Characterization and storage stability of astaxanthin esters, fatty acid profile and α-tocopherol of lipid extract from shrimp (*L. vannamei*) waste with potential applications as food ingredient

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ABSTRACT

In this work a lipid extract from shrimp waste was obtained and characterized. The most abundant fatty acids found were C16:0, C18:2n6c, C18:1n9c, C22:6n3, and C20:5n3. The extract contained all-*trans*-astaxanthin, two *cis*-astaxanthin isomers, 5 astaxanthin monoesters, and 10 astaxanthin diesters (7 ± 1 mg astaxanthin/g). C22:6n3and C20:5n3 were the most frequent fatty acids in the esterified forms. Appreciable amounts of α-tocopherol and cholesterol were also found (126 ± 11 mg/g and 65 ± 1 mg/g, respectively). Little lipid oxidation was observed after 120 days of storage at room temperature, revealed by a slight reduction of *ω*-3 fatty acids, but neither accumulation of TBARS nor formation of oxidized cholesterol forms was found. This is attributed to the antioxidant effect of astaxanthin and α-tocopherol, as their concentrations decreased as storage continued. The lipid extract obtained has interesting applications as food ingredient, owing to the coloring capacity and the presence of healthy components.

Keywords

Polyunsaturated fatty acids; α-tocopherol; astaxanthin; oxidation; food colorant; DHA; EPA; shrimp

1. INTRODUCTION

Large amounts of cephalothorax and cuticle waste, which may account for up to 50–60% of animal weight, are produced annually by the crustacean processing industry (Sachindra et al. 2005; Treyvaud Amiguet et al. 2012). This waste has the potential to be valorized to obtain food ingredients and additives such as chitin/chitosan, proteins, lipids, or carotenoids (Arancibia et al. 2014; Treyvaud Amiguet et al. 2012; Takeungwongtrakul et al. 2015; Takeungwongtrakul et al. 2012). Recent works of our research group have shown that the lipid extract obtained from shrimp cephalothoraxes and cuticles waste is a promising food ingredient with multiple technological applications (Montero et al. 2016; Gomez-Estaca et al. 2016; Gómez-Estaca et al. 2015). This is mainly because of its coloring capacity, provided by astaxanthin, which is improved when encapsulated by spray-drying or complex coacervation or incorporated into edible films. Moreover, astaxanthin is not the main component of the extract, as it is reported to range between 2 and 5 mg/g lipid extract (Takeungwongtrakul et al. 2015; Gómez-Estaca et al. 2016). Lipid extracts of similar nature have been reported to be rich in polyunsaturated fatty acids (Takeungwongtrakul et al. 2012; Chen et al. 2007), and also to contain tocopherols (Özogul et al. 2011) and cholesterol (Hernández-Becerra et al. 2014). Astaxanthin, polyunsaturated fatty acids (specially eicosapentaenoic (EPA) and docosahexaenoic acids (DHA)) and tocopherol may have health-promoting effects when consumed, mainly related to their antioxidant and/or anti-inflammatory activities. Inflammatory and oxidative mediated disorders including cancer, allergy, diabetes, neurodegenerative diseases and coronary heart diseases are the main disorders that are reduced thanks to consumption of these molecules (Özogul et al. 2011; Calder 2012; Zhang et al. 2014). For this reason, this ingredient can be considered not only a food colorant but also a functional compound for healthy food design. Multiple applications

on the use of ω-3 fatty acids-rich oils or astaxanthin for the development of functional foods can be found in the literature (Lopez-Huertas 2010; Taksima et al. 2015). Other compounds, however, such as the oxidized forms of cholesterol, are not desirable (Hernández Becerra et al. 2014).

Microalgae, seaweeds, and crustaceans are the most important sources of astaxanthin (Sowmya and Sachindra 2011). However, astaxanthin intake is low and almost limited to fish species (crustaceans and salmonids). Astaxanthin possesses strong antioxidant activity, which is higher than that of other carotenoids or α-tocopherol (Bauerfeind et al. 2014), emphasizing the interest on valorizing shrimp waste and developing new food ingredients from it. Indeed, the epidermis and the hepatopancreas have been found to be major sources of carotenoids in crustaceans, appearing colored or with massive, highly colored fat globules (Nègre-Sadargues et al. 2000). Astaxanthin is commonly found in nature either conjugated with proteins or esterified with one or two fatty acids (monoester and diester forms) (Breithaupt 2004; Yang et al. 2015). Work on the characterization of astaxanthin molecular species can be found for the alga *H. pluvialis* (Miao et al. 2013) and for some crustacean species, such as *L. vannamei*, *E. superba*, and *P. borealis* (Breithaupt 2004; Grynbaum et al. 2005; Yang et al. 2015).

Due to the interest and applications of the shrimp lipid extract obtained from cephalothoraxes and cuticles waste, which was previously demonstrated in our previous works, the objective of the present one has been to study in deep the composition of such extract, with special emphasis on studying the astaxanthin molecular species, and also its stability when stored at room temperature.

2. MATERIALS AND METHODS

2.1. Chemicals

All-*trans*-astaxanthin, cholesterol, fatty acid standards, and 1,1,3,3-tetraethoxypropane were from Sigma-Aldrich Química, S.A. (Madrid, Spain). DL-α-tocopherol was obtained from Supelco (Madrid, Spain) All HPLC-grade solvents, including methanol and methyl t-butyl ether (MTBE), were obtained from Labscan Ltd. (Dublin, Ireland). All other reagents were of analytical grade and supplied by Panreac Química S.A. (Madrid, Spain).

