

Marc David Grynbaum  
 Petra Hentschel  
 Karsten Putzbach  
 Jens Rehbein  
 Manfred Krucker  
 Graeme Nicholson  
 Klaus Albert

Institute of Organic Chemistry,  
 University of Tuebingen,  
 Tuebingen, Germany

## Unambiguous detection of astaxanthin and astaxanthin fatty acid esters in krill (*Euphausia superba Dana*)

HPLC atmospheric pressure chemical ionization (APCI)/MS, GC MS, HPLC diode array detection (DAD), and NMR were used for the identification of astaxanthin and astaxanthin fatty acid esters in krill (*Euphausia superba Dana*). Matrix solid phase dispersion was applied for the extraction of the carotenoids. This gentle and expeditious extraction technique for solid and viscous samples leads to distinct higher enrichment rates than the conventional liquid–liquid extraction. The chromatographic separation was achieved employing a C<sub>30</sub> RP column that allows the separation of shape-constrained geometrical isomers. A methanol/*tert*-butylmethyl ether/water gradient was applied. (*all*-E) Astaxanthin and the geometrical isomers were identified by HPLC APCI/MS, by coelution with isomerized authentic standard, by UV spectroscopy (DAD), and three isomers were unambiguously assigned by microcoil NMR spectroscopy. In this method, microcoils are transversally aligned to the magnetic field and have an increased sensitivity compared to the conventional double-saddle Helmholtz coils, thus enabling the measurement on small samples. The carotenol fatty acid esters were saponified enzymatically with Lipase type VII from *Candida rugosa*. The fatty acids were detected by GC MS after transesterification, but also without previous derivatization by HPLC APCI/MS. C14:0, C16:0, C16:1, C18:1, C20:0, C20:5, and C22:6 were found in astaxanthin monoesters and in astaxanthin diesters. (*all*-E) Astaxanthin was identified as the main isomer in six fatty acid ester fractions by NMR. Quantitation was carried out by the method of internal standard. (13-*cis*) Astaxanthin (70 µg/g), 542 µg/g (*all*-E) astaxanthin, 36 µg/g unidentified astaxanthin isomer, 62 µg/g (9-*cis*) astaxanthin, and 7842 µg/g astaxanthin fatty acid esters were found.

**Key Words:** Astaxanthin; Astaxanthin fatty acid esters; *Euphausia superba Dana*; Krill; HPLC APCI/MS

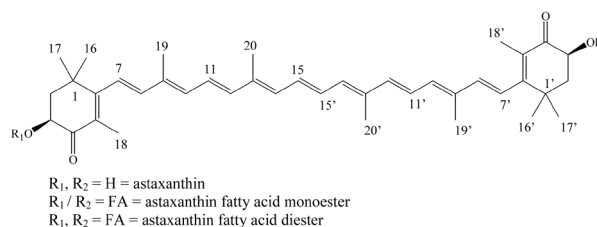
Received: April 6, 2005; revised: May 31, 2005; accepted: June 1, 2005

DOI 10.1002/jssc.200500152

### 1 Introduction

Carotenoids are one of the most important groups of natural pigments occurring in plants and animals. The first publication on these pigments dates back to 1817 and dealt with red pepper [1]. Carotenoids occur in plants, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They also appear in some nonphotosynthetic bacteria, yeasts, and molds, where they may carry out a protective function against damage by light and oxygen. Animals seem to be incapable of synthesizing carotenoids *de novo*; those carotenoids which are present are of dietary source. Carotenoids provide animals with bright coloration and serve as antioxidants and radical scavengers [2, 3].

**Correspondence:** Professor Klaus Albert, Universität Tübingen, Institut für Organische Chemie, Auf der Morgenstelle 18, D-72076 Tübingen, Germany. Fax: +49-7071-29-5875. E-mail: klaus.albert@uni-tuebingen.de.



