Electronic Supplementary Material (ESI) for Analyst.

Chromatographic, NMR and vibrational spectroscopic investigations of astaxanthin esters: application to "Astaxanthin-rich shrimp oil" obtained from processing of Nordic shrimps

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Experimental

Extraction of astaxanthin from shrimp oil

20-30 mg of oil was dissolved in 5 ml hexanes. The SPE cartridge was primed with 5 mL hexanes and the sample was poured on the cartridge. It was subsequently washed by 5 mL hexanes and 2 x 5 mL 6 % diethyl ether in hexanes. Astaxanthin was eluted by 5 mL acetone containing 200 mg/L BHT. The acetone solution was evaporated under a flux of nitrogen and the remaining red substance was dissolved in 1 mL of mobile phase (hexanes/acetone 96:4). The synthesized astaxanthin esters were dissolved in 1 mL of mobile phase.

Saponification for total astaxanthin analysis was performed by dissolving the extracted astaxanthin fraction in 2 mL diethyl ether and adding 2 mL of a 2 % KOH solution in methanol. The reaction mixture was kept at 4 °C and in the dark for 12.5 minutes, after which 2 mL of a 10% NaCl solution in water was added. The aqueous layer was removed and the organic phase was extracted two more times with the NaCl solution. The organic layer containing the pigment was evaporated under a flux of nitrogen and the remaining red substance was dissolved in 1 mL of mobile phase (hexanes/acetone 82:18).