2.2. Obtaining the lipid extract from shrimp waste

Ten kilograms of frozen shrimp (*L. vannamei*), kindly provided by Angulas Aguinaga Burgos (Burgos, Spain), was thawed at room temperature and peeled manually. Shrimp waste (cephalothorax, cuticles, tails, and pleopods) was homogenized to a particle size of ≈5 mm. Aliquots of 10 g of the homogenate were mixed with ethyl acetate (50 mL each) and stirred for 30 min at room temperature in darkness; after extraction the samples were filtered through Whatman No. 1 filter paper and all the aliquots were mixed together.

2.3. Analysis of carotenoids

The carotenoid species contained in the lipid extract were quantified by RP-HPLC-DAD using a Develosil UG C30 column (5 μm particle size) (Nomura Chemical, Sojo, Japan) with a guard cartridge (Phenomenex, Macclesfield, UK) packed with ODS C18. Aliquots of the lipid extract were dried and dissolved in a mixture of methanol/MTBE at 25:75 (v:v) ratio, and filtered through a 0.45 μm syringe filter, and then a 20 μl volume was injected. For elution, the linear mobile phase gradient was methanol 4% H_2O:MTBE from 83:17 to 33:67 over 60 min at a flow rate of 1 mL/min, at 22 °C. Commercial astaxanthin and spectral data were used to assign all-*trans*-astaxanthin isomers; all-*trans*-astaxanthin was quantified by means of a calibration curve following

the method described by Gómez-Prieto et al. (2002). A DU-70 spectrophotometer (Beckman Instruments) was routinely used to check the concentration of the working standard solution, the concentration being calculated using the extinction coefficient 125,100 (Britton 1995). Mono- and diesters of astaxanthin were quantified from the same calibration curve owing to the absence of patterns. Tentative identification of the mono- and diesters of astaxanthin was accomplished by RP-HPLC-MS (Agilent 6530 Accurate-Mass Q-TOF LC/MS) according to the exact masses expected for each compound, in the same conditions as described for RP-HPLC-DAD.

2.4. Fatty acid profile

Lipid extract (10 mg) was derivatized into fatty acid methyl esters (FAMEs) in triplicate using 0.5 M sodium methoxide in anhydrous methanol and acetyl chloride in anhydrous methanol. FAMEs were extracted with 4 mL hexane and used for GC analysis (1µL). The fatty acid profile was determined in an Agilent 7820A gas chromatograph with FID detector. Separation was carried out in a Agilent HP-88 column (60 m, 0.32mm i.d, 0.25 µm film thickness ref. 112-8867) with split injection (40:1) and Helium at a constant flow of 1.2 mL/min. Detector temperature was set at 260ºC and injector temperature at 250ºC. The temperature profile of the oven was 125ºC for 1 min which then increased by 8ºC/min to 145ºC for 26 min, then increased by 2ºC to 220ºC for 5min. Identification was accomplished by comparison of the retention times with standards, and results were expressed as relative percentage of the identified fatty acids. The fatty acids identified accounted for 94% of the total amount of fat in weight.

2.5. α-Tocopherol and cholesterol quantification

The lipid extract was subjected to saponification and subsequent extraction with hexane. An aliquot was dried and dissolved in methanol and then injected in an Agilent 1100

high resolution liquid chromatograph equipped with a Supelcosil LC-F column with pre-column (ref. 59158) and coupled to an Agilent G1946D simple quadrupole mass spectrometer. The mobile phases used were isocratic 1:10 water:methanol at a flow rate of 1 mL/min. Identification and quantification were accomplished by comparison with patterns, which were subjected to the same preparation steps as the samples.

2.6. Thiobarbituric acid reactive substances

A modified version of the method described by Gudipati et al. (2010) was adopted. The lipid extract was dissolved in absolute ethanol, and aliquots were mixed with a reagent solution containing 15% trichloroacetic acid and 0.375% thiobarbituric acid, and then incubated in a water bath for 15 min at 90 °C. The reaction was stopped in an ice bath and the malondialdehyde–thiobarbituric acid complex was extracted with 1-butanol by vigorous agitation in a vortex mixer. The mixture was centrifuged (5000 × g/10 min/22 °C), and the absorbance of the upper phase was read at 532 nm. TBARS concentrations were calculated from a standard curve prepared with 1,1,3,3-tetraethoxypropane.

2.7. Stability study

Aliquots of the lipid extract in ethyl acetate were transferred into 25 mL amber glass vials and dried under nitrogen flow (resulting in approximately 50 mg lipid extract/vial), after which the vials were closed (in the presence of air) and stored at room temperature (24 ± 2 °C) for 120 days. At each sampling day (0, 10, 35, 50, and 120), in triplicate, the following analyses were performed: quantitative analysis of all-*trans*-astaxanthin, total astaxanthin monoesters and total astaxanthin diesters, fatty acid profile, cholesterol content, α-tocopherol content, and TBARS were performed as described above. The degradation rate constants (*k*) of all-astaxanthin, total astaxanthin monoesters, total

148 astaxanthin diesters, and total astaxanthin of the lipid extract were calculated according
149 to the following equation:

150 $$k = \frac{-Ln\left(C_1/C_0\right)}{t} \qquad (1)$$

151 where C_1 is the total amount of astaxanthin at each sampling date, C_0 is the initial
152 amount of astaxanthin, and t is the storage time.

153 **2.8. Statistical analysis**

154 All the data were submitted to analysis of variance by ANOVA at a significance level of
155 $p \leq 0.05$ using the SPSS® computer program (SPSS Statistical Software, Inc., Chicago,
156 IL, USA).