**Figure 1.** Chemical structure and numbering of astaxanthin and astaxanthin fatty acid esters.

In marine invertebrate animals, pigmentation is often due to astaxanthin (Fig. 1), a symmetric ketocarotenoid (3,3'-dihydroxy-β,β'-carotin-4,4'-dione). Crustaceans (shrimp, krill) and Salmonidae (salmon, rainbow trout) have bright red to pink coloring due to the accumulation of astaxanthin [4]. It is the most commonly used carotenoid in salmonid fish farming and is deposited in the supplied, unesterified

form in fish muscle [5–7]. Natural sources of astaxanthin such as krill or algae supply it in the esterified form. After hydrolysis of the esters it is deposited in the flesh of salmonids in the free form [6, 8].

Krill (*Euphausia superba*) is the most important zooplankton species and plays a key role in the Antarctic food web. It is a shrimp-like crustacean, which attains a size of 6 cm and feeds primarily on phytoplankton or sea ice algae. Krill is the staple food of many fish, birds, and mammals in the Southern Ocean. The biomass of Antarctic krill is considered to be larger than that of the earth's human population and krill swarms can occupy an area of 450 km<sup>2</sup>. The krill has been the subject of many investigations. In 1977, a total content of 15.7 µg/g biomass of cryptoxanthin, astaxanthin esters, hydroxy- $\zeta$ -carotene, dihydroxy- $\zeta$ -carotene, zeaxanthin, flavoxanthin, and astaxanthin was found by column chromatography and UV/VIS spectroscopy [9]. The carotenoid content has been investigated as a function of age, structure, and sex [10]. Carotenoid concentrations of 50 µg/g (male) and of 152 µg/g (female) were found and additionally small amounts of  $\beta$ -carotene and an unidentified polyoxyxanthophyll were described. Yamaguchi *et al.* [11] indicated that the carotenoid content of krill and krill meals is composed almost exclusively of astaxanthin and its monoesters and diesters (30–40 µg/g; 5–15% unidentified). The stability of carotenoid pigments has also been studied [12], and the diesters have been found to be the most stable amongst them. The greatest carotenoid concentration was found in the cephalothorax and the carapace (71.3 and 59.8 µg/g) [13]. Maoka *et al.* [14] separated the two enantiomers (3*S*,3'*S*-/3*R*,3'*R*-) and the *meso*-astaxanthin (3*S*,3'*R*/3*R*,3'*S*), finding 3*R*,3'*R*- to be the most abundant (62–71%). They found up to 908 µg/g in krill eyes. The relative composition of astaxanthin, its monoesters and diesters remains constant at 5, 49, and 40%, respectively, but the total amount of astaxanthin varies with the season and the stage of sexual maturity [15].

The lipid, sterol, and fatty acid composition of Antarctic krill has been investigated separately from the carotenoids [16]. Phosphatidylcholine, phosphatidylethanolamine, triacylglycerol, free fatty acids, and sterols have been identified. The major fatty acids were C14:0, C16:0, C16:1(n-7), C18:1(n-9), C18:1(n-7), C20:5(n-3), and C22:6(n-3).

Recently, Takaichi *et al.* [17] determined the fatty acids present in astaxanthin esters by mild MS. The extract was separated by HPLC on a C<sub>18</sub> column, but the peaks are poorly resolved on their chromatograms. The peaks were collected and subjected to field desorption MS. Only five fatty acids were detected in astaxanthin, namely, dodecanoate, tetradecanoate, hexadecanoate, hexadeceno-

ate, and octadecenoate. Surprisingly, polyunsaturated fatty acids were not found in the respective fractions.

The present study uses HPLC coupled to atmospheric pressure chemical ionization (APCI) to study the carotenoid and carotenol fatty acid ester composition of Antarctic krill *Euphausia superba* Dana. Matrix solid phase dispersion (MSPD) was used for the extraction of the carotenoids, and C<sub>30</sub> sorbent was used for the separation. The identification of the carotenoids and its fatty acid esters was achieved by HPLC APCI/MS and microcoil NMR. The major advantage of HPLC APCI/MS is the direct detection of the fatty acid esters without the need of derivatization or fraction collection beforehand.