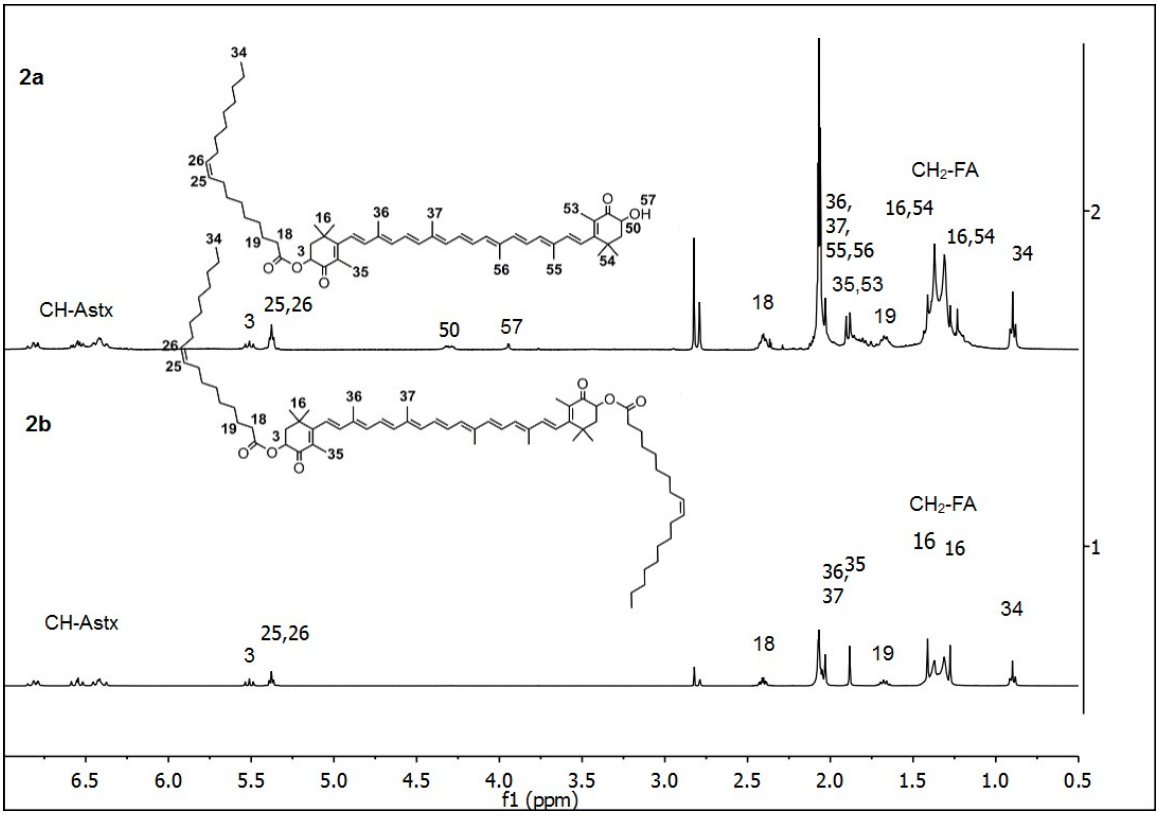

Fig. S1 ^{1}H NMR spectra of ASTX-C18:1n9 mono- (**2a**) and diester (**2b**)

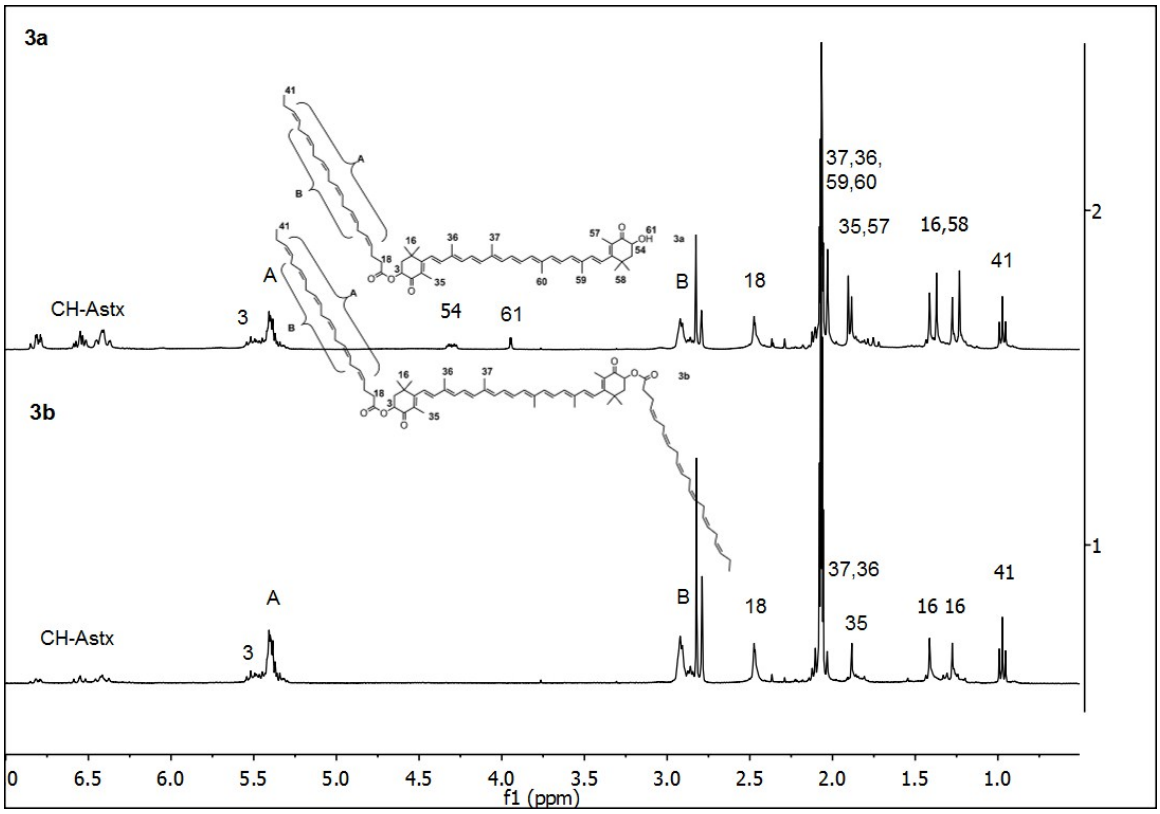

Fig. S2 ^{1}H NMR spectra of ASTX-DHA mono- (**3a**) and diester (**3b**)