157 **3. RESULTS AND DISCUSSION**

158 **3.1. Chemical characterization of the lipid extract**

159 From every 100 g of shrimps, 55 g were muscle and 45 g were waste; the moisture
160 content of the waste was 74.2 ± 0.9%. A lipid extract with an intense red color and a
161 characteristic shrimp odor was obtained from the waste fraction, the extraction yield
162 being 2.1 ± 0.6 g/100 g and 7.9 ± 2.5 g/100 g in wet and dry basis, respectively. The
163 most abundant compound in weight was fat, as the fatty acids fraction accounted for
164 804 ± 8 mg/g lipid extract. The following compounds in importance were α-tocopherol
165 (126 ± 1 mg/g), cholesterol (65 ± 1 mg/g), and astaxanthin (7 ± 1 mg/g); the sum of
166 these four compounds represented 100% of the lipid extract.

167 The fatty acid composition of the lipid extract during storage time is shown in Table 1.
168 With regard to the data at day 0, the most abundant fatty acids were palmitic (C16:0),
169 linoleic (C18:2n6c), oleic (C18:1n9c), DHA (C22:6n3), and EPA (C20:5n3) acids.

Saturated fatty acids (SFAs) accounted for ≈31% of the total amount of fatty acids identified, whereas MUFAs and PUFAs represented ≈25 and ≈44%, respectively. These results agree with those reported by Takeungwongtrakul et al. (2012), suggesting low variations in composition in this species as function of the region of capture. Given the low PUFA/SFA and *ω*-6/*ω*-3 ratios found (Table 1), it is obvious that the inclusion in the diet of the lipid extract obtained in the present work would help to reduce both of them. Excessive amounts of *ω*-6 PUFAs and a very high *ω*-6/*ω*-3 ratio promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of *ω*-3 PUFAs (a low *ω*-6/*ω*-3 ratio) exert suppressive effects (Simopoulos 2002). For example, the *ω*-6/*ω*-3 ratio is nowadays estimated to range between 15 and 16.7, whereas suppressive effects on various diseases have been observed at ratios ranging from 2 to 5 (Simopoulos 2002). Fatty acids influence inflammation through a variety of mechanisms; many of these are mediated by, or associated with, the fatty acid composition of cell membranes. Eicosanoids produced from arachidonic acid have roles in inflammation, whereas EPA and DHA give rise to resolvins which are anti-inflammatory and inflammation resolving. The contents of arachidonic acid and of EPA and DHA in cell membranes can be altered through oral administration of marine oils (Calder 2012).

The carotenoid profile analyzed by HPLC-DAD and the detailed data for the identification of astaxanthin molecular species by HPLC-MS are shown in Figure 1 and Table 2, respectively. A total of 18 astaxanthin derivatives, including all-*trans*-astaxanthin, two *cis*-astaxanthin isomers, 5 astaxanthin monoesters, and 10 astaxanthin diesters, were tentatively identified. Peak number 1 was identified as all-*trans*-astaxanthin, according to its retention time and spectrum, after injection of the corresponding pattern; this was further confirmed by HPLC-MS. Peaks 2 and 3 were

identified as two *cis*-astaxanthin isomers, owing to their retention time (Lin et al. 2005) and the fact that they showed the same mass spectra as all-*trans*-astaxanthin (Table 2). Peaks 4 to 8 and 9 to 18, which showed similar UV spectra to that of all-*trans*-astaxanthin, with tiny differences in the maximum absorption length, were attributed to fatty acid mono- and diesters of astaxanthin, respectively. Among monoesters found in the present work, one of them was composed of a saturated fatty acid (palmitic acid, C16:0), another one of a monounsaturated fatty acid (oleic acid, C18:1n9c), and the other three of polyunsaturated fatty acids, two ω-3 (DHA, C22:6n3, and EPA, C20:5n3) and one ω-6 (linoleic acid, C18:3n3). It is worth noting that, among monoesters, the DHA-astaxanthin one was by far the most abundant. Among astaxanthin diesters, 3 contained EPA and the other 7 DHA, and it was especially interesting that the double EPA and DHA diesters were present. The fatty acid composition of the mono- and diesters of astaxanthin is congruent with that of the whole lipid extract determined by GC, as the most abundant fatty acids reported (Table 1) are also the main constituents of the esterified astaxanthin forms (mainly oleic, palmitic, DHA, and EPA). The carotenoid profile obtained in the present work was quite similar to those reported by Lin et al. (2005) for shrimp (*P. hardwickii*) shell waste, Yang et al. (2015) for whole shrimp (*L. vannamei*), and Grynbaum et al. (2005) for krill (*E. superba*), showing all-*trans*-astaxanthin, *cis* isomers, and mono- and diesters of astaxanthin. The same was also true for *P. borealis*, although no *cis* isomers were found (Breithaupt 2004). The number, relative proportion, and composition of astaxanthin molecular species found in the literature and in the present work differed, probably owing to differences in species, site and season of catch, raw material used (whole shrimp, waste), extraction methods, etc. As an example, Yang et al. (2015) found that free astaxanthin, astaxanthin monoesters, and astaxanthin diesters in *L. vannamei* accounted for ≈33%, ≈59%, and

≈8% of the total amount, respectively, whereas these values were ≈2%, ≈20%, and ≈78%, respectively, for *P. borealis* (Breithaupt 2004). As Figure 3 shows, all-*trans*-astaxanthin, astaxanthin monoesters, and astaxanthin diesters in the present work accounted for ≈16%, ≈41%, and ≈43% of the total amount, respectively.