As carotenoids are very sensitive to both light and air, an optimized combination of analytical sample preparation, separation, and detection techniques has to be used. MSPD, a very gentle and expeditious extraction technique for solid and viscous samples, was used for the extraction of the krill's carotenoids and its esters. It is convenient to work with, decreases solvent use by up to 98%, and reduces sample turnaround time by 90% compared to conventional extraction techniques like liquid–liquid extraction [18–20]. Another advantage of MSPD is the higher enrichment of the analytes, which is very important for successful NMR measurements.

After MSPD, the carotenoids were separated on C<sub>30</sub> sorbent. Triacetyl phases have been especially developed for the separation of shape-constrained natural compounds, such as carotenoid stereoisomers, and the sample loading capacity of these phases is superior to that of the conventionally employed C<sub>18</sub> materials. Detailed NMR studies investigating the structural parameters of C<sub>30</sub> and interactions responsible for its specific separation behavior have already been carried out [21–23]. Online HPLC APCI/MS enables coupling of MS and LC with flow rates up to 1 mL/min and has a higher sensitivity than ESI for weakly polar compounds like carotenoids. APCI/MS allows the unambiguous identification and assignment of different carotenoids in positive as well as in negative ionization mode [24–26]. Carotenol fatty acid esters can also be detected [27, 28]. Obviously, a distinction between stereoisomers is not possible by MS. For unambiguous structure elucidation, NMR spectroscopy is absolutely necessary. The chemical shifts and coupling constants of the stereoisomers are slightly changed [20]. By the introduction of a *cis* double bond, the symmetry is lost and the “inner” protons of the *cis* bond are shifted to the deep field while the “outer” protons are shifted to higher field. Solenoidal NMR probes overcome the limited sensitivity of conventional saddle-shaped NMR probes and their sensitivity is threefold higher [29, 30]. They have successfully been employed not only for online coupled capillary HPLC NMR measurements [30–32], but also for stopped-flow

measurements [33]. Mass-limited samples can also be inserted into the NMR probe by syringe flow-injection. They are dissolved in very small volumes that are matched to the active volume of the microcoil NMR probe (1.5  $\mu\text{L}$ ), thus further enhancing concentration and sensitivity.

## 2 Materials and methods

### 2.1 Materials and standards

For HPLC, solvents (LiChrosolv gradient grade) from Merck (Darmstadt, Germany) and deionized water from a Milli-Q water purification system (Millipore AS, Bedford, MA, USA) were used. (*all-E*) Lutein, (*all-E*) zeaxanthin, (*all-E*) canthaxanthin, (*all-E*)  $\beta$ -carotene, and (*all-E*) lycopene were donated by BASF (Ludwigshafen, Germany). (*all-E*) Astaxanthin and  $\beta$ -apo-8'-carotenal were purchased from Sigma (Steinheim, Germany). For convenience, the trivial names of carotenoids are used throughout the text instead of the complex IUPAC nomenclature [34]. Antarctic krill were fished on an expedition carried out by the Alfred Wegener Institute for Polar and Marine Research in the Wedell Sea, deep frozen with liquid nitrogen, and stored at  $-40^\circ\text{C}$  until analysis. The extraction of the carotenoids from krill was performed utilizing MSPD with a silica based octadecyl sorbent material ( $\text{C}_{18}$  end-capped) from IST (Hengoed Mid Glam, UK) and butylated hydroxytoluene as antioxidant from Sigma. Before analysis, the extract was sterile filtered through a syringe filter from Carl Roth (Karlsruhe, Germany). Iodine-catalyzed isomerization of authentic (*all-E*) astaxanthin was performed similarly to Zechmeister [35]: An iodine solution was added to an (*all-E*) astaxanthin solution and the mixture was exposed to UV-light for 20 min. Lipase type VII from *Candida rugosa* and cholesterol esterase from *Pseudomonas fluorescens* for ester hydrolysis as well as bile salts were purchased from Sigma. Disodium hydrogen phosphate, potassium dihydrogen phosphate, calcium chloride, and sodium chloride were obtained from Merck. NMR measurements were carried out in acetone- $\text{d}_6$  (99.8%) from Deutero GmbH (Kastellaun, Germany).

