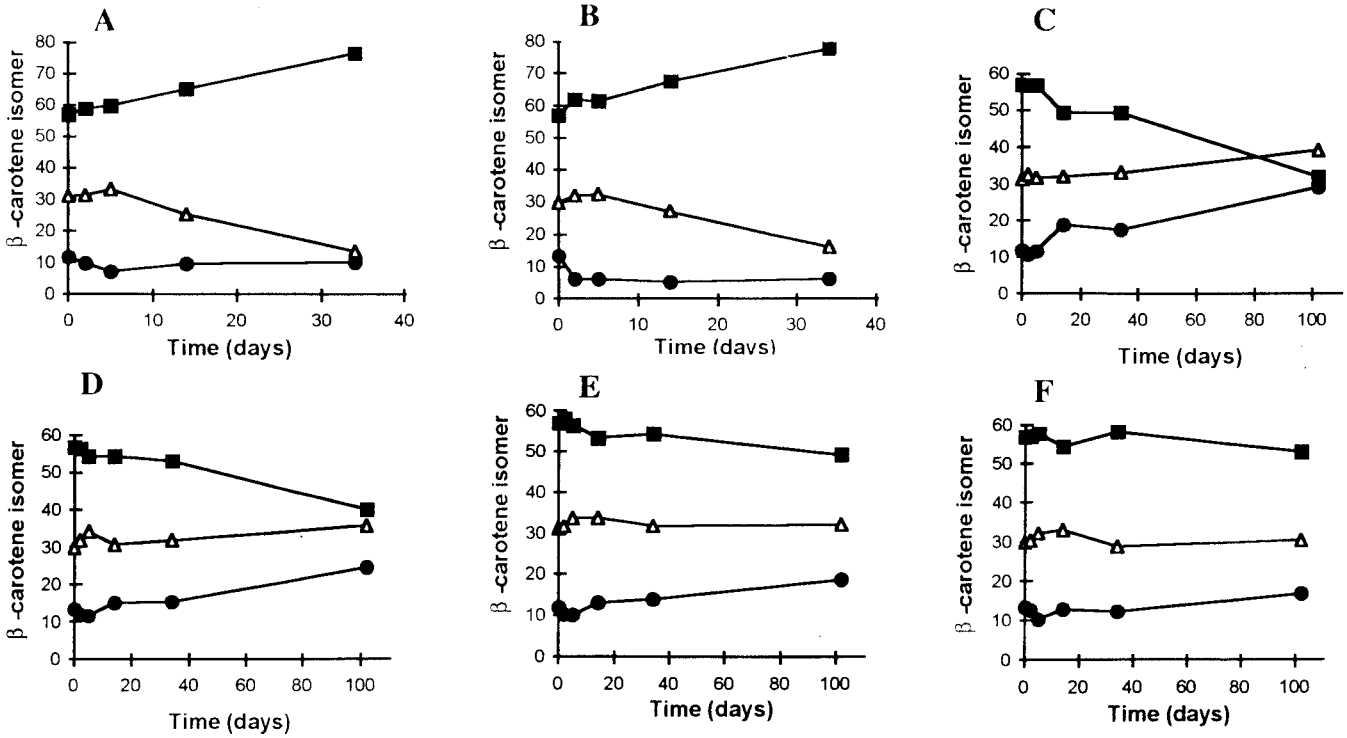

Figure 5. Evolution of the isomeric composition of *β*-carotene in powders of *D. salina*, stored under different conditions, with TBHQ (added to the algal biomass before spray-drying): (A) TBHQ 3000 ppm, light plus air; (B) TBHQ 9000 ppm, light plus air, (C) TBHQ 3000 ppm, dark plus air, (D) TBHQ 9000 ppm, dark plus air, (E) TBHQ 3000 ppm, dark plus N_2, (F) TBHQ 9000 ppm, dark plus N_2. *β*-Carotene isomers: (●) other-*cis* (all *cis*-isomers except 9- and 9,9′-*cis*); (■) *all-trans*; (△) 9-*cis*.

In contrast, TBHQ showed a much greater efficiency in preventing the isomerization of *β*-carotene. The evolution of the isomeric composition of *β*-carotene in TBHQ-based powders, under the three different storage conditions, is presented in Figure 5. Different patterns were obtained depending on the storage conditions. Under light plus air, *cis* → *trans* isomerization was observed (Figure 5A,B). The 9-*cis*-isomer was more affected than the other-*cis*-isomers, the concentration of which remained relatively constant. After 34 days of exposure, the concentration of 9-*cis*-isomer was reduced to 50% of the initial level. This result is in contrast with the *trans* → *cis* isomerization reported for *β*-carotene beadlets in dispersion exposed to light and oxygen (Pesek and Warthesen, 1988). However, the identification of *cis*-isomers was based only on the variation of the A_{340}/A_{450} ratio of the absorption spectrum of the remaining *β*-carotene. A different system, analyzed by the same authors, using cold, water-soluble *β*-carotene powder in dispersion, showed almost no variation of the A_{340}/A_{450} ratio. This result suggests that isomerization may be related to the system itself as well as the environmental conditions.