With regard to the astaxanthin content in absolute terms, as already mentioned in the introduction section, absolute comparison among works is very difficult, with values varying greatly, from 0.06 to 440 mg/g lipid extract (Sowmya and Sachindra 2012; Sachindra et al. 2005); accordingly, the value obtained in the present work (7 ± 1 mg/g) is in agreement with the citations. Similarly, the high α-tocopherol content found (126 ± 1 mg/g lipid extract) is in accordance with the literature, as both shrimp flesh and by-products such as cephalothorax are known as good sources of tocopherols (Özogul et al. 2011; Sanches-Silva et al. 2011). The extract also contained an appreciable amount of cholesterol (65 ±1 mg/g), which is an expected value, as Sriket et al. (2007) reported that the cholesterol concentration is usually greater than 100 mg/100 g of raw shrimp. As cholesterol is a lipophilic molecule, its concentration in the lipid extract obtained from the shrimp cephalothorax is not surprising. The importance of cholesterol intake with regard to the incidence of hypercholesterolemia is a topic of scientific debate. Studies reveal that a decrease in cholesterol intake had lesser effects on cholesterol blood levels than reducing saturated fatty acids intake (McNamara 1995), so the high PUFA content in the extract could lessen the noxious effects of cholesterol in the human organism.

3.2. Stability of the lipid extract

The stability of the lipid extract was evaluated by determination of changes in the fatty acid profile, TBARS, astaxanthin (all-*trans*-astaxanthin, total monoesters, and total

diesters), α-tocopherol, and cholesterol during storage. The analysis of the fatty acid profile pointed to some degradation of all the *ω*-3 fatty acids present in the extract, namely linoleic (C18:3n3), eicosapentaenoic (C20:5n3), docosapentaenoic (C22:5n3), and docosahexaenoic acids (C22:6n3), with a concomitant increase in the proportion of other fatty acids, not only SFA (palmitic (C16:0), margaric acid (C17:0), behenic acid (C22:0)), but also MUFA (palmitoleic acid, C16:1, and vaccenic acid, C18:1n7c) (Table 1). This behavior is attributed to the unsaturated nature of *ω*-3 PUFAs, which makes them more susceptible to lipid oxidation than less unsaturated lipids (Jacobsen et al. 2008). The partial degradation of PUFAs gave rise to a progressive decrease in the PUFA/SFA ratio, which fell from 1.39 to 1.30, as well as a progressive increase in the *ω*-6 /*ω*-3 PUFA ratio, from 1.30 to 1.44. However, having in mind the storage conditions (presence of oxygen, room temperature, long storage period), the degradation of PUFAs can be considered low, and the resulting PUFA/SFA and *ω*-6/*ω*-3 PUFA fatty acid ratios are still good from a nutritional point of view. The low oxidation rate is confirmed by the fact that no accumulation of TBARS was found (data not shown). The evolution of the cholesterol content during storage was monitored in order to infer the formation of oxidized cholesterol forms. As the cholesterol content was not significantly modified ($p \leq 0.05$) (Figure 2), the formation of oxidized cholesterol forms is not considered to be important.

The explanation for the low oxidation of the lipid extract lies in the high astaxanthin and α-tocopherol contents, whose antioxidant activity is well known (Higuera-Ciapara et al. 2006; Özogul et al. 2011; Bauerfeind et al. 2014). This is supported by the work by Cai et al. (2013), who found that the remaining bioactive compounds present in pine nut oils obtained from kernels submitted to increasing roasting times affected their radical scavenging activity and oxidation stability. Carotenoids, and especially astaxanthin, are

very good singlet oxygen quenchers and free radical scavengers, preventing the oxidation of lipids by making themselves available for reactions with radicals instead of lipids and undergoing decomposition (Sowmya and Sachindra 2012). Indeed, astaxanthin and α-tocopherol contents decreased as storage continued (Figures 3 and 4, respectively), showing at day 120 ≈12% and ≈30% of the amounts initially present, respectively. If we consider the degradation of astaxanthin in greater depth, it can be seen in Figure 3 that all the species studied (all-*trans*-astaxanthin, total monoesters, and total diesters) were gradually degraded. The content of *cis*-astaxanthin isomers has not been represented as they practically disappeared after 15 days of storage, owing to their instability, as compared to all-*trans*-astaxanthin (Hernandez-Marin et al. 2013). The evolution of the relative content of each astaxanthin species during storage (Figure 3) suggests that esterified forms are less stable than the free form, as the proportion of all-*trans*-astaxanthin to total astaxanthin increased as storage continued. Astaxanthin degradation fitted well to a first-order kinetic (r^2 = 0.813, 0.956, 0.951, and 0.946 for all-*trans*-astaxanthin, monoesters, diesters, and total astaxanthin, respectively), with degradation rate constants of –0.015, –0.0307, –0.0303, and –0.0268 for all-*trans*-astaxanthin, monoesters, diesters, and total astaxanthin, respectively, also pointing to the lower stability of the esterified forms of astaxanthin as compared to all-*trans*-astaxanthin. Yang et al. (2015), who also found astaxanthin esters to be more unstable than all-*trans*-astaxanthin when whole shrimps (*L. vannamei*) were subjected to thermal treatment, attributed this behavior to the release of free astaxanthin after hydroxylation of astaxanthin esters, owing to the numerous works reporting the higher stability of esterified carotenoids, including astaxanthin, as compared to the free forms (Miao et al. 2013; Schweiggert et al. 2007; Lorenz and Cysewski 2000). However, a recent study has pointed to the higher instability of astaxanthin esterified forms under light-induced

autoxidation owing to alteration of the *cis-trans* equilibrium, the *cis* forms being less stable than the *trans* ones (De Bruijn et al. 2016). As that study was performed working with a laboratory-constructed mono- or diesterified palmitate astaxanthin ester, further work would be needed to confirm this explanation in natural oils and fats.

