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Seasonal proximate and fatty acid variations of some seaweeds from the northeastern Mediterranean coast

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Abstract

The seasonal nutritional value of red (*Jania rubens*, *Laurencia papillosa*, *Spyridia filamentosa* and *Dasya rigidula*) and brown macroalgae (*Padina pavonia* and *Stypopodium schimperi*) was evaluated as a dietary supplement for human and animal nutrition based on proximate and fatty acid profiles. The protein content varied from 0.80% (*L. papillosa*) to 3.41% (*J. rubens*) of wet weight with the highest values in winter. The highest lipid levels were recorded in *S. schimperi* (2.03% in spring, 2.16% in summer), the lowest in *S. filamentosa* (0.08% in spring). The ash content of *J. rubens* (46.11–51.63%) was significantly higher than that of the other species (2.28–16.57%). Analysis of the fatty acid composition showed that these seaweed species are very rich in n-3 fatty acids.

DHA – docosahexaenoic acid,
EPA – eicosapentaenoic acid,
FA – fatty acid,
EFAs – essential fatty acids,
FAME – fatty acid methyl ester,
FID – flame ionization detector,
GC – gas chromatography.

1. Introduction

Seaweeds are reported to be rich in soluble dietary fibres, proteins, minerals, vitamins, antioxidants, phytochemicals and polyunsaturated fatty acids, with a low caloric value (Mohamed et al. 2012). Seaweeds have been consumed as food in Asian countries since ancient times. Nowadays, seaweeds are used as a raw material, especially in Japan, in the manufacture of many food products, such as jam, cheese, wine, tea, soup and noodles (Nisizawa et al. 1987). In Western countries, they serve mainly as a source of polysaccharides (alginates from brown seaweeds, agar, and carrageenans from red seaweeds) for nutritional and pharmaceutical uses (Indegaard & Ostgaard 1991, Mabeau & Fleurence 1993, Bárbara & Cremades 1993, Burtin 2003). Over the past few decades, the consumption of seaweed products has increased in European countries. Currently, approximately 15–20 edible algae strains are being marketed for consumption in Europe. These seaweed varieties differ greatly in their quality, colour, consistency and nutrient content (Dawczynski et al. 2007). In addition, their nutrient contents are affected by external factors such as geographical location, environment, season and sampling conditions (Nelson et al. 2002, Kostetsky et al. 2004, Renaud & Luong-Van 2006).

Commercially available varieties of marine macroalgae are commonly referred to as 'seaweeds'. Macroalgae are classified as red algae (Rhodophyta), brown algae (Phaeophyta) or green algae (Chlorophyta), depending on pigmentation, morphological and anatomical characters (Manivannan et al. 2009). Red and brown algae are mainly used as human food sources (Dawczynski et al. 2007). Seaweeds can be an important source of n-3 fatty acids, such as eicosapentaenoic acid – EPA (C20:5n3) and docosahexaenoic acid – DHA (C20:6n3) (Khotimchenko et al. 2002). These fatty acids are thought to reduce the risk of heart disease, thrombosis and atherosclerosis (Mishra et al. 1993).

Many studies have been performed worldwide to characterize algae species according to their chemical composition (Norziah & Ching 2000, Kamenarska et al. 2002, Sánchez-Machado et al. 2002, 2004, Rupérez 2002, Al-Masri et al. 2003, Abdallah et al. 2006, Manivannan et al. 2009). In view of the currently increasing demand for seaweed products, the aim of

this work was to determine the seasonal variations of proximate and fatty acid profiles of some marine seaweeds from the north-eastern Mediterranean coast.

2. Material and methods

2.1. Sample collection

Sampling was carried out on the north-eastern Mediterranean coast of Turkey in 2008 (Figure 1). To determine seasonal variations, samples were collected in spring (April–May), summer (July), autumn (October) and winter (December). The six species selected were sampled in at least two seasons. Four of the species – *Jania rubens* (Linnaeus) Lamouroux, *Laurencia papillosa* (Agardh) Greville, *Spyridia filamentosa* (Wulf.) Harv. and *Dasya rigidula* (Kütz.) Ardiss. – belong to Rhodophyta (red seaweeds), while the other two – *Padina pavonia* (Linnaeus) Gaillon and *Stypopodium schimperi* (Buchinger ex Kütz.) Verlaque et Boudour – belong to Phaeophyta (brown seaweeds).

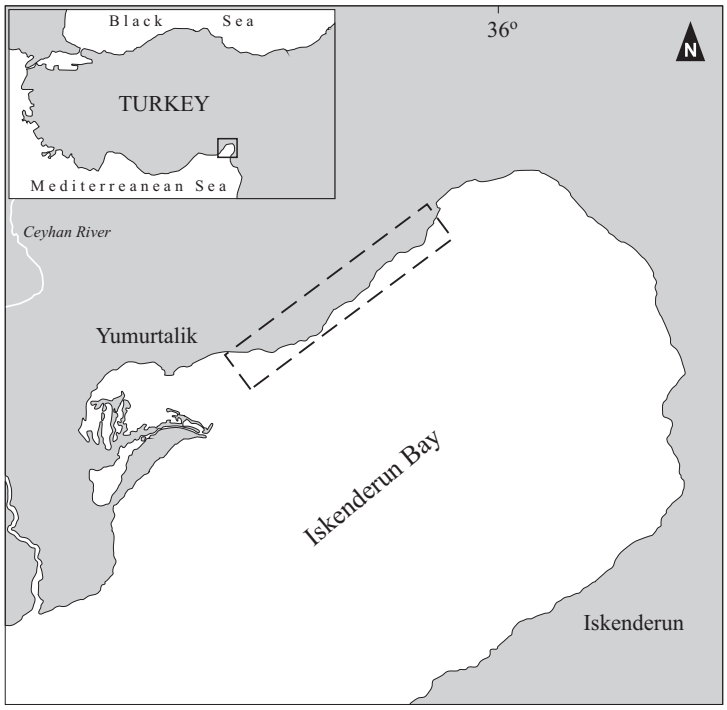

Figure 1. Map of the study site

Each seaweed sample was washed thoroughly with seawater to remove all unwanted impurities, adhering sand particles and epiphytes. Then, the sample was again washed thoroughly, this time with distilled water, to remove all the salt from the surface. The water was drained off and the seaweed spread on blotting paper to remove excess water. Subsequently, the samples were packaged in polyethylene bags and stored at −86°C prior to chemical analysis.

