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Stability Studies on Astaxanthin Extracted from Fermented Shrimp Byproducts

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To the best of our knowledge, stability studies on astaxanthin contained in carotenoproteins extracted from lactic acid fermented shrimp byproduct have never been reported. Carotenoprotein powder, containing 1% free astaxanthin, was subjected to oxidation factors of illumination, oxygen availability, and temperature, using synthetic astaxanthin as a control. The individual effects as well as first and second degree interactions were studied on natural and synthetic free astaxanthin stability. Air and full light were the two individual factors with the highest effects on astaxanthin oxidation. Sixty-two and 46% natural and synthetic astaxanthin, respectively, oxidized when exposed to air for 8 weeks of storage, whereas 35 and 28% of natural and synthetic astaxanthin, respectively, oxidized under full light. Ninety-seven and 88% of natural and synthetic astaxanthin, respectively, oxidized under a combination of full light, air, and 45 °C at 8 weeks of storage. Storage in the dark, nonoxygen, and 25 °C were the treatments that efficiently minimized astaxanthin oxidation. Natural astaxanthin from fermented shrimp byproduct presented moderate stability levels. Although natural astaxanthin oxidized faster than the synthetic pigment, its stability may improve by antioxidant and polymer addition.

KEYWORDS: Astaxanthin; oxidation; carotenoproteins; stability; carotenoids; shrimp byproduct

INTRODUCTION

The most abundant carotenoid in crustaceans is astaxanthin (**Figure 1**), present as diester, monoester, and free forms (*1*). This red-orange carotenoid has been referred to as a powerful biological antioxidant with beneficial health effects in both experimental animal and clinical studies; it has promising potential for human health and nutritional applications (*2*). Its expected health benefits include treatment of cancer, diabetes, ocular deterioration, enhancement of the immune system, and anti-inflammation, among others (*2*). Moreover, astaxanthin can be used as a colorant additive in foods and in medicines due to its antioxidant capacity (*3*). However, aquaculture is the most promising application, as it provides the red-orange color in salmon muscles (*4*).

Astaxanthin is probably bound to lipids and chitin through ester and imine bonds, respectively. Furthermore, it can also be attached to proteins through imine bonds (*5*). Molecules formed by the association of carotenoids with a protein are known as carotenoproteins (*6*); these complexes show a wide range of colors, from blue and green in live crustaceans such as shrimp, crayfish, and lobster, to red-orange occurring when the protein moiety is either denatured or partially separated from the carotenoid moiety (*5*−*7*). The development of a red-orange color is observed when cooking crustaceans (*8*). Hence, astaxanthin can be obtained from crustacean byproducts (*3*) such as shrimp byproducts (cephalothorax and exoskeleton), representing approximately 45% (nonedible portion) of the shrimp total weight, which represents a pollution problem (*9*).

However, methods to recover astaxanthin from shrimp waste must consider that this carotenoid is prone to oxidation (*9*). Methods such as lactic acid fermentation of shrimp byproducts can stabilize astaxanthin prior to extraction with organic solvents, thereby increasing extraction yields of this pigment (*9*). Previous studies reported the production of carotenoprotein powder, rich in astaxanthin stabilized through lactic acid fermentation of shrimp byproducts (*5*, *9*). To minimize astaxanthin chemical oxidation, it is important to consider that oxidation reactions of carotenoids are accelerated by heat (high temperatures) and the presence of oxygen and light (*10*).

The chemical stability of astaxanthin contained in shrimp has been studied. However, these studies have been limited to studying astaxanthin oxidation when processing (i.e., drying, packing, and storing) commercial shrimp products (*11*, *12*) and fish such as rainbow trout (*13*, *14*) and Atlantic salmon (*15*). Also, procedures to extract pigments from crawfish waste have been reported to promote astaxanthin oxidation mainly due to the combined effects of high temperatures and oxygen (*16*). To the best of our knowledge, to date, this is the first time a study assesses the effect of oxidation factors on the stability of free astaxanthin contained in carotenoprotein powder extracted from shrimp byproducts. Consequently, results from this work could have a significant impact for optimizing storage conditions of a natural source of astaxanthin (crustacean carotenoproteins), with a potential to be used as a food ingredient and/or a natural health product (*5*).