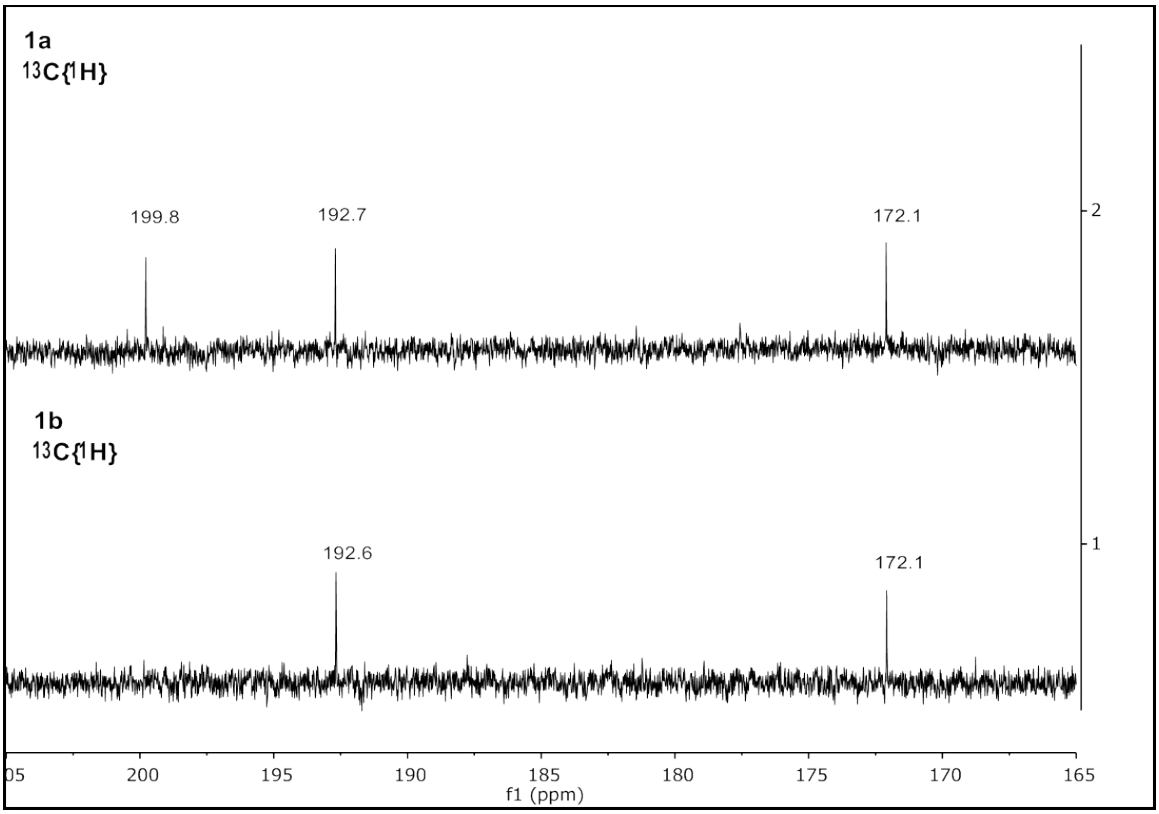

Fig. S3 Carbonyl region of $^{13}C\{^{1}H\}$ NMR spectra of ASTX-DHA mono- (**1a**) and diester (**1b**)