2.2. Proximate composition

The seaweed samples were analysed in triplicate for their proximate composition. The lipid content was determined by the Bligh & Dyer (1959) method. Approximately 10 g of sample was homogenized with a 1:2 mixture of chloroform and methanol and left in a dark place overnight after adding $CaCl_2$. The chloroform layer was removed and then vaporized in an evaporator and finally in an oven at 105°C for 30 minutes. The lipid content was calculated using the differences between the flask weights. Three replicates were used.

Total crude protein was determined by the Kjeldahl method (protein conversion factor: 6.25) (AOAC 1998a). Approximately 1 g of seaweed was digested for 2 hours with concentrated sulphuric acid (20 ml) and two Kjeltec catalyst tablets. After digestion, 75 ml of water was added. The distillation was performed using a Kjeldahl Distilling Unit. 25 ml of boric acid was poured into a 250 ml conical flask, which was placed in the distilling unit on the flask platform; the digested materials were then added to the unit. 50 ml 40% sodium hydroxide was added to the digest and the steam valve turned on. The boric acid solution in the flask receiving the distilled ammonia changed colour from red to green. After that, the contents of the flask were titrated against standard hydrochloric acid until the grey colour end point.

The moisture and ash contents of seaweeds were determined by the AOAC (1998b) method. The moisture content was determined by oven-drying of 5 g of seaweed at 105°C until a constant weight was obtained. The ash content was determined by placing samples in a muffle furnace at 550°C for 24 h and then weighing the residue.

2.3. Fatty acid methyl ester (FAME) analysis

The fatty acid profiles of the fat extracted from the seaweed samples were determined by gas chromatography (GC) of FAMEs. Boron trifluoride/methanol was used to prepare the FAMEs (AOAC 1990).

The fatty acids were analysed qualitatively and quantitatively using a GC Clarus 500 with autosampler (Perkin Elmer, USA) equipped with

a flame ionization detector and a fused silica capillary SGE column (30 m × 0.32 mm ID × 0.25 μm BP20 0.25 UM, USA). The oven temperature was 140°C, held for 5 min, raised to 200°C at 4°C min^{-1} and held at 220°C at 1°C/min; the injector and the detector temperatures were set at 220°C and 280°C respectively. The sample size was 1 μl and the carrier gas was controlled at 16 psi. a 1:100 split ratio was used. The fatty acids were identified by comparing the retention times of FAMEs in the samples with a standard 37 component FAME mixture. Two replicate GC analyses were performed and the results were expressed as the GC area (%) for each fatty acid as the mean value ± standard deviation.

2.4. Statistical analysis

All the data obtained separately for each sampling season were subjected to analysis of variance (one-way ANOVA) at the 5% confidence level using the Duncan multiple range test.

3. Results

The seasonal variations in the proximate compositions of seaweeds based on wet weight (w.w.) are shown in Table 1 (the results are also expressed on a dry weight basis (%) in parentheses). The protein content varied among the species from 0.80% w.w. (*L. papillosa*) to 3.41% w.w. (*J. rubens*). Protein contents in all the species were generally low in summer. The lipid contents of seaweed species were generally $< 2\%$ w.w., with the highest value (2.16% w.w.) being found for the brown alga *S. schimperi* in summer. The lipid level was the lowest in *S. filamentosa* (0.08% w.w.) in spring. The ash content of *J. rubens* was significantly higher (51.63%) than in all the other species. Distinctly low levels of ash from 2.28 to 16.57% were found in the remaining species. The moisture content of seaweeds fluctuated significantly ($p < 0.05$) across all seasons, except in the case of *S. filamentosa* and *J. rubens.* The highest moisture content was obtained from *L. papillosa* (90.07%) in spring and summer, whereas *J. rubens* contained the lowest amount of water (37.98–42.78%) in all seasons.

The fatty acid compositions of the species examined are summarized in Table 2. The fatty acids detected in macroalgae were myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1 n-9), linoleic acid (C18:2n-6), linolenic acid (C18:3n3), arachidonic acid (C20:4n6), cis-5,8,11,14,17-eicosapentaenoic acid (EPA, C20:5n-3) and cis-4, 7,10,13,16,19-docosahexaenoic acid (DHA, C22:6 n-3). The fatty acid compositions of each species ranged from 28.86–60.43% saturated (SFA), to 12.52–32.94% monounsaturated (MUFAs) and 5.54–41.42% polyunsaturated fatty acids (PUFAs).

4. Discussion

Although all the species displayed variations in the total lipid content in all the seasons (Table 1), no significant seasonal variation in lipid content was observed in them, apart from *J. rubens* and *P. pavonia* in autumn and *S. filamentosa* in spring ($p < 0.05$). The lipid contents of the species, expressed as wet weight, turned out to be very low. The highest lipid content was found in *S. schimperi* (2.03% in spring, 2.16% in summer). The lowest lipid levels were exhibited by *S. filamentosa* in spring (0.08%) and by *J. rubens* in autumn (0.11%). Similar results were reported in previous studies (Polat & Ozogul 2008, 2009, Matanjun et al. 2009). Herbreteau et al. (1997) found that total lipid contents of seaweeds were always less than 4%. This generalized evaluation is supported by the findings of Sánchez-Machado et al. (2004) and Shanmugam & Palpandi (2008). Expressed as dry weight (%), the lipid contents of the six species ranged from 0.19% to 11.58%. The highest dry weight lipid content was recorded in *S. schimperi* (11.58% in spring, 9.98% in summer), and the lowest in *J. rubens* (0.19% in autumn, 0.35% in spring).

The protein content of seaweed varieties varies greatly and depends on such factors as season and environmental growth conditions (Mishra et al. 1993, Fleurence 1999). In this study, the protein contents of macroalgae changed significantly ($p < 0.05$) during all the seasons. On a wet weight basis, *L. papillosa* had the lowest level of protein, whereas *J. rubens* had a higher protein content throughout the year (apart from summer) (Table 1). Similar protein contents for these macroalgae classes were reported in other studies (Fleurence 1999, Kolb et al. 1999, Rupérez & Saura-Calixto 2001, Polat & Ozogul 2008, 2009). On a dry weight basis, the results showed that *J. rubens* had the lowest level (2.90%) of protein in summer, whereas *D. rigidula* contained an appreciable amount of protein (23.50%) in autumn.