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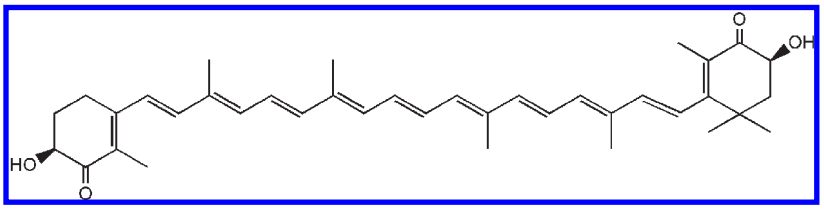

Figure 1. Chemical structure of astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione).

Table 1. Individual Effect of Oxidative Factors (Block Experimental Design) on Astaxanthin Concentration (mg g^{-1})[a]

	block I: illumination[b]						block II: temperature[c]						block III: oxygen availability[d]					
	natural astaxanthin			synthetic astaxanthin			natural astaxanthin			synthetic astaxanthin			natural astaxanthin			synthetic astaxanthin		
time (weeks)	darkness	reduced light	full light	darkness	reduced light	full light	5 °C	25 °C	45 °C	5 °C	25 °C	45 °C	air	reduced oxygen	nonoxygen	air	reduced oxygen	nonoxygen
0	986	950	973	975	1002	992	1005	1000	954	993	986	1005	956	948	1001	967	970	985
1	985	922	958	972	951	988	1002	992	950	990	982	999	912	930	998	952	938	983
2	982	900	939	973	925	975	1000	992	946	992	980	996	815	921	999	946	936	984
3	983	885	881	971	893	955	1001	990	940	987	981	993	749	898	996	900	932	981
4	980	852	844	972	880	921	998	991	937	988	977	995	653	778	995	773	893	983
5	981	836	804	969	867	893	1000	988	934	987	978	990	559	757	997	676	877	979
6	978	801	733	967	850	811	996	990	931	985	975	988	488	645	994	591	862	980
7	978	774	692	968	841	754	997	986	928	982	976	987	405	617	992	551	851	978
8	975	731	636	966	832	714	995	987	926	982	974	983	362	599	990	520	743	976
astaxanthin oxidation (%)[e]	1	23	35	1	17	28	1	1	3	1	1	2	62	37	1	46	23	1

[a] Data are expressed as means of three replicates. [b] Constants: temperature, 25 °C; and oxygen availability, nonoxygen. [c] Constants: illumination, darkness; and oxygen availability, nonoxygen. [d] Constants: illumination, darkness; and temperature, 25 °C. [e] After 8 weeks of storage.

The objective of this study was to study the effect of oxidation factors, such as temperature, oxygen, and light, on the stability of free astaxanthin contained in carotenoproteins extracted from previously stabilized lactic acid-fermented shrimp wastes. Furthermore, stability results were compared to those obtained with a commercial synthetic astaxanthin product.

MATERIALS AND METHODS

Materials. Carotenoprotein powder was obtained from lactic acid-fermented shrimp wastes (*Litopenaeus vannamei*) following the procedure described by Armenta and Guerrero-Legarreta (*5*). It contained approximately 1% free astaxanthin. Carophyll pink (DSM, Heerlen, The Netherlands) was used as a source of synthetic astaxanthin, containing approximately 8% free carotenoids. All analytical organic solvents and chemicals used were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

Stability of Astaxanthin. Astaxanthin, from lactic acid-fermented shrimp byproduct (natural) and from Carophyll pink (synthetic), was subjected to three factors and three levels of oxidation: illumination (darkness, reduced light, and full light), oxygen availability (air, reduced oxygen, and nonoxygen), and temperature (5, 25, and 45 °C). The effect of these factors was studied through an experimental design without interactions (block design). Additionally, the interaction of each factor was assessed through an experimental design where all factors (with two levels) and interactions were considered through a complete factorial design. The two factor levels were full light and darkness for illumination, air and nonoxygen for oxygen availability, and 25 and 45 °C for temperature. In both cases (block and full-factorial designs), the response variable was astaxanthin concentration measured every 1 (block design) and 2 weeks (full factorial design) throughout 8 weeks of storage.