Lu et al. (2014), who studied the stability of krill (*E. superba*) oil stored at 20 or 40 °C for up to 42 days, found similar results to those of the present work, namely a degradation of astaxanthin and tocopherols and low lipid oxidation. A higher oxidation rate was found by Takeungwongtrakul et al. (2015), working with lipids from shrimp (*L. vannamei*) hepatopancreas stored at 28–30 °C, in spite of the presence of astaxanthin. It is worth noting that the astaxanthin content of the lipid extract obtained in that work is lower than that found in the present one (≈2 mg/g vs. 7 mg/g), which is the most feasible explanation for the differences between the two works. Unfortunately, those authors did not report information on the tocopherol content. However, they demonstrated the antioxidant activity of astaxanthin, as the addition of commercial astaxanthin reduced lipid oxidation in comparison with the non-added sample.

In conclusion, shrimp waste of very low commercial value was valorized for the production of a lipid extract rich in healthy components such as astaxanthin, PUFAs, and α-tocopherol, that after storage for 120 days at room temperature suffered negligible lipid oxidation and only slight changes in fatty acid composition, although astaxanthin and α-tocopherol were gradually degraded. The lipid extract obtained has interesting applications as a food ingredient, owing to the coloring capacity of astaxanthin and the presence of healthy components.

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The authors state that there is not conflict of interest.

455

456

Captions

Figure 1. Carotenoid profile of the lipid extract from shrimp waste as determined by HPLC-DAD. The peaks were tentatively identified by HPLC-MS and results presented in Table 2.

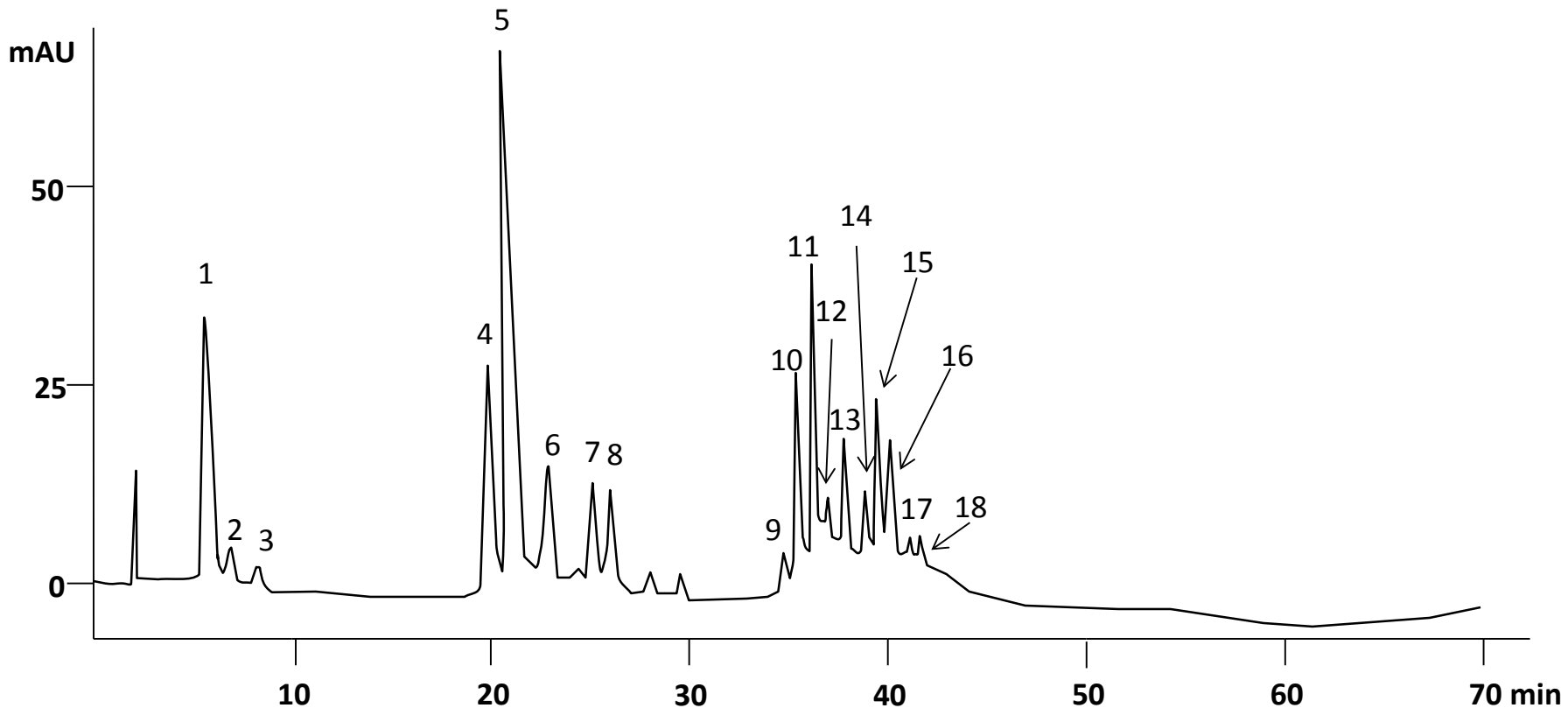
Figure 2. Cholesterol content (mg/g) of the lipid extract as a function of storage time.