All seaweeds contain large amounts of both macro-minerals (Ca, Mg, Na, P and K) and trace elements (Zn, I and Mn) (Matanjun et al. 2009, Polat & Ozogul 2009). Seaweeds generally contain 8–40% of minerals, and the essential minerals and trace elements needed for human nutrition are present in seaweeds (Mabeau & Fleurence 1993). The mineral contents of seaweeds are reported to vary according to such factors as species, geographical origin, seasons, environmental and physiological variations (Mabeau & Fleurence 1993, Kaehler & Kennish 1996). In this study, significant seasonal differences in the content of crude ash (from 2.28% for *L. papillosa* to 51.63% for *J. rubens* in spring) were found ($p < 0.05$) in all the species except *S. filamentosa*.

Table 1. Seasonal variations in the proximate composition of seaweeds on the basis of wet weight [%]*

Seaweeds	Component	Seasons			
		Spring	Summer	Autumn	Winter
	Protein				
J. rubens		2.83 ± 0.35^{b} (4.56)	1.66 ± 0.05^{a} (2.90)	2.22 ± 0.04^{b} (3.73)	3.41 ± 0.17^{c} (5.61)
P. pavonia		1.97 ± 0.46^{a} (8.86)	1.65 ± 0.15^{a} (5.80)	1.81 ± 0.08^{a} (8.00)	n.s.
L. papillosa		1.22 ± 0.27^{a} (12.29)	0.80 ± 0.33^{a} (7.10)	1.16 ± 0.24^{a} (4.75)	n.s.
S. schimperi		2.68 ± 0.06^{a} (15.29)	2.37 ± 0.24^{a} (10.95)	n.s.	n.s.
D. rigudula		n.s.	2.61 ± 0.02^{a} (14.57)	2.90 ± 0.47^{a} (23.50)	n.s.
S. filamentosa		1.98 ± 0.20^{a} (10.55)	2.81 ± 0.03^{b} (15.65)	n.s.	n.s.
	Lipid				
J. rubens		0.22 ± 0.01^{a} (0.35)	0.33 ± 0.07^{a} (0.58)	0.11 ± 0.01^{b} (0.19)	0.23 ± 0.05^{a} (0.38)
P. pavonia		0.86 ± 0.19^{a} (3.87)	0.87 ± 0.09^{a} (3.06)	0.65 ± 0.13^{b} (2.87)	n.s.
L. papillosa		0.21 ± 0.07^{a} (2.12)	0.26 ± 0.04^{a} (2.31)	0.23 ± 0.01^{a} (0.94)	n.s.
S. schimperi		2.03 ± 0.21^{a} (11.58)	2.16 ± 0.52^{a} (9.98)		n.s.
D. rigudula		n.s.	0.34 ± 0.05^{a} (1.90)	0.31 ± 0.04^{a} (2.51)	n.s.
S. filamentosa		0.08 ± 0.04^{a} (0.43)	0.23 ± 0.01^{b} (1.28)	n.s.	n.s.
	Moisture				
J. rubens		37.98 ± 2.49^{a}	42.78 ± 2.22^{a}	40.53 ± 0.12^{a}	39.24 ± 1.35^{a}
P. pavonia		77.77 ± 0.75^{a}	71.56 ± 1.61^{b}	77.37 ± 0.26^{a}	n.s.
L. papillosa		90.07 ± 0.22^{a} (9.93)	88.74 ± 0.60^{b}	75.59 ± 0.56^{c}	n.s.
S. schimperi		82.47 ± 0.52^{a}	78.35 ± 1.24^{b}	n.s.	n.s.
D. rigudula		n.s.	82.09 ± 1.31^{a}	87.66 ± 0.72^{b}	n.s.
S. filamentosa		81.23 ± 0.21^{a}	82.05 ± 0.29^{a}	n.s.	n.s.

Table 1. (*continued*)

Seaweeds	Component	Seasons			
		Spring	Summer	Autumn	Winter
	Ash				
J. rubens		51.63 ± 2.64^{a} (83.25)	46.11 ± 1.53^{b} (80.58)	46.02 ± 2.47^{ab} (77.58)	48.82 ± 0.88^{a} (80.35)
P. pavonia		9.71 ± 0.90^{a} (43.68)	$3.68 \pm 0.98b^{c}$ (48.10)	11.81 ± 1.06^{c} (52.19)	n.s.
L. papillosa		2.28 ± 0.15^{a} (22.96)	3.73 ± 0.34^{b} (33.13)	16.57 ± 0.69^{c} (67.88)	n.s.
S. schimperi		2.73 ± 0.14^{a} (15.57)	3.75 ± 0.42^{b} (17.32)	n.s.	n.s.
D. rigudula		n.s.	7.27 ± 0.88^{a} (40.59)	3.00 ± 0.56^{a} (24.31)	n.s.
S. filamentosa		5.61 ± 0.15^{a} (29.89)	5.98 ± 0.41^{a} (33.31)	n.s.	n.s.

*Different letters (a–d) in the same row indicate significant differences ($p < 0.05$); n.s.: no sample.

4.1. Fatty acid profiles

The results of the fatty acid composition analysis showed that macroalgae are very rich in n-3 fatty acids (Table 2). The fatty acid compositions of each species were in the following ranges: saturated (SFA) – 28.86–60.43%, monounsaturated (MUFAs) – 12.52–32.94% and polyunsaturated fatty acids (PUFAs) – 5.54–41.42%. The percentages of SFA in *S. schimperi* and *P. pavonia* were found to be lower than in the other species in all the seasons. The highest MUFA content was obtained for *D. rigidula* in autumn (32.94%), whereas *J. rubens* had the lowest MUFA level in spring.*D. rigidula* contained the lowest amount of PUFA (< 9%), while *J. rubens* contained a high level of PUFA (> 22%) throughout the year.

