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The seasonal variation in the chemical composition of the kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta*

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Abstract The seasonal chemical profiling of kelp species has historically either being carried out on only a single species or the data dates back over 60 years. This research highlights a detailed chemical composition profile of the four kelp species *Laminaria digitata*, *Laminaria hyperborea*, *Saccharina latissima* and *Alaria esculenta* over a 14-month period. These kelp species were selected due to their identified potential for cultivation. They were chemically characterised to identify seasonal variations and predict best harvest times. Components of interest included the carbohydrates cellulose, laminarin, alginate and mannitol as well as proteins, ash, metals, moisture, polyphenolics, total carbon and nitrogen content. The highest yields of lamianrin and mannitol coincided with the lowest yields in ash, protein, moisture and polyphenols. The implications of these observations for use of kelp species as a fermentation substrate are discussed.

Keywords *Laminaria* spp · *Saccharina latissima* · *Alaria esculenta* · Chemical composition analysis · Seasonal variation · Seaweed biofuels

Introduction

Historically, seaweed biomass has been used around the world for human consumption, as a fertiliser, soil improver and an animal food additive (Indergaard and Minsaas 1991; Tseng 1987). In 2010, wild harvest of macroalgae accounted for only 4.5 % of total production whilst over 19 million tonnes of seaweed were produced through cultivation (FAO 2012). Cultivation has been traditionally centred in Asia accounting for 99 % of total worldwide production (FAO 2012), where the majority of macroalgal biomass has been used for human consumption and the production of alginate, agar and carrageenan (Zemke-White and Ohno 1999; McHugh 2003; Glicksman 1987).

There is now a growing interest in countries outside those who have traditional cultivated macroalgae for phycolloid and food production. This includes Europe where new interest has developed around ultivating macroalgae for biofuels (Bruhn et al. 2011; Adams et al. 2011b; Hughes et al. 2012) and for high-end products such as biopharmaceuticals (Querellou et al. 2010). Furthermore, as kelps form some of the most diverse and productive habitats in cold-temperate regions (Smale et al. 2013) seaweed cultivation can be an important economic proposition for coastal regions with reduced access to alternative commercial activities (Valderrama 2012).

As components of seaweed undergo seasonal fluctuations, with maximum levels of components seldom coinciding at the same time (Black 1950b), a more complete understanding of these seasonal variations is needed to identify not only suitable species but also best harvest times. In addition, direct comparison of the chemical composition of different kelp species over one season dates back over 60 years (Black 1950b, 1948). As the biochemical composition of seaweed is influenced not only by the type of species but also by its environmental conditions, its maturity, its gender and also the season (Ito and Hori 1989; Murakami et al. 2011), sampling of different species over longer periods needs to be conducted to fully understand their impact on biomass composition.

Brown seaweed biomass has been described as carbohydrate rich, with the main carbohydrate, alginate, accounting for up to 40 % of the dry matter in *Laminaria hyperborea* (Horn et al. 1999). The highest alginate content in kelp species

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have been reported to occur in summer months (Rosell and Srivastava 1984). Cellulose, another structural component of seaweed tissue, has been reported to a lesser extent in kelp species, accounting for up to 8 % of the dry weight (Black 1950b). Variability of cellulose content has also been described in the literature, but the variations within a season were less pronounced than variations within the plant issue (Black 1950a). In addition to the structural carbohydrates alginate and cellulose, laminarin, mannitol and fucoidan contents have also reported to comprise a significant proportion of the carbohydrate pool of brown seaweeds (Black et al. 1951; Usov et al. 2001). The storage carbohydrates mannitol and laminarin in Laminariales have also been found to accumulate during summer and autumn (Adams et al. 2011a; Rosell and Srivastava 1984) and utilised during winter as an energy source for new tissue growth (Zimmerman and Kremer 1986).

Protein content in brown seaweeds is generally lower (3–15 % of the dry weight) compared to red and green seaweed (Fleurence 1999). Contrary to carbohydrate profiles, protein contents were found to be highest in winter and lowest during summer (Fleurence 1999; Adams et al. 2011a), where it has been suggested that this build-up of nitrogen reserves is to sustain the rapid growth rates of seaweeds into the summer months (Chapman and Craigie 1977).