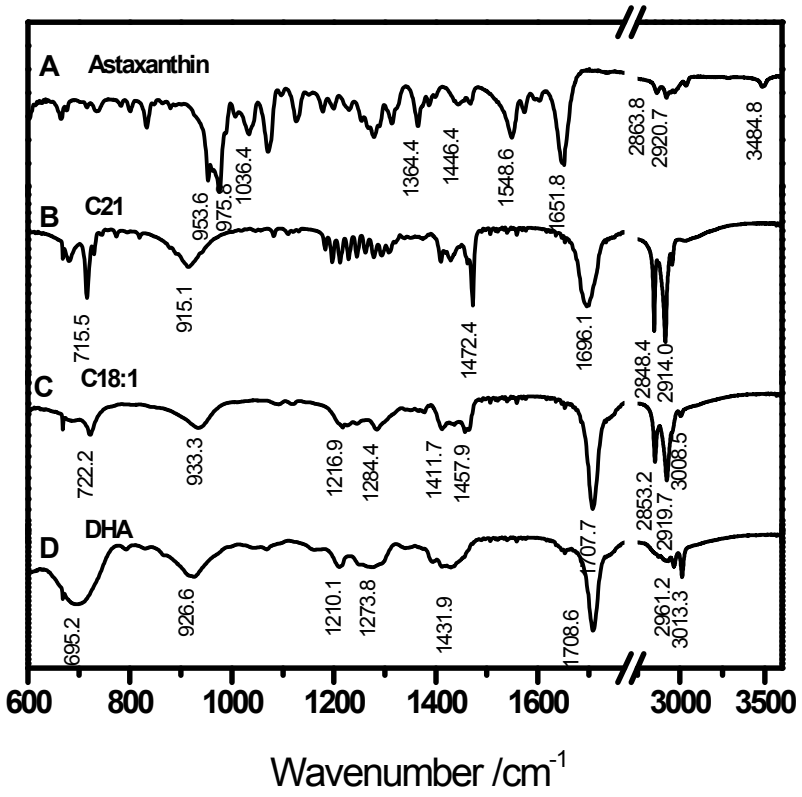

Fig. S4 FTIR spectra of astaxanthin (synthetic), C21, C18:1n-9, and DHA fatty acids