The highest proportions of fatty acids in macroalgae were calculated for myristic acid (C14:0, 2.49–7.89%), palmitic acid (C16:0, 21.88–45.15%), palmitoleic acid (C16:1, 3.82–15.34%), stearic acid (C18:0, 0.53–5.75%), oleic acid (C18:1 n-9, 4.0–17.38%), linoleic acid (C18:2n-6, 0.93–16.68%), linolenic acid (C18:3n3, 0.14-6.42%), arachidonic acid (C20:4n6, 0.94–9.83%), cis-5,8,11,14,17-eicosapentaenoic acid (EPA, C20:5n-3, 0.99–24.47%) and cis-4, 7,10,13,16,19-docosahexaenoic acid (DHA, C22:6 n-3, 0.15–3.82%). These results are in agreement with previous studies on fatty acid distributions in macroalgae (Polat & Ozogul 2008, 2009). It was

Table 2. Fatty acid profiles of *Jania rubens*, *Padina pavonia* and *Laurencia papillosa*

Fatty acids	*Jania rubens*				*Padina pavonia*			*Laurencia papillosa*		
[%]	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Spring	Summer	Autumn
C10:0	0.03 ± 0.0^{a}	–	0.07 ± 0.02^{b}	0.03 ± 0.0^{a}	0.04 ± 0.01^{a}	–	–	–	–	–
C11:0	3.53 ± 0.19^{a}	0.92 ± 0.22^{b}	3.61 ± 0.33^{a}	3.02 ± 0.11^{a}	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.03 ± 0.0^{b}	–	0.23 ± 0.03^{b}	–
C12:0	0.10 ± 0.0^{a}	0.22 ± 0.03^{b}	0.29 ± 0.0^{b}	0.14 ± 0.0^{a}	0.15 ± 0.02^{a}	0.04 ± 0.01^{b}	0.06 ± 0.02^{b}	1.3 ± 0.21^{a}	0.52 ± 0.05^{b}	0.47 ± 0.19^{b}
C13:0	0.13 ± 0.03^{a}	0.10 ± 0.01^{a}	0.17 ± 0.0^{b}	0.13 ± 0.0^{a}	–	0.03 ± 0.0^{a}	0.05 ± 0.01^{a}	–	–	–
C14:0	2.57 ± 0.21^{a}	2.79 ± 0.05^{a}	2.64 ± 0.03^{a}	2.49 ± 0.02^{a}	5.42 ± 0.29^{a}	3.96 ± 0.08^{b}	4.43 ± 0.18^{b}	6.66 ± 0.02^{a}	6.12 ± 0.83^{a}	4.95 ± 0.4^{b}
C15:0	0.76 ± 0.01^{a}	0.85 ± 0.04^{a}	1.12 ± 0.01^{b}	1.01 ± 0.04^{b}	0.44 ± 0.07^{a}	0.38 ± 0.01^{a}	0.44 ± 0.02^{a}	0.95 ± 0.02^{a}	1.35 ± 0.18^{b}	1.08 ± 0.03^{ac}
C16:0	29.85 ± 3.15^{a}	27.61 ± 1.09^{a}	33.78 ± 0.47^{b}	32 ± 0.45^{b}	24.46 ± 2.6^{a}	25.17 ± 0.66^{a}	23.30 ± 1.02^{a}	35.27 ± 0.79^{a}	34.67 ± 0.56^{a}	28.23 ± 0.14^{b}
C17:0	0.13 ± 0.01^{a}	0.20 ± 0.01^{b}	0.23 ± 0.03^{b}	0.16 ± 0.02^{c}	0.14 ± 0.04^{a}	0.11 ± 0.02^{a}	0.08 ± 0.01^{a}		0.63 ± 0.17^{a}	0.74 ± 0.02^{a}
C18:0	0.75 ± 0.08^{a}	1.69 ± 0.37^{b}	1.75 ± 0.11^{b}	0.89 ± 0.01^{a}	1.67 ± 0.63^{a}	1.75 ± 1.0^{a}	0.61 ± 0.06^{b}	1.96 ± 0.07^{a}	2.46 ± 0.08^{b}	3.03 ± 0.03^{c}
C20:0	0.12 ± 0.04^{a}	0.16 ± 0.01^{a}	0.12 ± 0.01^{a}	0.10 ± 0.0^{a}	0.34 ± 0.2^{a}	0.16 ± 0.0^{b}	0.26 ± 0.09^{a}	–	–	1.06 ± 0.03^{a}
C21:0	–	–	–	–	–	–	0.16 ± 0.09^{a}	–	–	–
C22:0	0.05 ± 0.01^{a}	–	–	–	–	–	–	–	–	–
C23:0	1.79 ± 0.59^{b}	0.67 ± 0.11^{a}	2.6 ± 0.04^{c}	2.04 ± 0.0^{b}	–	–	–	–	–	–
C24:0	0.27 ± 0.01^{a}	–	2.64 ± 0.0^{c}	0.18 ± 0.01^{b}		0.04 ± 0.01^{a}		–	–	–
Σ SFA	**40.08**	**35.21**	**49.02**	**42.19**	**32.71**	**31.70**	**29.42**	**46.14**	**45.98**	**39.56**
C14:1	–	–	–	0.05 ± 0.0^{a}	–	0.05 ± 0.03^{a}	0.07 ± 0.04^{a}	–	–	–
C15:1	–	–	–	0.06 ± 0.0^{a}	–	0.03 ± 0.0^{a}	0.05 ± 0.01^{a}	–	–	–
C16:1	5.15 ± 0.34^{a}	7.51 ± 0.5^{b}	8.28 ± 0.21^{c}	7.35 ± 0.12^{b}	7.16 ± 0.14^{a}	6.75 ± 0.06^{b}	7.74 ± 0.15^{a}	8.64 ± 0.11^{a}	5.67 ± 1.08^{b}	14.35 ± 0.61^{c}
C17:1	0.57 ± 0.08^{a}	0.09 ± 0.01^{b}	0.60 ± 0.01^{a}	0.65 ± 0.0^{a}	0.88 ± 0.07^{a}	0.24 ± 0.0^{b}	0.18 ± 0.02^{b}		0.41 ± 0.05^{a}	0.49 ± 0.01^{a}
C18:1 (n-9)	4.36 ± 0.18^{a}	6.74 ± 0.86^{b}	5.26 ± 0.22^{b}	4.08 ± 0.18^{a}	15.51 ± 1.17^{a}	14.02 ± 0.54^{a}	11.57 ± 0.42^{b}	8.29 ± 0.22^{a}	13.01 ± 0.76^{b}	8.07 ± 0.09^{a}
C20:1	0.56 ± 0.04^{a}	0.44 ± 0.04^{b}	0.69 ± 0.05^{a}	0.88 ± 0.01^{c}	0.96 ± 0.04^{a}	0.51 ± 0.31^{a}	0.16 ± 0.09^{b}	–	0.86 ± 0.53^{a}	0.84 ± 0.03^{a}
C22:1 (n-9)	1.26 ± 0.06^{a}	0.41 ± 0.04^{b}	0.53 ± 0.03^{b}	0.74 ± 0.0^{c}	–	–	0.10 ± 0.01^{a}	–	–	–
C24:1	0.62 ± 0.01^{a}	–	0.81 ± 0.01^{b}	0.6 ± 0.02^{a}	–	0.06 ± 0.02^{a}	–	–	–	–