Experimental Units. These were prepared by placing 100 mg of lyophilized carotenoprotein containing 1% free natural astaxanthin (1 mg), obtained from fermented shrimp waste, in 4.5 mL transparent glass vials sealed with rubber caps. The experimental units with synthetic astaxanthin were prepared by placing 12.5 mg of Carophyll pink that contained 8% free astaxanthin; thus, there was 1 mg of astaxanthin in each 4.5 mL glass vial. Oxygen removal was performed by purging the rubber-capped vials with nitrogen gas, and then, the oxygen content was measured by inserting a needle oxygen sensor (zirconium detector) connected to an oxygen headspace analyzer (PBI Checkmate, Totac Inc., Higham, MA). For nonoxygen and reduced oxygen samples, the oxygen contents within the glass vials were < 0.05 and 7−8%, respectively. Other samples were exposed to atmospheric air, which contained 20.9% oxygen. Regarding illumination, darkness was achieved by using amber glass vials covered with aluminum foil (0 lx). Reduced light samples were placed in a space exposed to an illumination of approximately 150 lx. Samples exposed to full light were exposed to light with an intensity of approximately 600 lx. Illumination levels were achieved using a 50 W artificial fluorescent lighting at a distance (from the samples) of approximately 3.5 and 1.5 m for 150 and 600 lx, respectively. Illumination levels were measured by using a Foot Candle/Lux Meter (Extech Instruments Corp., Waltham, MA), which measures light levels in lux units (1 lm of light m^{-2}).

Experimental Designs. For the design without interactions, the effects of illumination, temperature, and oxygen availability were studied through three independent blocks (**Table 1**). Data were subjected to an analysis of variance (ANOVA) for each variable condition, with the length of the study (time) and the pigment source (natural or synthetic) as variation sources with a first-order effect.

For the design with interactions, a complete factorial experimental design was applied, as shown in **Table 2**. All experiments were conducted in triplicate. Data were analyzed by ANOVA and Duncan's multiple range test at $p < 0.05$ (SPSS 13.0, SPSS Inc., Chicago, IL).

Analysis of Astaxanthin. Astaxanthin was quantified chromatographically by high-performance liquid chromatography (HPLC) at a wavelength of 474 nm (*5*). Whole samples from the experimental units (originally containing 1 mg of astaxanthin) were solubilized in 25 mL of distilled water. One milliliter of the solution was diluted 1:1 (v/v) with acetone, obtaining 0.02 mg mL^{-1} final pigment concentration. The HPLC system (Waters, Milford, MA) was equipped with a Waters Symmetry^{MR} C_{18} reverse-phase column and a precolumn. Samples were filtered through a 0.45 μm membrane (Gelman Acrodisc GHP No. 13, Krackeler Scientific Inc., Albany, NY) and analyzed by HPLC according to the procedure reported by Guillou et al. (*17*), using a mobile phase of acetonitrile/chloroform/methanol/water/propionic acid (71:22:4:2:1 by volume) under

Table 2. Interaction Effect of Oxidative Factors (Full-Factorial Experimental Design) on Astaxanthin Concentration (mg g^{-1})[a]

	astaxanthin															
	natural								synthetic							
	full light				darkness				full light				darkness			
	air		nonoxygen		air		nonoxygen		air		nonoxygen		air		nonoxygen	
time (weeks)	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C	25 °C	45 °C
0	981	968	975	974	968	971	963	983	991	995	996	976	970	974	990	991
2	705	657	914	855	803	728	955	976	754	701	963	831	879	865	985	985
4	555	353	889	632	606	530	952	979	613	583	807	794	703	649	987	981
6	347	110	755	510	423	262	948	964	561	305	775	678	606	440	980	977
8	159	31	634	455	366	144	950	952	267	118	717	545	520	305	981	975
astaxanthin oxidation (%)[b]	84	97	35	53	62	85	1	3	73	88	28	44	46	69	1	2

[a] Data are expressed as means of three replicates. [b] After 8 weeks of storage.

isocratic conditions with a flow of 1 mL min^{-1}. The mobile phase was previously filtered through a Millipore[MR] 0.45 μm membrane (resistant to organic solvents) and later degassed (with nitrogen) before and during its use in the HPLC. The HPLC was also equipped with a 7125 Rheodyne injector with a loop of 50 μL, pump system Waters 626, Waters control unit 600S (Controller Millipore[MR]), and a programmable diode array detector (Waters 994). We built a calibration curve using an astaxanthin standard of 97% purity (CaroteNature, Lupsingen, Switzerland) from 0 to 200 mg L^{-1} at intervals of 25 mg mL^{-1} (a nine-point calibration curve).