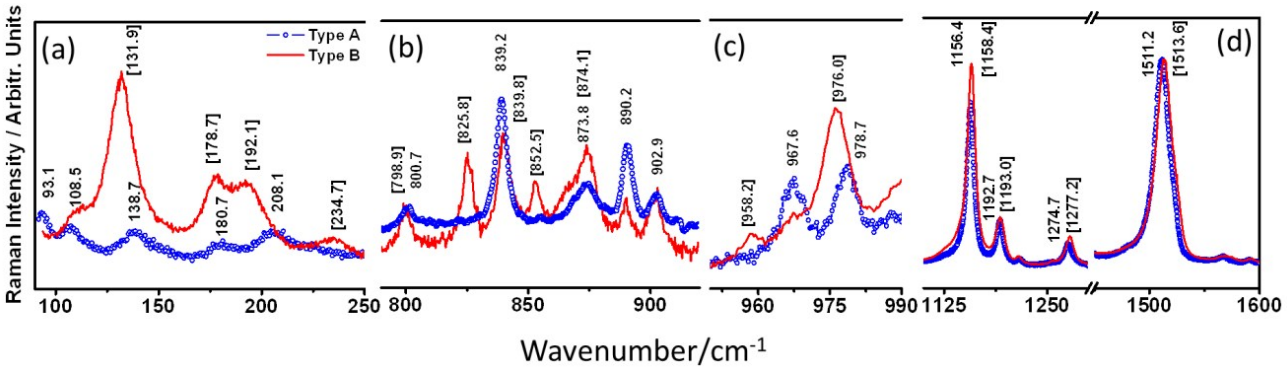

Fig. S5 Raman spectra of type A and type B crystal structure of astaxanthin

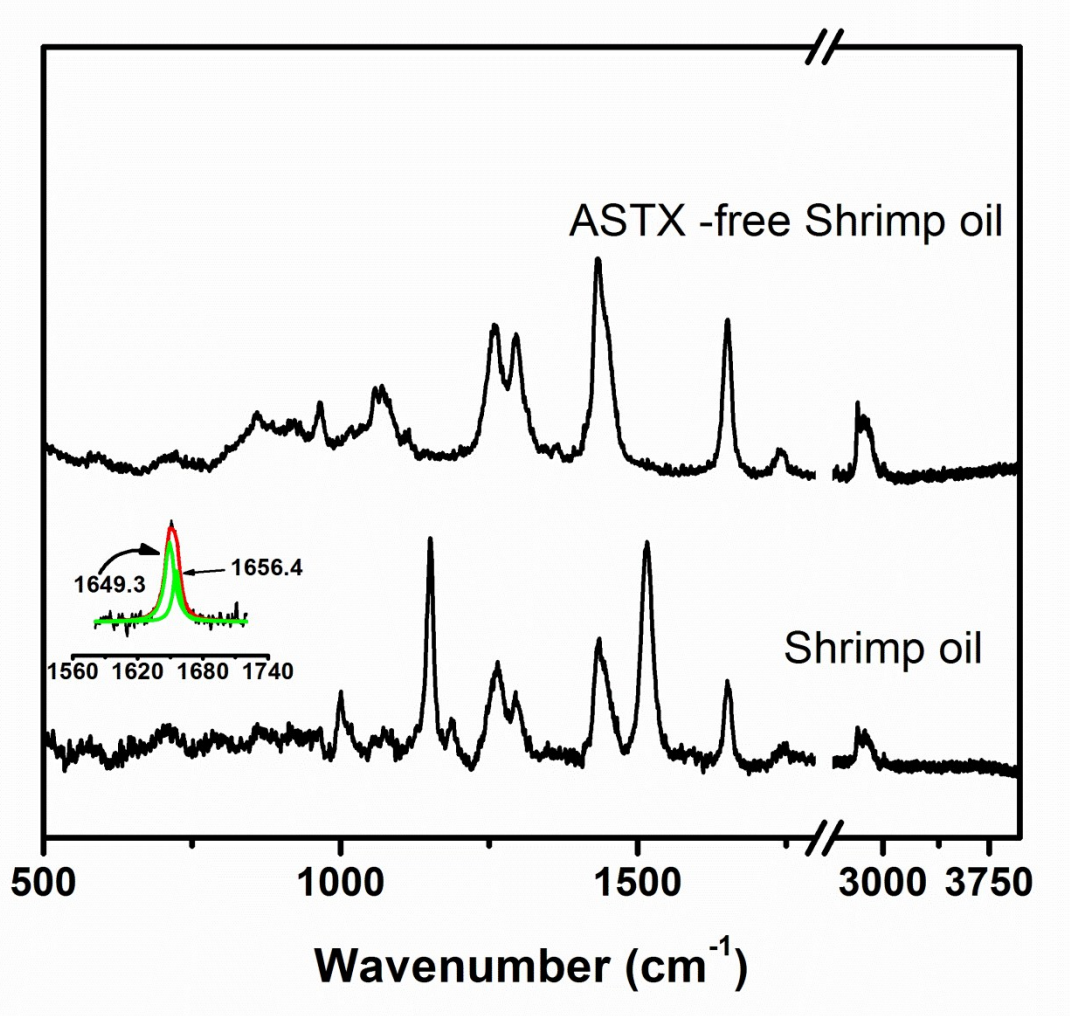

Fig. S6 Raman spectra of Astaxanthin-rich shrimp oil and Astx-free shrimp oil

Fig. S7 Astaxanthin-rich shrimp oil

Table S1 Total fatty acid profile of shrimp oil

Fatty acid	%	RSD % n=26
12:0	0,22	36,32
14:0	3,63	17,16
15:0	0,32	9,42
16:0	11,63	8,73
17:0	0,19	10,96
18:0	2,28	14,21
20:0	0,14	6,45
21:0	0,04	9,03
22:0	0,04	37,44
24:0	0,01	125,54
14:1n-7	0,06	20,53
14:1n-5	0,20	8,22
15:1n-5	0,02	57,36
16:1n-9	0,20	7,54
16:1n-7	12,28	11,14
16:1n-5	0,22	16,38
17:1	0,03	8,32
18:1n-9	12,53	5,12
18:1n-7	3,96	11,90
18:1n-5	0,62	9,14
20:1n-11	1,13	25,93
20:1n-9	7,72	17,64
20:1n-7	2,15	17,09
20:1n-5	0,09	51,67
22:1n-11+13	10,15	23,00
22:1n-9	3,13	20,69
22:1n-7	0,36	4,81
24:1n-9	0,37	5,11
24:1n-7	0,03	84,03
16:2n-6	0,04	18,61
16:2n-4	0,33	22,33
18:2n-9	0,05	34,21
18:2n-6 cis	0,73	26,83
18:2n-6 trans	0,01	179,65
18:2n-4	0,25	27,72
20:2 NMIi*	0,07	129,55
20:2 NMIj*	0,06	110,75
20:2n-9	0,03	66,91
20:2n-6	0,39	39,89
22:2 NMIi*	0,07	21,35
22:2 NMIj*	0,02	134,59

22:2n-6	0,03	72,67
16:3n-4	0,15	41,42
16:3n-3	0,04	15,00
18:3n-6	0,10	36,51
18:3n-4	0,12	10,42
18:3n-3	0,49	13,22
20:3n-6	0,05	14,80
20:3n-3	0,16	40,08
16:4n-3	0,06	23,10
16:4n-1	0,23	37,92
18:4n-3	1,18	11,08
18:4n-1	0,11	8,66
20:4n-6	0,44	15,99
20:4n-3	0,39	22,61
22:4n-6	0,13	18,71