Table 2. (*continued*)

Fatty acids	*Jania rubens*				*Padina pavonia*			*Laurencia papillosa*		
[%]	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Spring	Summer	Autumn
Σ MUFA	**12.52**	**15.19**	**16.17**	**14.41**	**24.51**	**21.66**	**19.87**	**16.93**	**19.95**	**23.75**
C18:2 (n-6)	1.05 ± 0.03^{a}	16.68 ± 0.03^{c}	1.51 ± 0.24^{b}	0.93 ± 0.01^{a}	11.94 ± 1.73^{a}	7.61 ± 0.51^{b}	6.13 ± 0.03^{c}	5.04 ± 0.15^{a}	2.66 ± 0.6^{b}	4.54 ± 0.19^{a}
C18:3 (n-6)	0.10 ± 0.04^{a}	0.12 ± 0.06^{a}	0.10 ± 0.03^{a}	0.09 ± 0.0^{a}	0.46 ± 0.08^{a}	1.10 ± 0.01^{b}	1.32 ± 0.04^{b}	0.31 ± 0.01^{a}	0.39 ± 0.0^{a}	
C18:3 (n-3)	1.11 ± 0.21^{a}	2.48 ± 0.85^{b}	0.97 ± 0.15^{a}	0.68 ± 0.1^{c}	5.36 ± 1.03^{a}	5.31 ± 0.16^{a}	6.42 ± 0.35^{a}	1.08 ± 0.28^{a}	–	1.15 ± 0.02^{a}
C20:2 cis	1.60 ± 0.12^{a}	0.45 ± 0.02^{b}	0.55 ± 0.01^{b}	0.67 ± 0.01^{b}	0.1 ± 0.03^{a}	0.19 ± 0.01^{b}	0.23 ± 0.01^{b}		–	0.35 ± 0.02^{a}
C20:3 (n-6)	0.85 ± 0.23^{a}	0.86 ± 0.08^{a}	0.59 ± 0.1^{b}	0.64 ± 0.03^{b}	1.23 ± 0.05^{a}	1.24 ± 0.04^{a}	1.23 ± 0.1^{a}	0.86 ± 0.0^{a}	0.55 ± 0.05^{b}	0.26 ± 0.01^{c}
C20:3 (n-3)	–	–	0.1 ± 0.01^{a}	–	–	–	–	–	–	–
C20:4 (n-6)	4.43 ± 0.07^{a}	6.61 ± 0.13^{b}	5.41 ± 0.06^{c}	6.31 ± 0.0^{b}		8.65 ± 0.1^{a}	8.82 ± 0.51^{a}	8.17 ± 0.1^{a}	9.83 ± 0.0^{b}	6.02 ± 0.34^{c}
C20:5 (n-3)	24.47 ± 0.94^{a}	13.86 ± 0.39^{b}	12.18 ± 0.02^{b}	21.15 ± 0.3^{c}	3.82 ± 0.26^{a}	1.88 ± 0.01^{b}	2.10 ± 0.03^{c}	18.42 ± 0.83^{a}	13.91 ± 0.32^{b}	7.31 ± 0.38^{c}
C22:2	–	–	–	–	–	0.07 ± 0.01^{a}	–	–	–	
C22:6 (n-3)	0.53 ± 0.05^{a}	0.36 ± 0.02^{b}	0.81 ± 0.11^{c}	0.46 ± 0.04^{b}	0.28 ± 0.08^{a}	0.13 ± 0.01^{b}	–	–		2.44 ± 0.23^{a}
Σ PUFA	**34.14**	**41.42**	**22.22**	**30.93**	**23.19**	**26.18**	**26.25**	**33.57**	**27.26**	**22.46**
PUFA/SFA	**0.85**	**1.17**	**0.45**	**0.73**	**0.70**	**0.82**	**0.89**	**0.72**	**0.59**	**0.56**
Σ n-6	**6.43**	**24.27**	**7.61**	**7.97**	**13.63**	**18.60**	**17.50**	**14.07**	**13.35**	**11.21**
Σ n-3	**26.11**	**16.70**	**14.06**	**22.29**	**9.46**	**7.32**	**8.52**	**19.50**	**13.91**	**10.09**
n6/n3	**0.24**	**1.45**	**0.54**	**0.35**	**1.44**	**2.54**	**2.05**	**0.75**	**0.95**	**1.11**
DHA/EPA	**0.02**	**0.02**	**0.06**	**0.02**	**0.07**	**0.06**			**0.33**	
Unknown	**13.26**	**8.18**	**12.59**	**12.47**	**19.59**	**20.46**	**24.46**	**3.36**	**6.81**	**14.23**

*Different letters (a–c) in the same row indicate significant differences ($p < 0.05$).

Table 3. Fatty acid profiles of *Stypopodium schimperi*, *Dasya rigidula* and *Spyridia filamentosa*.