RESULTS AND DISCUSSION

The nine-point calibration curve with astaxanthin standard had an $r^2 = 0.99$. A retention time = 5.063 min was observed for the astaxanthin standard (*trans*-astaxanthin) and free astaxanthin contained in carotenoprotein and Carophyll pink powders. The sum of peak areas of *trans*-astaxanthin, 9-*cis*-astaxanthin (6.66 min), and 13-*cis*-astaxanthin (7.1 min) was considered as the total area for the calculation of free astaxanthin concentration in both carotenoprotein and Carophyll pink (**Figure 2**). Both natural and synthetic astaxanthin had a relatively similar *cis* and *trans* isomer ratio (5:95 and 7:93 of *cis*:*trans* for natural and synthetic, respectively). The natural (from lactic-fermented shrimp carotenoproteins) had 5:95 *cis* and *trans* isomer ratio. The chromatographic behavior of the free astaxanthin isomers was similar to that reported by Lin et al. (*18*). It has been reported that the use of certain organic solvents may promote the conversion of *trans*-astaxanthin into *cis*-astaxanthin (*19*). Nevertheless, petroleum ether and acetone had a low isomerization effect when compared with other organic solvents such as dichloromethane, chloroform, methanol, and acetonitrile (*19*). This relates to the organic solvents system used to extract carotenoproteins from lactic acid-fermented shrimp byproducts, which was composed of petroleum ether:acetone:water (15:75:10, by volume) (*9*).

The present study was based on the effect of three factors (illumination, temperature, and oxygen availability) on the oxidation of natural and free astaxanthin (from carotenoprotein and Carophyll pink, respectively). Carotenoproteins extracted from lactic acid-fermented shrimp byproducts contain diester, monoesters, and free forms of astaxanthin. However, only the amount of free astaxanthin was considered as this nonesterified astaxanthin is more prone to oxidation than esterified forms of the pigment (*20*). Hence, oxidation levels of natural free astaxanthin can be compared to those obtained when using synthetic free astaxanthin contained in Carophyll pink.

Individual Effect of Oxidative Factors (Block Design). Table 1 shows the results of astaxanthin stability from the experimental design without interactions (block design). In general, the astaxanthin concentration decreased throughout 8 weeks.

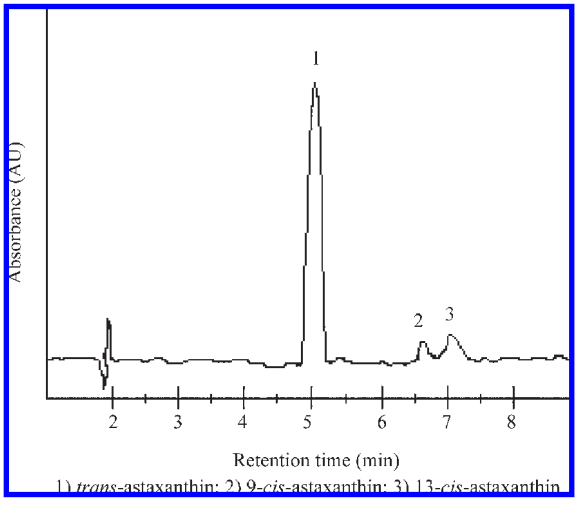

Figure 2. Typical chromatogram of free astaxanthin from natural (carotenoprotein) and synthetic sources.

However, the magnitude of the reduction was different for each of the factors studied.

The absence of light (darkness) is important to preserve the chemical stability of astaxanthin as this pigment is sensible to light (*14*). When samples were stored under darkness, after 8 weeks of storage, the concentration of astaxanthin diminished 1% for both the natural and the synthetic pigment (with constants of 25 °C and nonoxygen; **Table 1**). Samples exposed to reduced light had higher astaxanthin degradation, 23 and 17% for natural and synthetic astaxanthin, respectively, after 8 weeks of storage. When exposed to reduced light, the difference between the two types of pigments (natural and synthetic) was not significant ($p < 0.05$) until the fourth week of storage. Within the block of illumination, most astaxanthin was lost in samples exposed to full light; after 8 weeks of storage, 35 and 28% of natural and synthetic astaxanthin oxidized as compared to the starting pigment concentration.