### 2.2 Sample preparation

#### 2.2.1 Extraction

The krill (0.5 g) was ground with 1.5 g of  $\text{C}_{18}$  (end-capped) MSPD material and butylated hydroxytoluene into a dry homogeneous powder. This takes between 5 and 10 min, depending on the amount of water in the sample. The force has to be adjusted to the sample as well: too much will destroy the silica particles and produce a high back pressure, too little will not be sufficient to generate a dry homogeneous powder. For quantitation, the sample was spiked with 10  $\mu\text{L}$  of a  $\beta$ -apo-8'-carotinal solution (13.03 mg/mL). The mixture was loaded into an empty

SPE column and pressed between two frits. The column was washed with 10 mL of deionized water and the carotenoids were extracted with 4 mL of *tert*-butylmethyl ether (TBME). After evaporation of TBME under a nitrogen stream, the extract was stored at  $-30^\circ\text{C}$  until it was analyzed. Before analysis, the extract was redissolved in 300  $\mu\text{L}$  of TBME, sterile filtered, and the filter was washed three times with 500  $\mu\text{L}$  of TBME. The solvent was evaporated again under nitrogen stream, and the extract was redissolved in 100  $\mu\text{L}$  of ethanol, guaranteeing a high concentration of the carotenoids.

#### 2.2.2 Enzyme-catalyzed ester hydrolysis

The enzymatic assay was similar to the protocol developed by Breithaupt [36]. In brief, 10 mL of phosphate buffer (0.1 M, pH 7.4), 30 mg of bile salts, and 250  $\mu\text{L}$  of a calcium chloride/sodium chloride (75 mM/3 M) solution were preincubated with the dried MSPD extract for 30 min at  $37^\circ\text{C}$ . Then, 100  $\mu\text{L}$  of a suspension of lipase (50 mg/mL) in a 5 mM calcium chloride solution was added and the mixture was incubated at  $37^\circ\text{C}$  for 2 h. The carotenoids were extracted with chloroform, which was immediately evaporated under nitrogen stream. The residue was redissolved in TBME, washed with water, dried with sodium sulfate, filtered, and the TBME was evaporated under nitrogen stream. Finally, the hydrolyzed extract was redissolved in 100  $\mu\text{L}$  of ethanol and subjected to HPLC analysis.

#### 2.2.3 Transesterification of the carotenol fatty acid esters

Two hundred microliters of 15% acetyl chloride in methanol was added to the extract and heated to  $110^\circ\text{C}$  for 30 min. Then 100  $\mu\text{L}$  of water was added and the esters extracted with 400  $\mu\text{L}$  of *n*-hexane. The extract was dried with sodium sulfate and concentrated for GC MS analysis.

### 2.3 Chromatography

Analyses were carried out on an HP1100 system (Agilent Technologies, Waldbronn, Germany) using a UV detector (DAD) monitoring at 455 nm. The separations were performed on a  $250 \times 4.6$  mm ProntoSil  $\text{C}_{30}$  stainless steel column (Bischoff, Leonberg, Germany). The average pore diameter was 120  $\text{\AA}$  and the particle size was 3  $\mu\text{m}$ . A  $\text{C}_{18}$  cartridge (Spark Holland BV, AJ Emmen, the Netherlands) was used as guard column. The separation of the carotenoids from the MSPD extract was achieved using a mobile-phase gradient elution program at a flow rate of 1 mL/min. Two mixtures of methanol, *tert*-butylmethyl ether, and water were used as eluents (A – 83:15:2; v/v/v; B – 8:90:2; v/v/v). The elution started isocratically at 100% A for 20 min, followed by a linear gradient to 40% A within 170 min. To verify the repeatability of the results, 50  $\mu\text{L}$  of the MSPD extracts were injected five times.