Fatty acids	*Stypopodium schimperi*		*Dasya rigidula*		*Spyridia filamentosa*	
[%]	Spring	Summer	Summer	Autumn	Spring	Summer
C10:0	0.02 ± 0.01^{a}	–	–	–	0.09 ± 0.01^{a}	0.16 ± 0.12^{a}
C11:0	0.02 ± 0.01^{a}	0.07 ± 0.01^{b}	1.45 ± 0.11^{a}	0.25 ± 0.01^{b}	2.76 ± 0.16^{a}	0.51 ± 0.47^{b}
C12:0	0.07 ± 0.01^{a}	0.13 ± 0.04^{b}	0.85 ± 0.09^{a}	0.39 ± 0.01^{b}	0.21 ± 0.01^{a}	0.21 ± 0.13^{a}
C13:0	0.03 ± 0.0^{a}	–	–	–	–	–
C14:0	5.47 ± 0.03^{a}	4.63 ± 0.17^{b}	7.35 ± 0.15^{a}	7.89 ± 0.65^{a}	3.98 ± 0.17^{a}	4.11 ± 0.15^{b}
C15:0	0.37 ± 0.23^{a}	0.7 ± 0.23^{a}	1.17 ± 0.28^{a}	0.77 ± 0.03^{b}	0.54 ± 0.02^{a}	0.56 ± 0.07^{a}
C16:0	21.88 ± 0.05^{a}	23.01 ± 0.04^{b}	45.15 ± 0.22^{a}	43.45 ± 1.09^{a}	39.65 ± 0.83^{b}	42.41 ± 0.18^{b}
C17:0	0.16 ± 0.02^{a}	0.15 ± 0.04^{a}	0.54 ± 0.24^{a}	–	–	0.09 ± 0.03^{a}
C18:0	0.53 ± 0.05^{a}	0.55 ± 0.06^{a}	3.2 ± 0.36^{a}	1.10 ± 0.01^{b}	1.41 ± 0.06^{a}	5.75 ± 0.39^{b}
C20:0	–	–	0.72 ± 0.06^{a}	–	–	0.2 ± 0.0^{a}
C21:0	–	–	–	–	–	–
C22:0	–	0.1 ± 0.01^{a}	–	–	0.45 ± 0.03^{a}	–
C23:0	0.15 ± 0.01^{a}	0.2 ± 0.01^{a}	–	0.64 ± 0.04^{a}	0.69 ± 0.15^{a}	0.26 ± 0.08^{b}
C24:0	0.16 ± 0.01^{a}	0.23 ± 0.02^{b}	–		2.4 ± 0.24^{a}	1.08 ± 0.13^{a}
Σ SFA	**28.86**	**29.77**	**60.43**	**54.49**	**52.18**	**55.34**
C14:1	0.08 ± 0.02^{a}	0.09 ± 0.07^{a}	–	–	–	–
C15:1	0.09 ± 0.01^{a}	0.05 ± 0.01^{a}	–	–	–	–
C16:1	3.82 ± 0.02^{a}	5.19 ± 0.21^{b}	10.59 ± 0.13^{a}	15.34 ± 0.72^{b}	12.09 ± 0.3^{a}	9.86 ± 0.14^{b}
C17:1	0.47 ± 0.0^{a}	0.19 ± 0.03^{b}	0.65 ± 0.0^{a}	0.22 ± 0.01^{b}		0.31 ± 0.13^{a}
C18:1 (n-9)	13.9 ± 0.19^{a}	15.42 ± 0.01^{b}	11.93 ± 0.84^{a}	17.38 ± 0.32^{b}	5.04 ± 0.03^{a}	4 ± 0.01^{b}
C20:1	–	0.09 ± 0.0^{a}	0.26 ± 0.01^{a}	–	–	–
C22:1 (n-9)	–	–	–	–	2.4 ± 0.24^{a}	–
C24:1	–	–	–	–	–	–

Table 3. (*continued*)

Fatty acids	*Stypopodium schimperi*		*Dasya rigidula*		*Spyridia filamentosa*	
[%]	Spring	Summer	Summer	Autumn	Spring	Summer
Σ MUFA	**18.36**	**21.03**	**23.43**	**32.94**	**19.53**	**14.17**
C18:2 (n-6)	1.26 ± 0.01^{a}	2.82 ± 0.02^{b}	2.02 ± 0.15^{a}	1.65 ± 0.16^{b}	1.18 ± 0.07^{a}	1.28 ± 0.2^{a}
C18:3 (n-6)	0.41 ± 0.02^{a}	a 0.97 ± 0.08^{b}	–	0.16 ± 0.0^{a}	–	0.40 ± 0.05^{a}
C18:3 (n-3)	2.40 ± 0.02^{a}	1.30 ± 0.03^{b}	0.34 ± 0.03^{a}	0.14 ± 0.01^{b}	0.52 ± 0.06^{a}	1.30 ± 0.01^{b}
C20:2 cis	6.91 ± 0.04^{a}	5.18 ± 0.0^{b}	–	–	0.23 ± 0.01^{a}	2.84 ± 0.47^{b}
C20:3 (n-6)	0.54 ± 0.02^{a}	0.44 ± 0.02^{a}	–	–	–	0.40 ± 0.07^{a}
C20:3 (n-3)	–	–	–	–	–	–
C20:4 (n-6)	1.33 ± 0.03^{a}	2.32 ± 0.15^{b}	1.85 ± 0.17^{a}	0.94 ± 0.14^{b}	5.09 ± 0.82^{a}	13.88 ± 0.03^{b}
C20:5 (n-3)	4.07 ± 0.1^{a}	2.37 ± 0.13^{b}	4.36 ± 0.35^{a}	2.65 ± 0.37^{b}	0.99 ± 0.14^{a}	1.36 ± 0.04^{b}
C22:2	0.38 ± 0.02^{a}	0.26 ± 0.01^{b}	–	–	–	–
C22:6 (n-3)	0.15 ± 0.0^{a}	0.22 ± 0.02^{b}	–	–	0.69 ± 0.15^{a}	3.82 ± 0.03^{b}
Σ PUFA	**17.45**	**15.88**	**8.57**	**5.54**	**8.70**	**25.28**
PUFA/SFA	**0.60**	**0.53**	**0.14**	**0.10**	**0.16**	**0.45**
Σ n-6	**3.54**	**6.55**	**3.87**	**2.75**	**6.27**	**15.96**
Σ n-3	**6.62**	**3.89**	**4.70**	**2.79**	**2.20**	**6.48**
n6/n3	**0.53**	**1.68**	**0.82**	**0.98**	**2.85**	**2.46**
DHA/EPA	**0.03**	**0.09**			**0.69**	**2.80**
Unknown	**35.33**	**33.32**	**7.57**	**7.03**	**19.59**	**5.21**

*Different letters (a-b) in the same row indicate significant differences ($p < 0.05$).

also observed that the proportion of these fatty acids changed significantly ($p < 0.05$) during the seasons (Table 2).