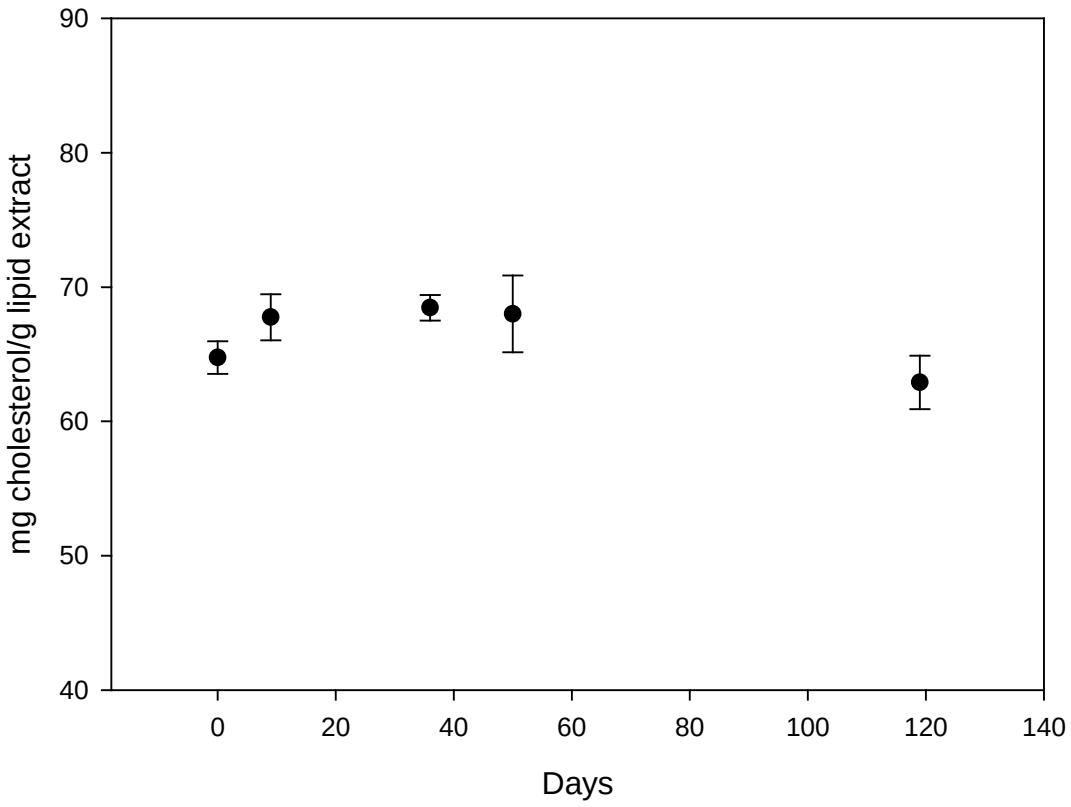
Figure 3. All-*trans*-astaxanthin, total astaxanthin monoesters, and total astaxanthin diesters (mg/g lipid extract), and percentage of each fraction with respect to total astaxanthin, as a function of storage time.

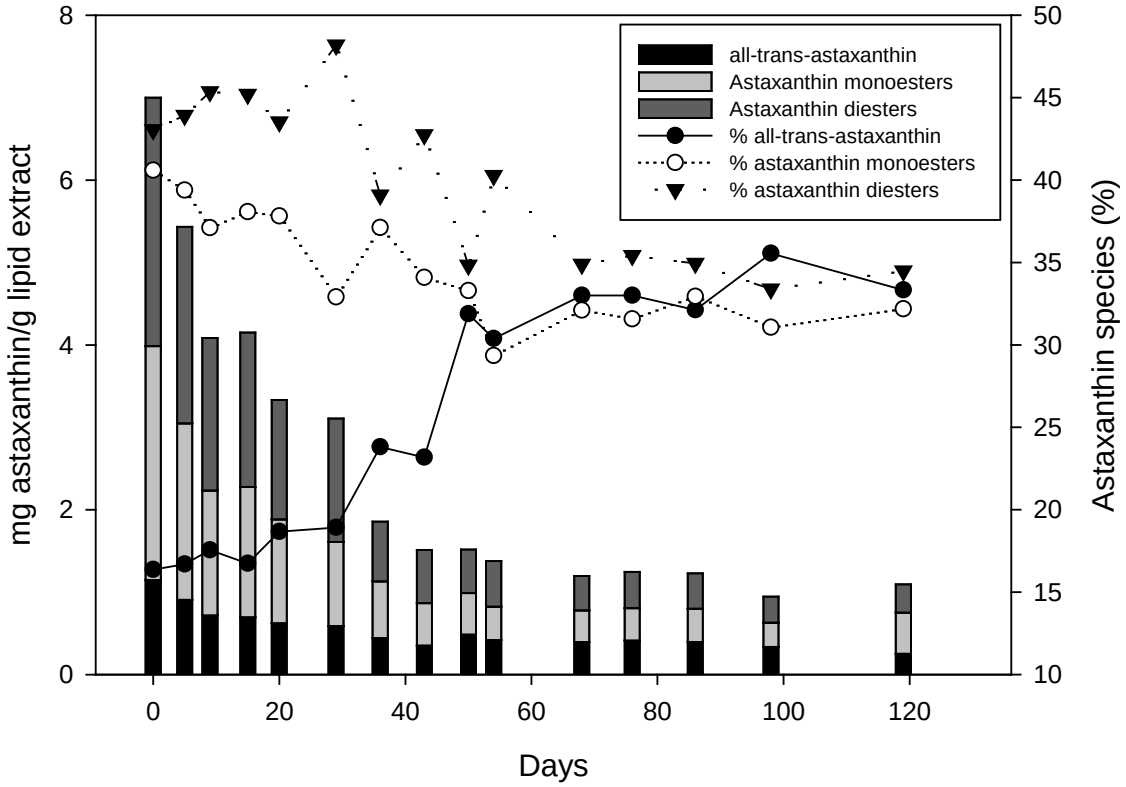
Figure 4. α-Tocopherol content (mg/g) of the lipid extract as a function of storage time.

Table 1. Fatty acid composition (% of total fatty acids identified) of the lipid extract from shrimp waste.

