Pak. J. Bot., 49(SI): 343-346, 2017.

PHYCOCHEMICAL INVESTIGATION OF BROWN ALGA *SARGASSUM TENERRIMUM* FROM KARACHI COAST

LAILA SHAHNAZ[1*], AMIR HAIDER[2] AND HINA SHEHNAZ[3]

[1]*Department of Botany, University of Karachi, Karachi, Pakistan*
[2]*Arysta Life Science Pakistan*
[3]*Department of Environmental Sciences, Sind Madressatul Islam University, Karachi-74000*
[*]*Corresponding author's email: lailashehnaz@hotmail.com*

Abstract

The thalli of brown alga *Sargassum tenerrimum* J. Agardh collected from upper and lower littoral deep rocky pools at Buleji, Manora and Paradise Point in the month of February 2008 to March 2009. The Gas chromatography–mass spectrometry (GC-MS) of the methylated fatty acids exhibited the presence of total 29 fatty acids, *i.e.* 12 saturated and 17 unsaturated fatty acids. The proportion of former acids was higher (70 %) than that of the later ones (30 %). Palmitic acid (C16:0) was present in the greatest quantity (34.12 %) followed by stearic acid (C18:0) which was present (13.01 %). Among unsaturated acids hexadecatrienoic acid (C16:3) was found in a highest amount (7.12 %). Cholesterol was identified by EIMS and ^{1}H-NMR.

Key words: *Sargassum*, Phaeophycota, Phycochemistry, Karachi

Introduction

A variety of brown seaweeds growing at the coastal areas of Pakistan which is spread over 880 Km, while the Karachi coast, mainly comprises of Buleji, Cape Monze, Hawkesbay, Manora, Nathiagali, Pacha, Paradise Point, and Sandspit covering 100 Km area and it is rich in algal vegetation (Shameel & Tanaka 1992). A variety of brown seaweeds have been phycochemically investigated by previous workers (Bano *et al*., 1987; Usmanghani *et al*., 1987a, 1987b; Ahmad *et al*., 1990; Shaikh *et al*., 1990a, 1990b; Ahmad *et al*., 1991; Atta–ur–Rahman *et al*., 1991; Shaikh *et al*., 1991a, 1991b; Shameel *et al*., 1991; Aslam *et al*., 1994; Shaikh & Shameel, 1999; Shahnaz & Shameel, 2007; Shaikh *et al*., 2009). The present work is a continuation of these studies. The *Sargassum tenerrimum* J. Agardh belongs to the family Sargassaceae (order Fucales, class Fucophyceae, phylum Phaephycota; *fide* Shameel, 2001; 2008; 2012) was studied for its fatty acids and sterols composition.

Materials and Methods

The fresh thalli of *Sargassum tenerrimum* J. Agardh were collected from three different areas of Karachi coast *i. e.* Buleji, Manora and Paradise Point during February 2008 – March 2009. The algal material was washed thoroughly in running water clear the specimens from epiphytes, epizoons, attached detritus material of animals and plants and sand particles. The clean thalli were again washed with distilled water and dried in dark at room temperature to avoid the disintegration of secondary metabolites under sunlight and high temperature. The dried algal material was cut into small pieces and ground. The pulverized specimens weighing 2 kg fresh weight were percolated with *n*-Hexane:chloroform (1:1, v/v) in a respirator at room temperature for a couple of weeks. The extract so obtained was evaporated under reduced pressure and partitioned between EtOAc and water (1:1, v/v) which on evaporation yielded 20 g of thick crude algal material. The crude substance was saponified, refluxed, esterified, methylated and then analyzed by GC-MS, this method has been described earlier for the detection of fatty acids (Shahnaz & Shameel, 2005). The method for the detection, isolation, purification and structure elucidation of sterols has also been described previously (Shahnaz & Shameel, 2006).