Within the block of oxygen availability, after 8 weeks of storage, approximately 62 and 46% of the natural and synthetic astaxanthin, respectively, was oxidized when samples were exposed to air (**Table 1**). Meanwhile, natural and synthetic astaxanthin degraded by approximately 37 and 23%, respectively, in reduced oxygen samples. Natural and synthetic astaxanthin concentrations showed a significant difference ($p < 0.05$) starting at the second and fourth week of storage for both air and reduced oxygen oxidative factors. Nonoxygen samples had the least astaxanthin degradation, as only 1% of pigments oxidized after 8 weeks of storage (**Table 1**). The importance of oxygen

removal for reducing astaxanthin degradation in the storage of dried shrimp has been reported by Niamnuy et al. (*12*), finding that astaxanthin oxidized the least when shrimp was stored under vacuum regardless of the storage temperature (4−25 °C).

This study showed that air affected astaxanthin stability more than full light. This differed from a previous finding where light produced more oxidized astaxanthin contained in steaks of rainbow trout (*14*). This difference may be attributed to astaxanthin being bounded to a matrix of proteins and lipids in the muscle of rainbow trout that is different than carotenoproteins recovered from shrimp byproducts. Also, the cited study (*14*) used ultraviolet light instead of visible light used in our study. Ultraviolet light, due to its short wavelength, is a radiation form with high energy. Thus, its effect on astaxanthin oxidation would be greater when compared to a form of radiation with lower energy such as visible light. Additionally, the present stability study was based only on the oxidation of free astaxanthin; monoester and diester forms of the pigment were not considered.

Regarding the temperature effect, at darkness and nonoxygen constants (**Table 1**), 5 and 25 °C presented the least oxidation effect (1%) on both natural and synthetic astaxanthin (**Table 1**). Although the difference was not significant ($p < 0.05$), natural astaxanthin had a higher oxidation (3%) than the synthetic version (2%) after 8 weeks of storage at 45 °C. Similar levels of astaxanthin oxidation were obtained at different temperatures (5, 25, and 45 °C), which may be due to the removal of oxygen (nonoxygen) and storage at darkness (**Table 1**). This result strongly suggests that high temperatures do not affect astaxanthin stability as long as oxygen and light are avoided. Yamauchi et al. (*21*) indicated that carotenoids have low levels of oxidation at relatively high temperatures when contact with oxygen and light is minimized. Niamnuy et al. (*12*) also found that when drying shrimp at high temperatures (100 °C), astaxanthin had less oxidation than when drying at lower temperatures (80 and 100 °C). These results may have been related to the shorter time required to dry shrimp at high temperatures, thus reducing the period time in which astaxanthin is exposed to oxygen.

The high oxidative individual effect of air and full light on astaxanthin was also assessed visually when preparing the correspondent solutions to be analyzed for HPLC. These highly oxidized samples presented a yellow color as compared to red-orange solutions prepared with samples with low levels of astaxanthin oxidation. Haila (*22*) mentioned that the loss of color is the most common and easy way to observe oxidation of carotenoids. An unoxidized astaxanthin solution has a red-orange color, and it changes gradually toward yellow hues depending on the oxidation levels.

In general, synthetic astaxanthin presented better stability levels than natural astaxanthin when exposed to highly oxidized factors such as air and full light. This difference was due to synthetic astaxanthin being relatively protected from oxidizing factors such as illumination, temperature, and oxygen availability as the carotenoid is encapsulated, usually in a gum or a polymer, which represents 92% of the product Carophyll pink. On the other hand, the natural astaxanthin used for this study (contained within a carotenoprotein) was not an encapsulated product; thus, it was more prone to oxidation. Nevertheless, it may be possible to improve the stability of the natural astaxanthin by encapsulating this carotenoid with the same materials as the synthetic version is prepared. The addition of antioxidants may be also considered to improve the stability of the natural astaxanthin (Carophyll pink contains 0.5−1.5% of ascorbyl palmitate). The specific factors that affected astaxanthin stability the most were air and full-light.

Finally, the specific factors under which astaxanthin had the least oxidation (under the block experimental design) were when storing at either 5 or 25 °C under the constants of darkness and nonoxygen atmosphere. Nevertheless, storing at 45 °C also showed low levels of oxidation (**Table 1**).