Table 2. Astaxanthin species tentatively identified by HPLC-MS. The peaks correspond to those shown in Figure 1.


mAU
50
25
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
10
20
30
40
50
60
70 min


90
80
70
60
50
40
mg cholesterol/g lipid extract
0
20
40
60
80
100
120
140
Days


mg astaxanthin/g lipid extract
Astaxanthin species (%)
Days
all-trans-astaxanthin
Astaxanthin monoesters
Astaxanthin diesters
% all-trans-astaxanthin
% astaxanthin monoesters
% astaxanthin diesters
0
2
4
6
8
10
15
20
25
30
35
40
45
50
0
20
40
60
80
100
120

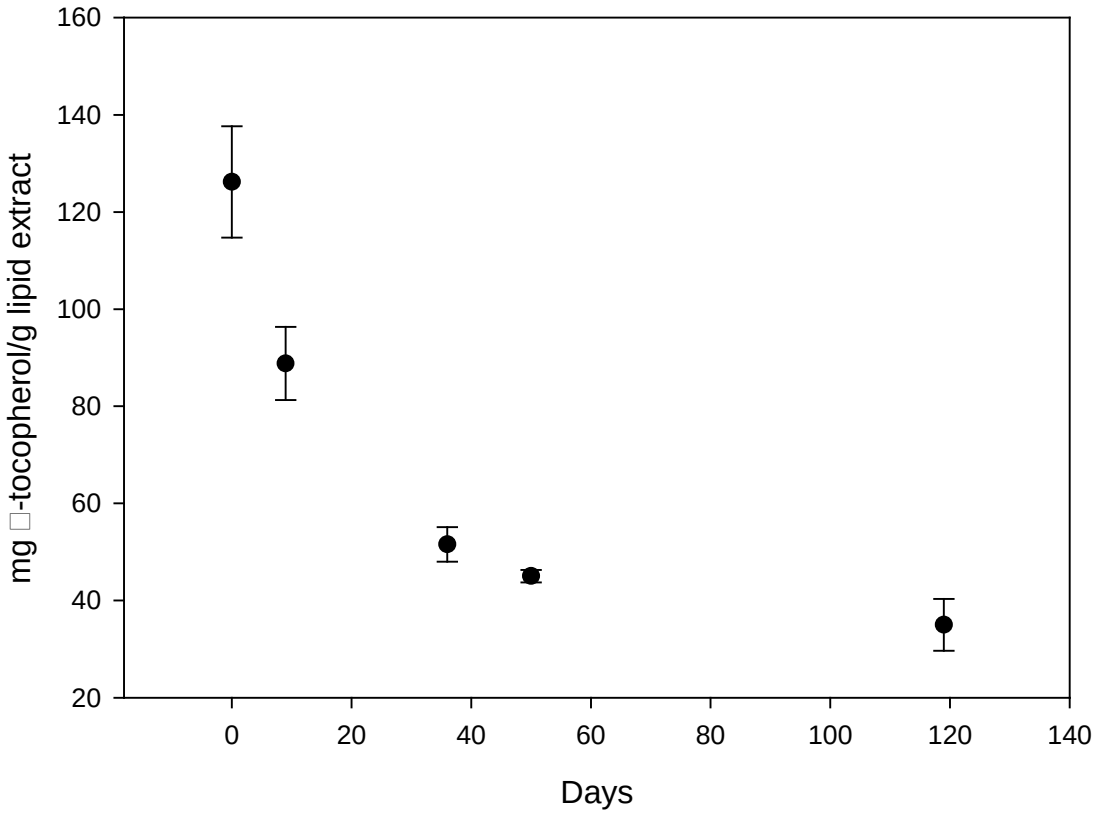

160
140
120
100
80
60
40
20
mg □-tocopherol/g lipid extract
0
20
40
60
80
100
120
140
Days

Table 1. Fatty acid composition (% of total fatty acids identified) of the lipid extract from shrimp waste.