Results

The total fatty acids (FAs) composition of *Sargassum tenerrimum* J. Agardh have been present in Table 1, which reveals the presence of 12 saturated and 17 unsaturated fatty acids. The former were present in much larger quantity (70%) than the latter (30%). Among unsaturated fatty acids (UFAs), nine were monoenoic, four dienoic and four trienoic fatty acids. However, monoenic and trienoic (15.63% & 10.37%) and dienoic accounts for 4% of total FAs. This alga exhibited a great diversity in its fatty acid constitution. Palmitic acid (C16:0) was found to be in the largest proportion (34.12 %) followed by stearic acid (C18:0) and myristic acid (C14:0) were found (13 % and 7.17%) respectively, as compared to all other fatty acids. Among unsaturated ones Hexadecatrienoic acid (C16:3) was detected in the highest quantity (7.12 %), followed by oleic (C18:1) acid (4.55 %). All the other fatty acids occurred in a very small amount. No polyenoic fatty acid could be detected. Analysis of the sterol fraction afforded cholesterol in pure form (Table 1).

Discussion

In previous studies on two different species of *Sargassum* from Karachi coast *S. boveanum* and *S. swartzii* by (Qasim, 1986; Shahnaz & Shameel, 2007) found saturated fatty acids (SFAs) in a greater amount (76.76% & 75.61%) than unsaturated (23.22% & 24.16%). Palmitic acid (C16:0) was present in highest amounts in both the species (65.30%) and (39.80 %). While pentadecylic acid (C15:0, 14.70%) and myristic acid (C14:0, 11.55%) were found next dominant saturated

fatty acids (SFAs) in *S. swartzii*. Among unsaturated fatty acid composition of both species the oleic acid (C18:1, 11.37%) in *S. boveanum* and hexadecatrienoic acid (C16:3, 10.70%) in *S. swartzii* was detected in greatest quantity. As reported earlier in (Shahnaz & Shameel, 2007) for *S. swartzii* our study also revealed that (C16:3, 7.12%) was the most abundant acid among all other trienoic fatty acids.

A study from Korea (Kim *et al*., 2010) on *Sargassum thunbergii* revealed the presence of 23 fatty acids (FAs), and the main fatty acids were found to be arachidonic acid (C20:4, 10.407 mg/g) > arachidic acid (C20:0, 9.87 mg/g) > palmitic acid (C16:0, 7.663 mg/g) > elaidic acid (C18:1 *trans* n=9, 7.474 mg/g) >linoleic acid (C18:2, *trans* n=6, 7.465 mg/g) > stearic acid (C18:0, 5.55 mg/g) > cis-5,8,11,14,17-eicosanoic acid (C20:5 *trans* n=3, 4.367 mg/g) of total acids. The *Sargassum thunbergii* showed nutraceutical importance against (reactive oxygen species) ROS-induced tissue damage due to the presence of polyunsaturated fatty acids (PUFAs).

In *Sargassum kjellmanianum* and *Sargassum thunbergii* of Bohai Sea, (n-6) PUFAs and (n-3) PUFAs present in highest amount respectively. Palimitic acid (C16:0), was observed to be the most abundant acid among all investigated SFAs in both species (Li *et al*., 2002). While eicosapentaenoic acid (C20:5, (n-3) and arachidonic acid (C20:4, (n-6) acids account for (17.5% and 10.2%) in *Sargassum thunbergii* and the Oleic acid C18:1 was the major FAs in brown algae (Li *et al*., 2002).

The tetradecanoic acid (C14:0), hexadecanoic acid (C15:0), oleic acid (C18:1), 8-heptadecene, and 3,7,11,15-tetramethyl- 2-hexadecen-1-ol were the main components of *Sargassum fulvellum* and *Sargassum thunbergii* and after conducting the various trials on rats it was observed that both species of *Sargassum* can be used in health care sectors as a potent source of antipyretic, analgesic, anti-edemic and anti-inflammatory constituents (Kang *et al*., 2008).

Van Ginneken *et al*., 2011 stated that the *S. natans* of tropical seas has a distinctive quality of the occurrence of docosahexaenoic acid (C22:6, n-3), this is a major component of fish fatty acids, beside this the palmitic (C16:0) and oleic acids (C18:1, n-9) were also found in large quantity. The *S. natans* species also revealed the (n-6) FA / (n-3) FA ratio of about 0.55 (Van Ginneken *et al.,* 2011), according to the recommended value of the World Health Organization (WHO), this ratio should be < 10 to reduce the risk of inflammatory, cardiovascular and nervous system disorders (Sa´ nchez-Machado *et al*., 2004).