Interaction Effect of Oxidative Factors (Full-Factorial Design). Astaxanthin oxidation in samples exposed to full light, air, and 25 °C lost approximately 84 and 73% of natural and synthetic astaxanthin, respectively, at 8 weeks of storage (**Table 2**). Oxidation levels between natural and synthetic pigment started to be significantly different ($p < 0.05$) at the second week of storage. Relative to the initial concentration of astaxanthin (zero weeks), samples exposed to the same conditions cited above (full light and 25 °C), but with nonoxygen, lost approximately 35 and 28% of natural and synthetic astaxanthin, respectively, at the eighth week of storage. Natural and synthetic carotenoid oxidation levels started to be significantly different ($p < 0.05$) at the second week of storage (**Table 2**).

Samples stored under full light, air, and 45 °C presented the highest oxidation levels in both sources of astaxanthin (natural and synthetic). Approximately 97 and 88% of natural and synthetic astaxanthin, respectively, oxidized at 8 weeks of storage (**Table 2**). Oxidation levels of natural and synthetic astaxanthin started to be significantly different ($p < 0.05$) at the second week of storage. The second most oxidative treatment was the combination of full light, air, and 25 °C, oxidizing 84 and 73% of natural and synthetic astaxanthin, respectively (**Table 2**).

Thirty-five and 28% of natural and synthetic astaxanthin, respectively, oxidized in samples stored for 8 weeks under full light, nonoxygen, and 25 °C (**Table 2**). These were significantly different ($p < 0.05$) when compared with oxidation levels observed under the same conditions of light and oxygen availability but at 45 instead of 25 °C (53 and 44% oxidation for natural and synthetic astaxanthin, respectively). This indicated that a high temperature (45 °C) promoted astaxanthin oxidation when samples were exposed to full light. In the case of samples stored under darkness, air, and 45 °C, natural astaxanthin oxidized by 85% at the eighth week of storage. Meanwhile, synthetic astaxanthin oxidized by 69%. Under the latter conditions, astaxanthin concentrations between the synthetic and the natural pigments started to be significantly different ($p < 0.05$) at the second week of storage. At the same conditions (darkness and air) but at 25 °C, natural and synthetic astaxanthin oxidized 62 and 46%, respectively, starting to show a significant difference ($p < 0.05$) in astaxanthin concentration between the pigment source (natural and synthetic) at the second week of storage (**Table 2**).

A minimal astaxanthin oxidation (1%) was observed when samples were stored under darkness, nonoxygen, and 25 °C. The absence of light and oxygen (darkness and nonoxygen, respectively) were the best conditions to preserve the chemical stability of both natural and synthetic astaxanthin (**Figure 3**). Although astaxanthin oxidation after 8 weeks of storage was slightly higher at 45 °C than at 25 °C, the difference was not significant ($p < 0.05$; **Table 2**). This indicates that astaxanthin can be stable at a relatively high temperature as long as oxygen and light are avoided during sample storage. It has been reported that when storing a shrimp product under a vacuumed atmosphere at low temperatures, astaxanthin can efficiently be protected from oxidation (*12*).

The combination of air, full light, and a high temperature (45 °C) were the variable factor levels that affected astaxanthin stability the most and thereby increased astaxanthin oxidation (**Figure 3**). The combination of darkness, air, and 25 °C was more oxidative ($p < 0.05$) than a treatment that combined full light,