## 2.4 HPLC MS

MS was performed on a Bruker Esquire 3000plus LC-MS<sup>(m)</sup> system (Bruker Daltonics, Bremen, Germany) equipped with an APCI interface and an ion trap (IT). The HPLC APCI/MS coupling was accomplished using an HP1100 system (Agilent Technologies) and 50  $\mu$ L of sample was injected into the system. Mass spectra were recorded in the mass range of 50–1400  $m/z$ . The detection was performed using APCI in the positive ionization mode. The voltage of the corona needle was set to 3.5 kV. Nitrogen was used as drying gas as well as carrier gas at a flow rate of 4 L/min with a nebulizer pressure of 65 psi. The ionization chamber temperature was set to 300°C and the dry gas temperature was held at 250°C. The compound stability was set to 75% and the trap drive level to 70%. The chromatographic conditions were the same as in the analytical separation described in Section 2.3.

## 2.5 GC MS

Analyses were performed on an HP 6890 system, coupled with an HP MD 5973 quadrupole mass spectrometer (Agilent Technologies). A fused-silica capillary Nordion SE54 (25 m  $\times$  0.32 mm, 0.25  $\mu$ m film thickness) was used for the separation. The carrier gas was helium with a constant flow rate of 1.3 mL/min. The injector temperature was set to 280°C and the column temperature program was as follows: the initial temperature of 100°C was held constant for 2 min and then increased by 4 K/min to the final temperature of 270°C.

## 2.6 NMR

All NMR experiments were recorded using a Bruker AMX 600 spectrometer (Bruker, Rheinstetten, Germany). Astaxanthin and two isomers were collected from two saponified MSPD extracts from the analytical HPLC separation, as well as selected ester peaks from three MSPD extracts. The solvent was evaporated under nitrogen stream and the fractions were lyophilized and stored at  $-30^\circ\text{C}$  until NMR analysis. For this, the fractions were redissolved in 15  $\mu$ L of acetone- $d_6$ .  $^1\text{H}$  NMR spectra of the carotenoids were recorded utilizing syringe flow injection to a  $^1\text{H}$  selective microcoil NMR probe (Protasis/MRM, Savoy, IL, USA) with an active volume of 1.5  $\mu$ L.

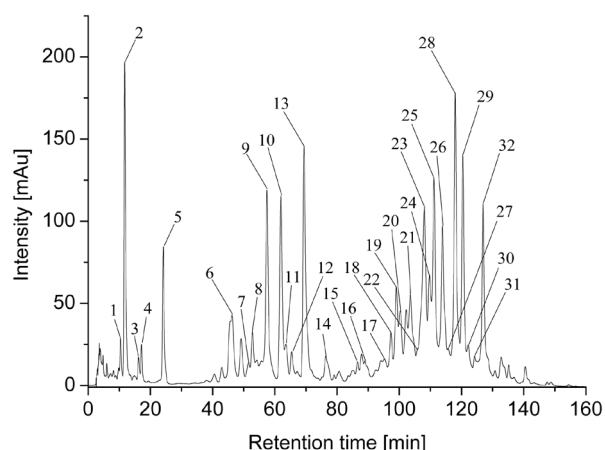
$^1\text{H}$  NMR spectra were recorded with the pulse program zg without solvent suppression and a pulse program to suppress the residual solvent signals (zgcprsp) from deuterated acetone- $d_6$  and water, using rectangular shaped pulses for low-power presaturation (rectangular pulses, length 100 ms). The temperature was set to 298 K. Transients (4K) were recorded with a spectral width of 6024 Hz and 16K time domain points. The relaxation delay was set to 1 s.  $^1\text{H}$ ,  $^1\text{H}$ -COSY spectra were recorded for an unequivocal peak assignment with the pulse program cosy. Two

hundred and fifty six transients with 2K complex data points and a spectral width of 6024 Hz were accumulated in the F2 dimension and 128 complex data points were accumulated in the F1 dimension.