The fatty acids composition of *S. duplicatum* and *S. binderi* studied in Malaysia exhibited the occurrence of docosahexanoic acid (C22:6, n-3), eicosapentanoic acid (C20:5, n-3), arachidonic acid (C20:4, n-6), linoleic acid (C18:2, n-6) and alpha-linolenic acid (C18:3, n-3) contents, *S. duplicatum* was revealed the higher quantity of fatty acids (0.76, 2.55, 13.64, 5.81 and 5.35%) than *S. binderi* (0.70, 1.82, 9.13, 6.37 and 4.39%, consequently). Among saturated fatty acids, palmitic (C16:0) was found to be the dominant fatty acid (24.88-25.09 %) of the total FAs in both the species of *Sargassum* (Noviendri *et al*., 2011). The species of *S. binderi* and *S. duplicatum* have n-6: n-3 ratio between 0.87 and 1.73, is beneficial for health against the cancer, coronary infarction, inflammatory and autoimmune diseases (Simopoulus, 2002).

It has been reported from *S. polycystum,* palmitic (C16:0, 37.97%) and oleic acids (C18:1, n-9, 24.21%) were found in higher amount among total FAs contents. Furthermore, it contained linoleic acid (C18:2, n-6, 8.44%), eicosatrienoic acid (C20:3, n-3, 6.38%) and stearic acid (C18:0, 4.20%), on the other hand *S. polycystum* showed a small quantity of docosahexaenoic acid (C22:6, n-3, 0.13%) (Matanjun *et al.,* 2009).

A study on the fatty acids (FAs) composition of *S. marginatum, S. thunbergii* and *S. confusum* collected from the tropical and temperate waters showed that palmitic acid (C16:0) was common fatty acid among all three species. Its proportion was highest (43.76%) in *S. marginatum* as compared to *S. thunbergii* (24.12%) and *S. confusum* (21.05%). The C18 and C20 groups of fatty acids were found to be the most abundant PUFAs among total FAs contents (Narayan *et al*., 2004).

The results of the present study for palmitic acid (C16:0) content of saturated fatty acids of *S. tenerrimum,* have found similar to other species of *Sargassum* reported from Pakistan and other parts of the world (Qasim, 1986; Li *et al*., 2002; Narayan *et al*., 2004; Shahnaz & Shameel, 2007; Matanjun *et al.,* 2009; Kim *et al*., 2010; Noviendri *et al*., 2011; Van Ginneken *et al*., 2011).

This study revealed the presence of 29 fatty acids (FAs). The present piece of work reflects several interesting observation unlike previously studied species of *S. tenerrimum* and *S. swartzii* from the Karachi coast by (Shaikh *et al.,* 2010; Shahnaz & Shameel, 2007). The former one contained, total seven fatty acids (FAs), three were saturated and four unsaturated acids. The proportion of unsaturated FAs were slightly higher (51.2%) than that of saturated ones (48.75%). Palmitic acid was present in less quantity (7.26%), which was detected in *S. swartzii* (39.80%) (Shahnaz & Shameel, 2007) and in the present study of *S. tenerrimum* (34.12%). The tetradecatrienoic acid was not present in both the studies except in (Shaikh *et al*., 2010). Our observations have shown oleic acid (C18:1) 4.55%, which is very close to the amount detected in *S. swartzii* (4.38%). While this acid was totally absent in (Shaikh *et al.,* 2010). It is quite interesting to note that all the unsaturated fatty acids isolated here were mono-, di-, and trienoic acids but no polyenoic acids could be analyzed in this study. However, no monoenoic in (Shaikh *et al.,* 2010) and dienoic acids in (Shahnaz & Shameel, 2007) could be detected.

The most dominant sterol content of brown algae is fucosterol, according to (Patterson, 1971; Majik *et al.,* 2015) a large number of brown algae also contain traces of cholesterol and biosynthetic precursor of fucosterol. The particular sterols of genus *Sargassum* C. Agardh were sargasterol and saringosterol (Tsuda *et al.,* 1958).

The sterol isolated from *S. tenerrimum* in the present work was cholesterol. Though it is not a common sterol of brown seaweeds. This sterol component has also been reported from *S. swartzii* (Shahnaz & Shameel, 2007), but it could not be isolated from previously studied species of *S. tenerrimum* (Shaikh *et al.,* 2010). Cholesterol has also been reported from *S. oligocystum* and *S. glaucescens* as well as *S. accinarium* with other sterol contents (Kanias *et al.,* 1992; Permeh *et al.,* 2012; Payghami *et al.,* 2015).