In dark plus air storage, *trans* → *cis* isomerization of *β*-carotene was observed (Figure 5C,D). This resulted in an increase of 9-*cis*- and other-*cis*-*β*-carotenes. The rate of isomerization was greater at lower concentrations of TBHQ. After 102 days, relative increases of 83 and 145% of the *cis*-isomers other than the 9-*cis* form were observed at TBHQ concentrations of 9000 and 3000 ppm, respectively. The increase in levels of 9-*cis*-isomer was lower: 19% at TBHQ concentrations of 9000 ppm and 26% at 3000 ppm. In dark plus N_2 conditions (Figure 5E,F), the overall isomeric composition of *β*-carotene was found to be relatively stable, even after 102 days of exposure when 60% of total *β*-carotene had degraded (see Figure 3). *β*-Carotene degradation was also studied by reversed-phase HPLC to check for the possible formation of epoxycarotenes during processing and/or subsequent storage. Epoxycarotenes such as *β*-carotene-5,6-epoxide and *β*-carotene-5,8-epoxide are not present in intact cells of *D. salina* but are common products resulting from the oxidation of carotenoids (Liebler and Kennedy, 1992). Neither of these epoxycarotenes was detected in the dried algal biomass, even when significant loss of *β*-carotene had occurred.

Under all three storage conditions, the relative level of α-carotene (a relatively minor component of *D. salina*) was found to increase with time (when calculated as a percentage of total recovered carotene). After 34 days of storage, the ratio of α-carotene:*β*-carotene increased by ∼15 and ∼34% at TBHQ concentrations of 3000 and 9000 ppm, respectively. α-Carotene thus appears to be more stable than *β*-carotene, possibly due to its chemistry (with one less conjugated double bond than *β*-carotene, it will act as a less effective antioxidant) or its cellular location/matrix.

Selected antioxidants, namely, TBHQ and α-tocopherol, were able to delay the degradation of *β*-carotene in spray-dried cells of *D. salina*. These were also found to delay the *cis* → *trans* isomerization when the powder was stored in light plus air. In contrast, antioxidants that had no effect on preventing the oxidation of *β*-carotene oxidation did not appear to slow the isomerization of *β*-carotene compared with the control powder.

The isomerization process was found to be dependent on the storage conditions when TBHQ was present in the powder. Whereas under light plus air, *cis* → *trans* isomerization was noted, under dark plus air, a reverse *trans* → *cis* isomerization process was observed. The rate of isomerization was found to be dependent on TBHQ concentration. A similar *trans* → *cis* isomerization pattern was observed under dark plus N_2, but the overall process appeared to be much slower. Recorded

isomerization patterns for direct photoexcitation and triplet-sensitized isomerization of β-carotene in solution are essentially the same (Kuki, 1994). Both *trans* → *cis* and 9-*cis* → *trans* isomerizations occur, with *cis* → *cis* being less favored. The isomerization of *all-trans*-β-carotene appears to favor the formation of central *cis*-isomers such as 13- and 15-*cis*-β-carotene as well as the peripheral *cis*-isomers, such as 9-*cis*. In contrast, thermal isomerization of 9-*cis*-β-carotene resulted in the formation of selected di-*cis*-isomers (e.g., 9,13′-, 9,15-, and 9,13-di-*cis*-β-carotene) rather than the *all-trans* form. Thermal isomerization of *all-trans*-β-carotene favored the formation of 13-*cis*-β-carotene as well as resulting in the formation of the 15-*cis* and 9-*cis* forms (Kuki, 1994). In this study, no significant increase in the levels of any individual *cis*-isomer was observed, although a general rise in the relative levels of mono- and di-*cis*-isomers other than the 9-*cis* form was observed in the dried powders, and as a result no specific pattern of isomerization could be discerned.

At the concentrations used in this study, TBHQ was the most efficient antioxidant to delay oxidation of β-carotene in cells of *D. salina*, as well as maintain its isomeric composition. The protection of β-carotene was greatly improved when the powders were stored under dark plus N_2. In comparison to freeze-drying (Orset and Young, unpublished data), spray-drying was a more productive and stable process in terms of preserving the algal carotenoid content and composition. β-Carotene from freeze-dried cells of *D. salina* was generally highly unstable and degraded rapidly. This was coupled with a high degree of batch-to-batch variation in carotenoid recovery. As with the spray-dried powders, TBHQ and α-tocopherol were efficient in minimizing the degradation of β-carotene. As an alternative, microencapsulation of β-carotene-rich cells of *D. salina* has proved to be an effective means of protecting β-carotene and its isomeric composition. Leach et al. (1998a,b) produced a stable formulation using maltodextrin/gum arabic (in a ratio of polymer/algal biomass of 3.5:1). The resulting powder maintained a stable isomeric composition for about one year (stored in the dark under an atmosphere of N_2); however, the high glycerol content of *Dunaliella* appears to be a factor that influences the efficacy of this process.

ABBREVIATIONS USED

BHT, butylated hydroxytoluene; TBHQ, *tert*-butylhydroquinone; HPLC high-performance liquid chromatography; SEM, scanning electron microscope; SD, standard deviation.

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