Another significant part of brown seaweed biomass is its ash content which can account for over 50 % of its dry weight (Moss 1952). The ash content of the kelp species *Laminaria digitata* consists largely of the ions—sodium, potassium, calcium and magnesium—with chloride and sulphate as the main counter-ions (Adams et al. 2011b). These four metal ions have also been reported to simulate the ash profile most closely in Laminariales, with highest levels of ash and metals measured during winter months (Adams et al. 2011a). In other members of the order Laminariales, macro-nutrients such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), P and Si have been found along with trace metals such as Fe, Zn, Mn, Al and Cu (Ross et al. 2008).

In comparison to carbohydrates, the metal, protein and polyphenol content of the Laminariales make up proportionally less of the seaweed tissue compared to that found in the Fucales (Van Alstyne et al. 1999) where a maximum of ~13 % of dry matter in *Ascophyllum nodosum* and *Fucus vesiculosis* during winter and lows of ~9 % in the summer have been reported (Ragan and Jensen 1978). Although polyphenols have been shown to possess a range of health-promoting activities (Zhang et al. 2006; Cardona et al. 2013), it is their inhibitory action towards microbial activities, largely due to inhibition of vital enzymes (Moen et al. 1997), which makes this component of seaweeds an important parameter to know if the biomass is used in for biological processes such as bioethanol fermentation.

This paper details the direct comparison of seasonal variation for a range of biochemical and chemical components for the species *L. digitata*, *L. hyperborea*, *Saccharina latissima* and *Alaria esculenta* and use seasonal variation analysis for identification of best harvest times. Results from these studies are also discussed to identify the potential of seaweed biomass as a resource for biofuel production.

Materials and methods

Seaweed collection and pre-treatment

Wild *Laminaria digitata*, *Laminaria hyperborea* and *Saccharina latissima* were collected eight times at low tides from the sheltered shores of the Clachan Sound on the Isle of Seil, Scotland (OS grid coordinates NM 78517 19700) between August 2010 and October 2011. The water in this narrow sea channel is shallow, with a swift current running through. As *Alaria esculenta* did not grow in sufficient quantities at Clachan Sound, wild species were collected three times also at low tide near Dunstaffnage (OS grid coordinates NM 88005 34499). Plant size and age for all species was not determined. Approximately 1 to 2 kg of tissue was collected each time for each of the different species. However, as harvests of *A. esculenta* were only possible three times in the intertidal zones between the months of March to July, after which species disappeared it is assumed that this species was not perennial. Holdfasts from all seaweeds were removed and remaining seaweed thalli macerated and homogenised using an industrial-sized mincer (Hobart, Model E4522) and frozen immediately at −20 °C, freeze dried and the dried material milled to <1-mm size using a coffee grinder.

Moisture, ash and mineral analysis

Water contents were evaluated seven times between August 2010 and September 2011 according to standardised methods after oven-drying seaweed biomass at 105 °C (Sluiter et al. 2008). Ash contents were determined from subsequent combustion of oven-dried biomass at 550 °C (Sluiter et al. 2005). Metal analysis was carried out on acid digested seaweed using ICP-MS for trace metal detection using a Thermo Scientific X-Series (II) and ICP-OES for the measurement of Ca, Na, K and Mg using a Perkin Elmer Optima DV4300. Results were expressed as mg kg^{-1} (dry weight).

Carbohydrate analysis

Laminarin and mannitol were analysed using a HPLC method after acid hydrolysis of 600±60 mg of freeze dried sample in 3 mL of 0.5 M sulphuric acid for 15 min at 121 °C. Solutions were diluted afterwards to 25 mL using deionised water, centrifuged and filtered through a 0.45-μm PTFE filter into HPLC glass vials. Acid hydrolysates were analysed for glucose and mannitol using a Phenomenex Resex ROA 150×

7.8 mm column, equipped with a micro-guard cation-H^+ column and operated at 60 °C using a 5-mM sulphuric acid mobile phase at 0.5 mL min^{-1}. Concentrations of mannitol and glucose were determined using Agilent ChemStation software (Rev. B.01.01 [164]) by comparison to glucose and mannitol standards. Laminarin and mannitol concentrations were expressed as a % of dry weight seaweed biomass (% d.w.).