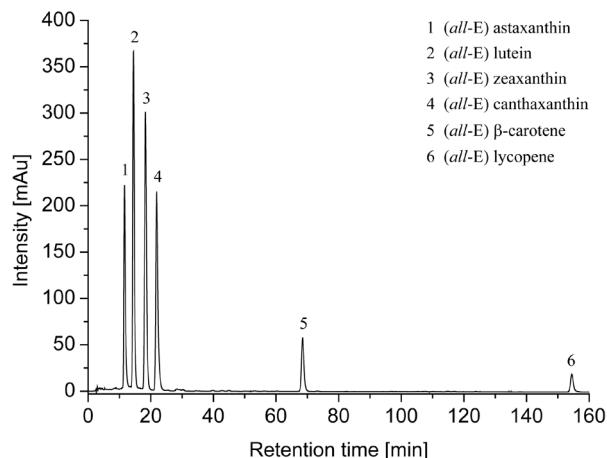
For all spectra, before Fourier transformation, a squared sine bell function was applied to the FID. Baseline correction and phasing were performed manually. The chemical shift axis was referenced with respect to acetone- $d_6$ ,  $\delta = 2.04$  ppm, for all  $^1\text{H}$  NMR spectra. All NMR data were processed with XWIN-NMR version 3.5 (Bruker).

## 3 Results and discussion

Extraction of the carotenoids from krill was performed by MSPD. The deep frozen krill were ground with  $\text{C}_{18}$  sorbent material into a dry homogeneous powder, which was loaded into an SPE cartridge and pressed between two frits to a compact column bed. Polar compounds were eluted with water and the carotenoids were eluted with TBME. After sterile filtration, the solvent was evaporated and the dry residue was redissolved in 100  $\mu$ L ethanol. The carotenoids were separated by HPLC on a  $\text{C}_{30}$  column (250  $\times$  4.6 mm, 3  $\mu$ m, 120  $\text{\AA}$ ), resulting in the chromatogram shown in Fig. 2. The chromatogram is divided into three parts. First, the more polar carotenoids elute. By UV spectroscopy (DAD) peak 2 is interpreted as (*all-E*) astaxanthin and peaks 1, 3, and 4 are interpreted as *cis* isomers. The absorption spectra of the two astaxanthin *cis* isomers (9-*cis* and 13-*cis*) are fairly similar to the one of the (*all-E*) isomer with only small differences [37–39]. In comparison to the (*all-E*) astaxanthin with a  $\lambda_{\text{max}}$  of 476 nm, the two isomers (peaks 1 and 4 in Fig. 2) show a small hypsochromic effect of 7 and 4 nm. The intensity of



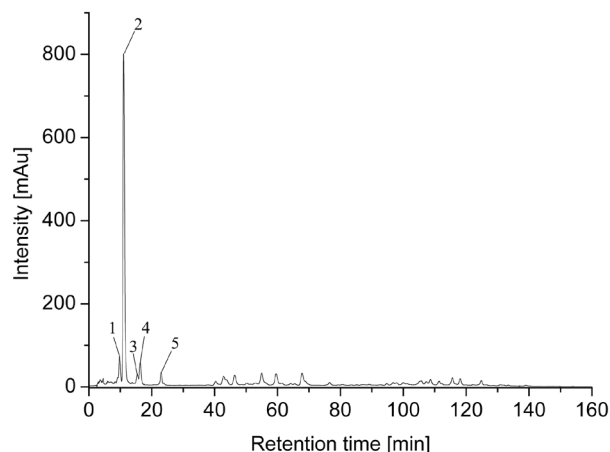
**Figure 2.** HPLC chromatogram (DAD, 455 nm) of the krill MSPD extract. Peaks 1–4 represent free astaxanthin, peak 5 represents  $\beta$ -apo-8'-carotinal (spiked), peaks 6–14 represent astaxanthin fatty acid monoesters, and peaks 15–31 represent astaxanthin diesters. For precise peak assignment, see Table 2.