Fatty acid	Day 0	Day 10	Day 35	Day 50	Day 120
C12:0	0.08 ± 0.00	0.07 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.09 ± 0.02
C14:0	1.97 ± 0.01	1.98 ± 0.01	2.02 ± 0.01	2.02 ± 0.00	2.04 ± 0.02
C15:0	1.61 ± 0.06	1.60 ± 0.00	1.63 ± 0.01	1.63 ± 0.01	1.63 ± 0.01
C16:0	21.20 ± 0.11	21.40 ± 0.07	21.82 ± 0.08	21.66 ± 0.08	21.83 ± 0.02
C17:0	1.07 ± 0.02	1.07 ± 0.00	1.08 ± 0.00	1.10 ± 0.01	1.13 ± 0.02
C18:0	4.32 ± 0.16	4.11 ± 0.02	4.00 ± 0.02	4.19 ± 0.03	4.27 ± 0.02
C20:0	0.34 ± 0.05	0.32 ± 0.00	0.33 ± 0.02	0.33 ± 0.00	0.34 ± 0.01
C21:0	0.17 ± 0.01	0.19 ± 0.01	0.18 ± 0.00	0.20 ± 0.01	0.19 ± 0.02
C22:0	0.28 ± 0.01	0.28 ± 0.01	0.30 ± 0.00	0.30 ± 0.01	0.32 ± 0.01
C23:0	0.15 ± 0.02	0.15 ± 0.01	0.14 ± 0.00	0.15 ± 0.00	0.16 ± 0.01
C24:0	0.16 ± 0.02	0.15 ± 0.01	0.14 ± 0.01	0.16 ± 0.00	0.17 ± 0.01
Σ SFA[1]	**31.34 ± 0.47**	**31.33 ± 0.14**	**31.72 ± 0.13**	**31.81 ± 0.15**	**32.15 ± 0.16**
C14:1	0.09 ± 0.00	0.10 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.09 ± 0.00
C16:1	3.94 ± 0.02	4.14 ± 0.11	4.04 ± 0.02	4.12 ± 0.07	4.15 ± 0.01
C18:1n9t	0.28 ± 0.03	0.29 ± 0.02	0.26 ± 0.02	0.28 ± 0.00	0.30 ± 0.01
C18:1n9c	15.91 ± 0.04	16.01 ± 0.05	16.24 ± 0.03	16.30 ± 0.06	16.42 ± 0.01
C18:1n7c	3.64 ± 0.03	3.66 ± 0.04	3.72 ± 0.02	3.75 ± 0.02	3.75 ± 0.01
C20:1	0.89 ± 0.02	1.07 ± 0.03	0.87 ± 0.01	0.89 ± 0.01	0.92 ± 0.01
C22:1n9	0.10 ± 0.00	0.10 ± 0.01	0.10 ± 0.00	0.11 ± 0.01	0.12 ± 0.01
C24:1	0.25 ± 0.03	0.19 ± 0.00	0.23 ± 0.03	0.22 ± 0.02	0.24 ± 0.04
Σ MUFA[2]	**25.09 ± 0.17**	**25.55 ±0.28**	**25.57 ± 0.15**	**25.77 ± 0.21**	**25.98 ± 0.10**
C18:2n6c	19.78 ± 0.10	19.82 ± 0.05	19.88 ± 0.17	19.92 ± 0.03	19.84 ± 0.06
C18:3n3	1.43 ± 0.02	1.42 ± 0.01	1.42 ± 0.01	1.40 ± 0.01	1.38 ± 0.01
C20:2	2.48 ± 0.05	2.50 ± 0.01	2.53 ± 0.01	2.48 ± 0.02	2.54 ± 0.06
C20:3n6	0.20 ± 0.00	0.20 ± 0.00	0.19 ± 0.01	0.20 ± 0.01	0.20 ± 0.00
C20:4n6	2.20 ± 0.00	2.17 ± 0.01	2.16 ± 0.00	2.12 ± 0.00	2.10 ± 0.01
C20:5n3	6.71 ± 0.01	6.55± 0.01	6.42± 0.01	6.35 ± 0.03	6.18 ± 0.03
C22:5n3	0.92 ± 0.01	0.92± 0.01	0.89 ± 0.01	0.89 ± 0.01	0.86 ± 0.01
C22:6n3	9.85 ± 0.10	9.54 ± 0.03	9.23 ± 0.02	9.07 ± 0.04	8.76 ± 0.04
Σ PUFA[3]	**43.57 ±0.29**	**43.12 ± 0.13**	**42.71 ± 0.24**	**42.43 ± 0.15**	**41.87 ± 0.22**
Σ PUFA*ω*-3[4]	**18.91 ±0.14**	**18.43 ± 0.04**	**17.95 ± 0.03**	**17.70 ± 0.05**	**17.19 ± 0.05**
Σ PUFA*ω*-6[5]	**24.66 ± 0.11**	**24.69 ± 0.06**	**24.76 ± 0.18**	**24.72 ± 0.09**	**24.68 ± 0.07**
Σ PUFA/Σ SFA[6]	**1.39**	**1.38**	**1.35**	**1.33**	**1.30**
Σ PUFA*ω*-6/Σ PUFA*ω*-3[7]	**1.30**	**1.34**	**1.38**	**1.40**	**1.44**

[1]Σ SFA: total amount of saturated fatty acids. [2]Σ MUFA: total amount of monounsaturated fatty acids. [3]Σ PUFA: total amount of polyunsaturated fatty acids. [4]Σ PUFA*ω*-3: total amount of *ω*-3 polyunsaturated fatty acids. [5]Σ PUFA*ω*-6: total amount of *ω*-6 polyunsaturated fatty acids. [6]Σ PUFA/Σ SFA: polyunsaturated/saturated fatty acids ratio. [7]Σ PUFA*ω*-6/PUFA*ω*-3: *ω*-6/*ω*-3 polyunsaturated fatty acids ratio.

Table 2. Astaxanthin species tentatively identified by HPLC-MS. The peaks correspond to those shown in Figure 1.

Peak no.	Rt (min)	Compound	$[M+Na]^+$	$[M+H]^+$	Formula
1	6.89	*All-trans* Ast	619.3758	597.3938	C40 H52 O4
2	7.35	*Cis* Ast	619.3758	597.3938	C40 H52 O4
3	8.90	*Cis* Ast	619.3758	597.3938	C40 H52 O4
4	19.90	Ast C20:5	903.5898	881.6079	C60 H80 O5
5	21.07	Ast C22:6	929.6054	907.6235	C62 H82 O5
6	22.96	Ast C18:2	881.6054	859.6235	C58 H82 O5
7	25.33	Ast C18:1	883.6211	861.6392	C58 H84 O5
8	26.42	Ast C16:0	857.6054	835.6235	C56 H82 O5
9	34.95	Ast C20:5 / C20:5	1187.8038	1165.8219	C80 H108 O6
10	36.37	Ast C22:6 / C22:6	1239.8351	1217.8532	C84 H112 O6
11	37.25	Ast C22:6 / C16:1	1165.8195	1143.8375	C78 H110 O6
12	37.96	Ast C22:6 / C18:2	1191.8351	1169.8532	C80 H112 O6
13	38.85	Ast C22:6 / C16:0	1167.8351	1145.8532	C78 H112 O6
14	39.20	Ast C22:6 / C15:0	1153.8195	1131.8375	C77 H110 O6
15	39.95	Ast C20:5 / C16:0	1141.8195	1119.8375	C76 H110 O6
16	40.08	Ast C22:6 / C20:5	1213.8195	1191.8375	C82 H110 O6
17	41.10	Ast C22:6 / C18:1	1193.8508	1171.8688	C80 H114 O6
18	41.98	Ast C20:5 / C18:1	1167.8351	1145.8532	C78 H112 O6

Note. Rt, retention time; Ast, astaxanthin.