22:4n-3	0,23	39,68
20:5n-3	9,90	13,56
21:5n-3	0,30	13,78
22:5n-6	0,09	42,57
22:5n-3	0,57	16,38
22:6n-3	9,44	9,94
Total	100,00	
Omega-3	22,75	5,82
Omega-6	2,00	14,04
SFA	18,26	8,49
MUFA	55,25	4,95
PUFA	26,26	5,78

* non-methylene interrupted fatty acid

[1]average values for shrimp oil acquired from ten different batches of production

Table S2 FTIR bands and assignments for astaxanthin

S.No	ASTX	Assignments
1.	953.6 (S) 962.3(S) 975.8 (S)	C-C stretching
2.	1034.6 (S)	CH deformation (out of plane)
3.	1364.4 (S)	Methyl rocking
4.	1446.6 (S)	Methyl symmetric deformation, CH deformation (in plane)
5.	1548.6(S)	Methyl asymmetric deformation, C=C stretching
6.	1651.8	C=O (ketonic)
6.	2867.0 to 3036.7	CH stretching
7.	3484.8 (S)	OH stretching

Table S3 FTIR bands and assignments for fatty acids

S.No	C18:1	C21	DHA	Assignments
1.	722.2(m)	715.5(s)	695.2 (s)	CH2 rocking
2.	933.3(m)	915.1(m)	926.6(s)	Out of plane OH stretch
3.	1284.4(w)	1276.7(w)	1273.8(m)	C-O stretch
4.	1411.7(w)	1409.4(w)	1410.7(m)	CH3 umbrella mode
5.	1457.9(w)	1472.4(s)	1431.9 (broad)	Bending vibrations of aliphatic CH2 and CH3 group
6.	1707.7(vs)	1696.1(vs)	1708.6(vs)	C=O stretch
7.		2848.4(vs)		Symmetric and asymmetric vibration of the aliphatic CH2 group
8.	2853.2(s) 2919.7(s)	2914.0(vs) 2954.5(w)	2961.2(m)	Symmetric and asymmetric vibration of the aliphatic CH3 group
9	3008.5(w)		3013.3(s)	C-H stretching of C=C