Table 1. Fatty acids determined as methyl esters present in the *Sargassum tenerrimum*.

Acids type	Systematic name	Common name	Molecular formula	Mol wt	Rel %age
I. Saturated fatty acids				**70.00**	
C12:0	*n*-Dodecanoate	Laurate	$C_{13}H_{26}O_2$	214	0.71
C13:0	*n*-Tridecanoate	Tridecanoate	$C_{14}H_{28}O_2$	228	0.19
C14:0	*n*-Tetradecanoate	Myristate	$C_{15}H_{30}O_2$	242	7.17
C15:0	*n*- Pentadecanoate	Pentadecylate	$C_{16}H_{32}O_2$	256	0.88
C16:0	*n*-Hexadecanoate	Palmitate	$C_{17}H_{34}O_2$	270	34.12
C17:0	*n*-Heptadecanoate	Margarate	$C_{18}H_{36}O_2$	284	2.11
C18:0	*n*-Octadecanoate	Stearate	$C_{19}H_{38}O_2$	298	13.01
C19:0	*n*- Nonadecanoate	Nonadecylate	$C_{20}H_{40}O_2$	312	4.52
C20:0	n-Eicosanoate	Arachidate	$C_{21}H_{42}O_2$	326	3.00
C23:0	Tricosanoate	Tricosanoate	$C_{24}H_{48}O_2$	368	0.87
C24:0	*n*-Tetracosanoate	Lignocerate	$C_{25}H_{50}O_2$	382	3.21
C30:0	Hentriacontanoate	Melissate	$C_{31}H_{62}O_2$	466	0.21
II. Monoenoic fatty acids				**15.63**	
C14:1	9-Tetradecenoate	Myristoleate	$C_{15}H_{28}O_2$	240	0.21
C15:1	Pentadecenoate	Pentadecylenate	$C_{16}H_{30}O_2$	254	2.09
C16:1	*9*- Hexadecenoate	Palmitoleate	$C_{17}H_{32}O_2$	268	2.23
C17:1	Heptadecenoate	Heptadecylenate	$C_{18}H_{34}O_2$	282	1.42
C18:1	9-Octadecenoate	Oleate	$C_{19}H_{36}O_2$	296	4.55
C19:1	Nonadecenoate	Nonadecylenate	$C_{20}H_{38}O_2$	310	0.59
C20:1	9-Eicosenoate	Gadoleate	$C_{21}H_{40}O_2$	324	2.22
C22:1	11-Docosenoate	Cetolate	$C_{23}H_{44}O_2$	352	0.20
C26:1	17-Hexacosenoate	Hexacosenoate	$C_{27}H_{52}O_2$	408	2.12
III. Dienoic fatty acids				**4.00**	
C13:2	12-Tridecadienoate	-	$C_{14}H_{24}O_2$	224	0.55
C14:2	7-Ethyl-3-methyl-2,6-undecadienoate	-	$C_{15}H_{26}O_2$	238	1.02
C16:2	Hexadecadienoate	Hexdecadienoate	$C_{17}H_{30}O_2$	266	0.93
IV. Trienoic fatty acids				**10.37**	
C15:3	6,10,14-Hexadeca-trienoate	Hiragonate	$C_{16}H_{26}O_2$	250	1.01
C16:3	Hexadecatrienoate	Hexadecatrienoate	$C_{17}H_{28}O_2$	264	7.12
C17:3	Heptadecatrienoate	Heptadecatrienoate	$C_{18}H_{30}O_2$	278	1.77
C29:3	Nonacosatrienoate	-	$C_{30}H_{54}O_2$	446	0.55
V. Sterols:					
C27	Cholesterol				

Rel % age = relative percentage, Mol wt = molecular weight.

Acknowledgements

We are extremely grateful to Prof. Dr. Mustafa Shameel (Late) for species identification and his guidance during the whole study period.

References

Ahmad, V.U., S. Bano, W. Shaikh, S. Uddin and M. Shameel. 1990. Isolation and structure determination of 1,1,6,6-tetrachloro-3, 4-diphenyl hexane from brown alga *Dictyota dichotoma. Pak. J. Sci. Ind. Res.,* 33: 428-430.