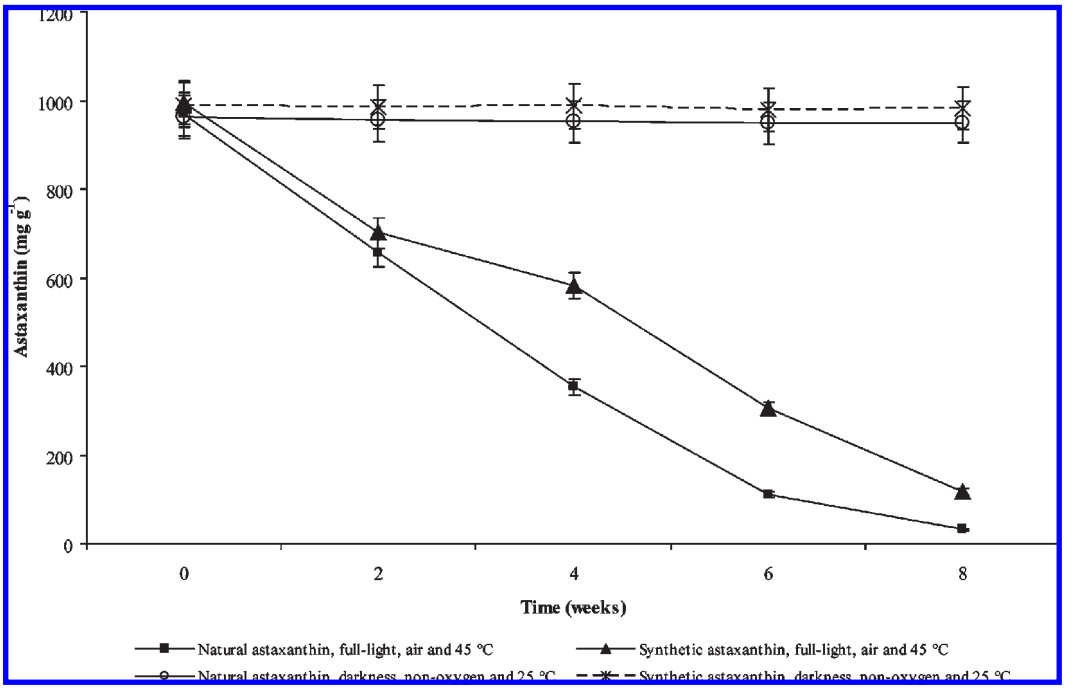

Figure 3. Selected treatments (with interaction of factors) with the greater and the lesser effects on astaxanthin oxidation.

nonoxygen, and 45 °C (**Table 2**); this is possibly due to the absence of oxygen in the latter treatment. A carotenoid oxidation mechanism is similar to the autoxidation of unsaturated fatty acids, it is accelerated by heat, and the reaction is catalyzed by the presence of oxygen and light (*10*). This means that heat affects carotenoid stability; however, its oxidative effect is triggered and accelerated when carotenoids are exposed to oxygen and full light. Frankel (*23*) considered oxygen as the factor with a major effect on carotenoid oxidation, adding that temperature affects the stability and the carotenoid oxidation rate when in combination with oxidative factors such as oxygen and light. Nonetheless, in the absence of air (nonoxygen), carotenoids can handle the effect of relatively high temperatures.

We assessed the effect of oxidative factors on the stability of natural astaxanthin recovered from lactic acid-fermented shrimp byproducts. Furthermore, we compared these stability results with those observed in a synthetic pigment. Synthetic astaxanthin was more stable than natural astaxanthin. As mentioned earlier, this was due to the lack of a carrier or excipient in the source of pigment that can act as a microcapsule and thereby reduce astaxanthin's exposure to oxidation factors. In Carophyll pink, astaxanthin has the advantage to be microencapsulated probably with gums or polymers. Yet, natural astaxanthin had a relatively similar oxidation pattern to the synthetic astaxanthin, at least during the first stages of exposure to the variable factors studied (illumination, temperature, and oxygen availability); this possibly as a natural astaxanthin was relatively protected by the protein fraction of the carotenoprotein complex.

This protective effect diminished relative to the storage time, especially in those treatments with exposure to air and 45 °C. The protective effect of the protein fraction on astaxanthin stability diminishes as proteins tend to denaturalize at high temperatures, increasing the carotenoids proneness to oxidation when they come in contact with oxygen and light (*21*, *23*).

The combination of full light, a relatively high temperature (45 °C), and oxygen (air) caused the most oxidation of astaxanthin (**Figure 3**). The conditions that protected the most astaxanthin from oxidation were absence of both light and oxygen (darkness and nonoxygen conditions, respectively) at either low or relatively high temperatures (5−45 °C) (**Figure 3**). Although natural astaxanthin from carotenoprotein presented relatively good chemical stability, more studies would be required to improve its stability. This could be achieved by microencapsulating the carotenoprotein with polymers and considering the addition of antioxidants. Significant results from this research could be used as a reference to determine optimal conditions to maximize the shelf life of a natural source of astaxanthin such as crustacean carotenoproteins. Consequently, an unoxidized natural astaxanthin would have a more efficient use in applications such as in nutraceuticals, cosmetics, and in salmon cultures.

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