The proportions of n-3 PUFAs ranged from 2.20% for *S. filamentosa* in spring to 26.11% for *J. rubens* in spring. The highest amounts of n-3 PUFAs were obtained from *J. rubens* independing on season, whereas *D. rigidula* and *S. filamentosa* exhibited the lowest values of n-3 PUFAs. The proportions of n-6 PUFAs ranged from 2.75% for *D. rigidula* in autumn to 24.27% for *J. rubens* in summer. *P. pavonia* had higher levels of n-6 PUFAs (13.63–18.60) throughout the year, whereas *D. rigidula* displayed the lowest values of n-6 PUFAs (2.75–3.87). The recommended maximum dietary ratio of n6/n3 PUFAs is 4.0 (HMSO, 1994). In this study, the n6/n3 PUFA ratio was found to be lower (0.24 for *J. rubens* and 2.85 for *S. filamentosa*, both in spring) than the recommended maximum value. The recommended minimum value of the PUFA/SFA ratio is 0.45 (HMSO 1994), which is lower than that obtained for all the species examined in this study throughout the year, except in the case of *D. rigidula* in summer (0.14) and autumn (0.10) and *S. filamentosa* in spring (0.16). The highest PUFA/SFA ratio was obtained for *J. rubens* in summer (1.17), followed by *P. pavonia* in autumn (0.89).

Essential fatty acids (EFAs) have two or more double bonds in their carbon chain. There are two groups of EFAs, the n-6 and the n-3, defined by the position of the first double bond in the molecule starting from the carbon atom at the methyl end of the chain. EFAs have to be provided in the food since they cannot be synthesized within the body. Besides omega-3 fatty acids, omega-6 fatty acids also play a crucial role in brain function, as well as normal growth and development. *P. pavonia* contained C18:3 n-6 (1.32% in autumn), which is one of the essential fatty acids.

Humans and other mammals lack the enzymes necessary to insert a cis double bond at position w6 or w3 in fatty acids. Since PUFAs (especially EPA and DHA) are effectively synthesized only by aquatic organisms, humans can obtain these essential components by consuming marine/freshwater products. EPAs with five double bonds serve as precursors of prostaglandins and thromboxans via the cyclooxygenase system. As a consequence, dietary omega-3 PUFA helps to reduce the risk of heart disease, decreases low density lipoprotein (LDL) cholesterol, and prevents osteoarthritis and diabetes (Burtin 2003, Matanjun et al. 2009). Seaweeds can be used as sources of n-3 fatty acids such as eicosapentaenoic acid (EPA). Significantly high proportions of EPA ($p > 0.05$) were obtained for *J. rubens* (24.47%) in spring and (21.15%) in winter, followed by *L. papillosa* (18.42%) in spring. *S. filamentosa* had the lowest value of EPA in both spring (0.99%) and summer (1.36%). Generally low proportions of

DHA were found in all the macroalgae species examined here in all seasons, ranging from 0.15% for *S. schimperi* in spring to 3.82% for *S. filamentosa* in summer. It has been reported that the lipid and fatty acid contents are influenced by the type of species, habitat conditions, season, genetic differences and locations (Nelson et al. 2002, Kostetsky et al. 2004, Khotimchenko et al. 2005). In the present study the biochemical contents of the seaweeds varied between species and seasons. Marinho-Soriano et al. (2006) found an inverse correlation between protein levels of seaweeds and water temperature. Similarly, in our results, protein levels were generally higher in winter and spring and lower in summer. Furthermore, Banerjee et al. (2009) reported a significant correlation between environmental parameters and the biochemical composition of seaweeds. They concluded that abiotic parameters such as light intensity, temperature, salinity etc. have a potential role in the biosynthetic pathways of seaweeds.

It can be concluded that seasonal changes in environmental conditions have a great effect on the biochemical composition of seaweed species. It can also be concluded that all six species of seaweeds examined in this study have high ash contents, appreciable protein contents, low lipid contents, and relatively high levels of polyunsaturated fatty acids. Knowledge of the biochemical variations that take place in seaweeds during the year provide useful data for culturing conditions and the timing of harvesting for special extracts. Further studies should be conducted with more species in order to determine their biochemical contents and their variations during the year.

References

AOAC (Association of Official Agricultural Chemists), 1990, *Official methods of analysis*, 15th edn., AOAC, Washington.

AOAC, 1998a, *Official method 955.04. Nitrogen (total) in seafood. Fish and other marine products*, [in:] *Official methods of analysis of AOAC International*, Arlington, VA, 6 pp.

AOAC, 1998b, *Official method 938.08. Ash of seafood. Fish and other marine products*, *Official methods of analysis of AOAC International*, Gaithersburg, Maryland, 6 pp.

Abdallah A. M., Abdallah M. A., Beltagy A., Siam E., 2006, *Contents of heavy metals in marine algae from Egyptian Red Sea coast*, Toxic. Environ. Chem., 88 (1), 9–22, http://dx.doi.org/10.1080/02772240500414911.

Al-Masri M. S., Mamish S., Budier Y., 2003, *Radionuclides and trace metals in eastern Mediterranean Sea algae*, J. Environ. Radio., 67 (2), 157–168, http://dx.doi.org/10.1016/S0265-931X(02)00177-7.

Banerjee K., Ghosh R., Homechaudhuri S., Mitra A., 2009, *Seasonal variation in the biochemical composition of red seaweed (Catenella repens) from Gangetic Delta, northeast coast of India*, J. Earth Syst. Sci., 118 (5), 497-505, http://dx.doi.org/10.1007/s12040-009-0045-2.

Bárbara I., Cremades J., 1993, *Guía de las algas del litoral gallego*, a Coruña: Concello da Coruña – Casa das Ciencias.

Bligh E. C., Dyer W. J., 1959, *a rapid method of total lipid extraction and purification*, Can. J. Biochem. Physiol., 37 (8) 913–917.

Burtin P., 2003, *Nutritional value of seaweed*, Electr. J. Environ. Agr. Food Chem., 2, 498–503.

Dawczynski C., Schubert R., Jahreis G., 2007, *Amino acids, fatty acids, and dietary fibre in edible seaweed products*, Food Chem., 103 (3), 891–899, http://dx.doi.org/10.1016/j.foodchem.2006.09.041.

Fleurence J., 1999, *Seaweed proteins: biochemical nutritional aspects and potential uses*, Trends Food Sci. Tech., 10 (1), 25–28.