Ahmad, V.U., S. Parveen, S. Bano, W. Shaikh and M. Shameel. 1991. Dolastane diterpenoids from the brown alga *Dictyota indica*. *Phytochem*., 30: 1015-1018.

Aslam, M., M. Ahmad, G.H. Rizwani, M. Anwar, V.U. Ahmad and M. Shameel. 1994. Fatty acids composition and antimicrobial activity of *Dictyopteris australis* (Phaeophyta). *Pak. J. Pharmacol.,* 11: 21-27.

Atta-ur-Rahman, Z. Shah, M.I. Choudhary, S.A. Abbas and M. Shameel. 1991. Stockerene - a noval linear metabolite from *Stockeyia indica*. *Fitoterapia,* 62: 77-80.

Bano, S., S. Perveen, V.U. Ahmad, Shafiuddin, N. Bano and M. Shameel. 1987. Chemical constituents of *Endarachne binghamiae* (Scytosiphonales, Phaeophyta). *Bot. Mar.,* 30: 371-372.

Kang, J.Y., M.N.A. Khan, N.H. Park, J.Y. Cho, M.C. Lee, H. Fujii and Y.K. Hong. 2008. Antipyretic, analgesic, and anti-inflammatory activities of the seaweed *Sargassum fulvellum* and *Sargassum thunbergii* in mice. *J. Ethnopharmacol.,*116: 187–190.

Kanias, G.D., H. Skaltsa, E. Tsitsa, A. Loukis and J. Bitis. 1992. Study of correlation between trace elements, sterols and fatty acids in brown algae of the Saronikos Gulf of Greece. *J. Anal. Chem.,* 344: 334-339.

Kim, J.A., C.S. Kong and S.K. Kim. 2010. Effect of *Sargassum thunbergii* on ROS mediated oxidative damage and identification of polyunsaturated fatty acid components. *Food & Chem. Toxicol*., 48: 1243-1249.

Li, X., X. Fan, L. Han and Q. Lou. 2002. Fatty acids of some algae from the Bohai Sea. *Phytochem*., 59: 157-161.

Majik, M.S., H. Adel, D. Shirodkar,S. Tilvi and J. Furtado. 2015. Isolation of stigmast-5,24-dien-3-ol from marine brown algae *Sargassum tenerrimum* and its antipredatory activity. *RSC Adv.*, 5: 51008-51011.

Matanjun, P., S. Mohamed, N. M. Mustapha and K. Muhammad. 2009. Nutrient content of tropical edible seaweeds, *Eucheuma cottonii, Caulerpa lentillifera* and *Sargassum polycystum*. *J. Appl. Phycol*., 21: 75-80.

Narayan B., K. Miyashita and M. Hosakawa. 2004. Comparative evaluation of fatty acid composition of different *Sargassum* (Fucales, Phaeophyta) species harvested from temperate and tropical waters. *J. Aqua. Food Prod. Technol*., 13: 53-70.

Noviendri D., I. Jaswir, H. M. Salleh, M. Taher, K. Miyashita and N. Ramli. 2011. Fucoxanthin extraction and fatty acid analysis of *Sargassum binderi* and *S. duplicatum. J. Medici Plants Res*., 5: 2405-2412.

Patterson, G. W. 1971. The distribution of sterols in algae. *Lipids*, 6: 120-127.

Payghami, N., S. Jamili, A. Rustaiyan, S. Saeidnia, M. Nikan and A.R. Gohari. 2015. Alpha-amylase inhibitory activity and sterol composition of the marine algae, *Sargassum glaucescens. Pharmacognosy Res.*, 7: 314-321.

Permeh, P., S. Saeidnia, A. Mashinchian-Moradi and A.R. Gohari. 2012. Sterols from *Sargassum oligocystum*, a brown algae from the Persian Gulf, and their bioactivity. *Nat. Prod. Res*., 26: 774-777.

Qasima, R. 1986. Studies on fatty acid composition of eighteen species of seaweeds from the Karachi coast. *J. Chem. Soc. Pak.,* 8: 223-230.

Sa´ nchez-Machado D.I., J. Lo´ pez-Cervantes, J. Lo´ pez-Herna´ ndez and P. Paseiro-Losada. 2004. Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chem*., 85: 39-444.

Shahnaz, L. and M. Shameel. 2005. Phycochemical studies on *Oscillatoria sancta* (Cyanophyta) from Karachi coast. *Int. J. Phycol. Phycochem*., 1: 193-198.