Table S4 FTIR bands and assignments for ASTX-Fatty acid esters

S.No	C18		C21		DHA		Assignments
	Mono	**Di**	**Mono**	**Di**	**Mono**	**Di**	
1.	720.3(w)	721.3(w)	719.3(m)	719.3(m)	705.8(s)	703.9(s)	CH2 rocking
2.	831.2(w)	832.1(w)	829.3(vw)		830.2(w)	830.2(w)	-----
3.	964.3(vs)	960.4(vs)	960.4(vs) 974.9(s)	961.4(vs)	960.4(vs)	966.2(vs)	ASTAXANTHIN (CH deformation)
4.	1073.2(m)	1085.7(w)	1077.1(w)	1094.4(w)	1077.1(m)	1086.8(w)	-C-O stretching
5.	1158.1(m)	1160.0(m)	1158.1(s)	1159.0(s)	1151.3(s)	1151.3(s)	-C-O-C stretching,
6.	1238.1(w)	1238.1(w)	1239.1(vw)	1242.0(w)	1238.1(w)	1238.1(w)	CH2 bending
7.	1365.4(m)	1365.4(m)	1364.4(w)	1372.1(w)	1364.4(m)	1368.3(w)	-C-H(CH3) Bending
8.	1450.2(m)	1450.2(m, broad)	1471.4(m)	1471.4(m)	1436.7(m)	1439.6(vw)	Bending of aliphatic CH2 and CH3 group
9.	1555.3(m)	1558.2(m)	1558.2(w)	1557.3(w)	1557.3(s)	1556.3(w)	Methyl asymmetric deformation, C=C stretching
10.	1657.9(s)* 1679.2(s)*	1675.9(vs)	1657.3(s) 1692.3(s)	1674.9(s)	1656.6(vs) 1679.7(m)	1677.8(vs)	C=O stretching (Ketonic, ASTX) C=O stretching (ASTX ester)
11.	1739.5(s)	1739.5(vs)	1740.5(s)	1744.3(s)	1740.5(vs)	1740.5(vs)	
12.	2853.2(s)	2852.2(s)	2849.4(vs)	2849.4(vs)	2870.6(vw)	2869.6(vw)	CH2 sym.stretching
13.	2922.6(vs)	2921.7(vs)	2916.9(vs)	2917.8(vs)	2920.7(vw)	2918.8(vw)	CH2 asym.stretching
14.					2962.2(vw)	2963.1(w)	CH3 asym.stretching
15.	3001.7(w)	3001.3(w)			3011.3(m)	3012.3(m)	=C-H(cis) stretching
16.		3340.2(vw) 3366.5				3400 (b,vw)	-C=O ester overtone
17.	3480.0(w)		3498.3(w)		3498.3(w)		OH-from ASTX(only in mono-esters)

Table S5 ^{1}H NMR chemical shifts assignation for shrimp oil and astaxanthin extract

Peak number	δ (ppm)	Assignation
1	5.37	-C**H**=C**H**-
2	4.17, 4.13, 5.27	glycerol
3	2.39	-OCO-C$\mathbf{H_2}$-CH2-CH=CH- DHA
4	2.32	-OCO-C$\mathbf{H_2}$-
5	2.09	CH3-C$\mathbf{H_2}$-CH=CH- PUFA
6	2.05	-C$\mathbf{H_2}$-CH=CH-
7	1.69	-OCO-CH2-C$\mathbf{H_2}$- EPA
8	1.60	-OCO-CH2-C$\mathbf{H_2}$-
9	0.96	-C$\mathbf{H_3}$ PUFA
10	0.88	-C$\mathbf{H_3}$