Herbreteau F., Coiffard L. J. M., Derrien A., De Roeck-Holtzhauer Y., 1997, *The fatty acid composition of five species of macroalgae*, Bot. Mar., 40 (1–6), 25–27, http://dx.doi.org/10.1515/botm.1997.40.1-6.25.

HMSO, 1994, *Nutritional aspects of cardiovascular disease*, Rep. Health Soc. Subj. No. 46, HMSO, London.

Indegaard M., Ostgaard K., 1991, *Polysaccharides for food and pharmaceutical uses*, [in:] *Seaweed resources in Europe: uses and potential*, M. D. Guiry & G. Blunden (eds.), John Wiley & Sons Ltd., Chichester, 169–183.

Kaehler S., Kennish R., 1996, *Summer and winter comparisons in the nutritional value of marine macroalgae from Hong Kong*, Bot. Mar., 39, 11–17, http://dx.doi.org/10.1515/botm.1996.39.1-6.11.

Kamenarska Z., Gasic M. J., Zlatovic M., Rasovic A., Sladic D., Kljajic Z., Stefanov K., Seizova K., Najdenski H., Kujumgiev A., Tsvetkova I., Popov S., 2002, *Chemical composition of the brown alga Padina pavonia (L.) Gaill. from the Adriatic Sea*, Bot. Mar., 45 (4), 33–345, http://dx.doi.org/10.1515/BOT.2002.034.

Khotimchenko S. V., Vaskovsky V. E., Titlyanova T. V., 2005, *Fatty acids of marine algae from the Pacific Coast of North California*, Bot. Mar., 45 (1), 17–22.

Kostetsky E. Y., Goncharova S. N., Sanina N. M., Shnyrov V. L., 2004, *Season influence on lipid composition of marine macrophytes*, Bot. Mar., 47, 134–139, http://dx.doi.org/10.1515/BOT.2004.013.

Kolb N., Vallorani L., Stocchi V., 1999, *Chemical composition and evaluation of protein quality by amino acid score method of edible brown marine algae Arame (Eisenia bicyclis) and Hijiki (Hijikia fusiforme)*, Acta Aliment., 28 (3), 213–222, http://dx.doi.org/10.1556/AAlim.28.1999.3.1.

Mabeau S., Fleurence J., 1993, *Seaweed in food products: Biochemical and nutritional aspects*, Trends Food Sci. Tech., 4, 103–107.

Manivannan K., Thirumaran G., Karthikai Devi G., Anantharaman P., Balasubramanian T., 2009, *Proximate composition of different groups of seaweeds from Vedalai coastal waters (Gulf of Mannar): southeast coast of India, Middle-East*, J. Sci. Res., 4 (2), 72–77.

Matanjun P., Mohamed S., Mustapha N. M., Muhammad K., 2009, *Nutrient content of tropical edible seaweeds, Eucheuma cottonii, Caulerpa lentillifera and Sargassum polycystum*, J. Appl. Phycol., 21 (1), 1–6 http://dx.doi.org/10.1007/s10811-008-9326-4.

Marinho-Soriano E., Fonseca P. C., Carneiro M. A. A., Moreira W. S. C., 2006, *Seasonal variation in the chemical composition of two tropical seaweeds*, Bioresource Technol., 97 (18), 2402–2406, http://dx.doi.org/10.1016/j.biortech.2005.10.014.

Mishra V. K., Temelli F., Ooraikul B., Shacklock P. F., Craigie J. S., 1993, *Lipids of the red alga, Palmaria palmata*, Bot. Mar., 36 (2), 169–174, http://dx.doi.org/10.1515/botm.1993.36.2.169.

Mohamed S., Hashim S. N., Rahman H. A., 2012, *Seaweeds: a sustainable functional food for complementary and alternative therapy*, Trends Food Sci. Tech., 23, 83–96.

Nelson M. M., Phleger C. F., Nichols P. D., 2002, *Seasonal lipid composition in macroalgae of the Northeastern Pacific Ocean*, Bot. Mar., 45, 58–65, http://dx.doi.org/10.1515/BOT.2002.007.

Nisizawa K., Noda H., Kikuchi R., Watanabe T., 1987, *The main seaweeds in Japan*, Hydrobiologia, 151/152, 5–29, http://dx.doi.org/10.1007/BF00046102.

Norziah M. H., Ching C. Y., 2000, *Nutritional composition of edible seaweed Gracilaria changgi*, Food Chem., 68, 69–76, http://dx.doi.org/10.1016/S0308-8146(99)00161-2.

Polat S., Ozogul Y., 2008, *Biochemical composition of some red and brown macro algae from the Northeastern Mediterranean Sea*, Int. J. Food Sci. Nutr., 59 (7–8), 566–572, http://dx.doi.org/10.1080/09637480701446524.

Polat S., Ozogul Y., 2009, *Fatty acid, mineral and proximate composition of some seaweeds from the northeastern Mediterranean coast*, Ital. J. Food Sci., 21, 317–324.

Renaud S. M., Luong-Van J. T., 2006, *Seasonal variation in the chemical composition of tropical Australian marine macroalgae*, J. Appl. Phys., 18, 381–387.

Rupérez P., Saura-Calixto F., 2001, *Dietary fibre and physicochemical properties of edible Spanish seaweeds*, Eur. Food Res. Tech., 212 (3), 349–354, http://dx.doi.org/10.1007/s002170000264.

Rupérez P., 2002, *Mineral content of edible marine seaweeds*, Food Chem., 79 (1), 23–26, http://dx.doi.org/10.1016/S0308-8146(02)00171-1.

Sánchez-Machado D. I., López -Cervantes J., López-Hernández J., Paseiro-Losada P., 2004, *Fatty acids, total lipid, protein and ash contents of processed edible seaweeds*, Food Chem., 85, 439–444, http://dx.doi.org/10.1016/j.foodchem.2003.08.001.

Sánchez-Machado D. I., López-Hernández J., Paseiro-Losada P., 2002, *High-performance liquid chromatographic determination of α-tocopherol in macroalgae*, J. Chrom., 976, 277–284, http://dx.doi.org/10.1016/S0021-9673(02)00934-2.

Shanmugam A., Palpandi C., 2008, *Biochemical composition and fatty acid profile of the green alga Ulva reticulata*, Asian J. Biochem., 3 (1), 26–31, http://dx.doi.org/10.3923/ajb.2008.26.31.