Shahnaz, L. and M. Shameel. 2006. Phycochemistry and bioactivity of some siphonaceous green algae from Karachi coast of Pakistan. *Int. J. Phycol. Phycochem.,* 2: 223-228.

Shahnaz, L. and M. Shameel. 2007. Phycochemistry and bioactivity of some brown algae from Karachi coast. *Int. J. Phycol. Phycochem.,* 3: 7-10.

Shaikh, W. and M. Shameel. 1999. Studies on the phycochemistry of *Cystoseira indica* (Fucales, Phaeophyta). *Pak. J. Mar. Biol.,* 5: 165-175.

Shaikh, W., M. Shameel and A. Zarina. 2009. Phycochemical studies on *Jolyna laminarioides* (Scytosiphonales) Phaeophycota. *Int. J. Phycol. Phycochem.,* 5: 135-142.

Shaikh, W., M. Shameel and A. Zarina. 2010. Fatty acid composition of *Sargassum tenerrimum* (Fucales, Phaeophycota). *Int. J. Phycol. Phycochem.,* 6: 29-32.

Shaikh, W., M. Shameel, A. Hayee-Memon, K. Usmanghani, S. Bano and V.U. Ahmad. 1990a. Isolation and characterization of chemical constituents of *Stoechospermum marginatum* (Dictyotales, Phaeophyta) and their antimicrobial activity. *Pak. J. Pharm. Sci.,* 3: 1-9.

Shaikh, W., M. Shameel, R. Khan, K. Usmanghani and V.U. Ahmad. 1990b. Phycochemical studies on *Spatoglossum variabile* (Dictyotales, Phaeophyta). *Pak. J. Bot.,* 22: 100-105.

Shaikh, W., M. Shameel, K. Usmanghani and V.U. Ahmad. 1991a. Phycochemical examination of *Padina tetrastromatica* (Dictyotales, Phaeophyta). *Pak. J. Pharm. Sci.,* 4: 55-61.

Shaikh, W., M. Shameel, V.U. Ahmad and K. Usmanghani. 1991b. Phycochemical studies on *Colpomenia sinuosa* (Scytosiphonales, Phaeophyta). *Bot. Mar.,* 34: 77-79.

Shameel, M. 2001. An approach to the classification of algae in the new millennium. *Pak. J. Mar. Biol.,* 7: 233-250.

Shameel, M. 2008. Change of divisional nomenclature in the Shameelian classification of algae. *Int. J. Phycol. Phycochem.,* 4: 225-232.

Shameel, M. 2012. Nomenclatural changes in the Shameelian classification of algae. *Int. J. Phycol. Phycochem.,* 8: 7-22.

Shameel, M. and J. Tanaka. 1992. A preliminary check list of marine algae from the coast and inshore waters of Pakistan. In: *Cryptogamic Flora of Pakistan*. Vol. 1, (Eds.): T. Nakaike and S. Malik, Nat. Sci. Mus., Tokyo, pp. 1-64.

Shameel, M., W. Shaikh and R. Khan. 1991. Comparative fatty acid composition of five species of *Dictyota* (Phaeophyta). *Bot. Mar.,* 34: 425-428.

Simopoulus , A.P. 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother*., 56: 365-379.

Tsuda, K., R. Hayatsu, Y. Kashida and S. Akagi.1958. Steroid studies IV. Studies on the constitution of sargasterol. *J. Am. Chem. Soc*., 80: 921 - 925.

Usmanghani, K., M. Shameel, S. Siddiqui and M. Alam. 1987a. Studies on the sterols of a brown seaweed *Iyengaria stellata* from Pakistan. *Pak. J. Bot.,* 19: 249-252.

Usmanghani, K., M. Shameel, S. Siddiqui and S.A. Siddiqui 1987b. Studies on the fatty acids of *Iyengaria stellata* (Scytosiphonales, Phaeophyta). *Bot. Mar.,* 30: 305-307.

Van Ginneken, V.J.T., J.P.F.G. Helsper, W. de Visser, H. van Keulen and W.A. Brandenburg. 2011. Polyunsaturated fatty acids in various macroalgal species from north Atlantic and tropical seas. *Lipids in Health & Diseases*, 10: 104-111.

(Received for publication 15 January 2016)