**Figure 3.** HPLC chromatogram (DAD, 455 nm) of a standard solution containing astaxanthin (1), lutein (2), zeaxanthin (3), canthaxanthin (4),  $\beta$ -carotene (5), and lycopene (6).

the *cis* band is greater since the *cis* double bond is nearer to the center of the molecule. This indicates that peak 1 must be the (13-*cis*) isomer and peak 4 the (9-*cis*) isomer; this empirical assignment will be verified by NMR spectroscopy. Peak 3 might be (15-*cis*) astaxanthin, as the UV spectrum was similar to the one of (*all-E*) astaxanthin ( $\Delta\lambda = 1$  nm), but this was not verified because the peak was too small for NMR analysis. Peak 5 is  $\beta$ -apo-8'-carotinal, which was used as internal standard for quantitation. It has passed through the same workup steps and does not interfere with the carotenoids extracted from krill. This means it is well suited for this purpose [40]. The fractions which elute later could be astaxanthin fatty acid esters; in this case, peaks 6–14 could be monoesters and peaks 15–32 could be diesters.

To identify carotenoids potentially contained in the krill by cochromatography, a carotenoid standard mixture was prepared and also subjected to HPLC under identical conditions (Fig. 3). All carotenoids are baseline separated and they elute according to their polarity in the following order: (*all-E*) astaxanthin (1; 11.6 min), (*all-E*) lutein (2; 14.5 min), (*all-E*) zeaxanthin (3; 18.3 min), (*all-E*) canthaxanthin (4; 21.9 min), (*all-E*)  $\beta$ -carotene (5; 68.5 min), and (*all-E*) lycopene (6; 154.5 min). (*all-E*) Astaxanthin was found to be the only one present in the extract. The other peaks were assumed to be carotenol fatty acid esters, the presence of which can be revealed by saponification. In addition, carotenoids that occur mainly in esterified form rather than in the free form can be revealed by that method. Under aerobic conditions, an alkali hydrolysis cannot be applied to esterified  $\alpha$ -ketols like astaxanthin, because they undergo oxidation to form the 2,3-didehydro-3-hydroxy-4-keto end group in the presence of base and oxygen [41]. For anaerobic saponification, a modified Schlenk tube with appendix can be used



**Figure 4.** HPLC chromatogram (DAD, 455 nm) of krill MSPD extract that was subjected to enzymatic saponification using lipase from *C. rugosa* type VII.

[42], but the procedure is rather involved. Enzymatic cleavage of alkali-unstable carotenol esters has been applied successfully [36, 43]. The HPLC chromatograms of the saponified product and the nonsaponified samples are compared. If the eluted compound is more polar, but has an unchanged UV/VIS spectrum, that indicates that an ester was present and has been hydrolyzed.

We successfully applied Lipase type VII from *C. rugosa* (Fig. 4) and cholesterol esterase from *P. fluorescens* (data not shown) for the hydrolysis of the astaxanthin monofatty acid esters and difatty acid esters. No new peaks from carotenoids that might naturally occur only in their esterified form appear in the chromatogram of the hydrolyzed extract. The assumed astaxanthin monofatty acid esters and difatty acid esters (peaks 6–14 and peaks 15–32, respectively) are almost completely hydrolyzed, while astaxanthin (peak 2) and the isomers (peaks 1, 3, and 4) dominate the chromatogram. Peak 2 shows the same UV/VIS spectrum as the esters with the absorption maximum at 476 nm. Due to the different solvent composition, the absorption maximum of the esters is slightly shifted to 478 nm. Peaks 1–4 are magnified by a factor of 9. This indicates that peaks 1, 3, and 4 are isomers of astaxanthin. The analytical proof was provided by iodine-catalyzed isomerization of the authentic standard. After isomerization under UV light, the mixture of (*all-E*) astaxanthin and the *cis* isomers was subjected to HPLC. The chromatogram showed the same isomer pattern as the krill extract (data not shown). Further MS and NMR experiments will show that these compounds are isomers and not products of decomposition.

Next, GC MS experiments were performed to identify the fatty acids that are esterified to astaxanthin. For this, the MSPD extract was transesterified with acetyl chloride to the corresponding ethyl ester and the fatty acid esters

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.