The structural component alginate was analysed colourimetrically after alkali extraction. Approximately 30± 3 mg of seaweed sample (or sodium alginate standard) was steeped in 10 mL of a 6 % (w v^{-1}) sodium carbonate solution in 25-mL volumetric flasks and placed in a shaking water bath (Grant, OLS 200) for 3 h at 60 °C. The final volume was then adjusted to 25 mL with deionised water. Alginate content of centrifuged extract (2 min at 16,200×*g*) was analysed according to a method described by Ramus (1977) using the cationic copper phthalocyanine dye Alcian Blue. A 100-μL volume of supernatant or alginate standard solution was combined with 800 μL of 0.5 M acetic acid, to which 100 μL of 0.5 % (*w* v^{-1}) Alcian Blue stock solution was then added, mixed briefly and incubated overnight at room temperature (RT). Dye precipitate was removed by centrifugation (2 min at 16,200×*g*) and the absorption of the supernatant measured at 610 nm. The quantity of dye removed from solution was determined using an alginate calibration curve in the range 100–900 μg L^{-1}. Alginate content was expressed as a % dry weight seaweed biomass.

For the determination of the cellulose content, a modified version of a National Renewable Energy Laboratory (NREL) method described by Sluiter et al. (2008) was used. Approximately 200±20 mg of freeze-dried seaweed was acid hydrolysed with 1 mL of a 72 % (*v* v^{-1}) sulphuric acid solution (ρ= 1.74 g mL^{-1}) for 60 min in a 20-mL glass pressure tube at 30 °C in a water bath. In the second stage, 16.8 m deionised water was added and the sample autoclaved for 15 min at 121 °C. Acid hydrolysates were pH adjusted to ~pH 3 with $BaCO_3$ powder. Total glucose (structural and non-structural) was analysed using the HPLC method described above. The cellulose content was determined by subtracting the non-structural laminarin content from the total glucan content.

Polyphenol analysis

The polyphenolic content of seaweed biomass was determined using a modified version of that described by (Nwosu et al. 2011). Approximately 100±10 mg of freeze-dried seaweed was extracted three times in 1 mL of a solvent mix consisting of acetonitrile/water/formic acid at a ratio of 50:49.8:0.2 (*v* v^{-1}) at RT using 2-mL micro centrifuge plastic tubes, attached onto a rotary wheel, rotating at 20 rpm for 1 h. After completion of each extraction stage, tubes were centrifuged for 2 min at 16,200×*g* and supernatants collected in 5-mL volumetric flasks, with final volume adjustment to 5 mL using fresh solvent. A Folin Ciocalteau colourimetric method was applied (Waterhouse 2001) to determine the total phenolic content of the extracts using gallic acid as a standard. The results were expressed as a % polyphenols of dried seaweed biomass.

Protein analysis

Extraction of total protein content was carried out using a combined acid and alkaline extraction method as developed for release of proteins from microalgal biomass (Slocombe et al. 2013). Protein extract was analysed colourimetrically using a Lowry assay and bovine serum albumin (BSA) as the standard. Results were expressed as % protein of dried biomass (d.w.).

Total organic nitrogen and carbon determination

Total carbon and nitrogen analysis was carried out according to the method described by (Slocombe et al. 2013) and analysed using a ANCAGSL20-20 stable isotope analyser (PDZ Europa, UK). Nitrogen-to-protein conversion factors (Jones' factor) were determined as described by González López et al. (2010).

Statistical analysis

Experimental error was determined for triplicate assays and expressed as standard deviation (SD). The significance of differences in the yields of each component in the species *L. hyperborea*, *L. digitata* and *S. latissima* across the season was determined by two-way analysis of variance (ANOVA). Due to having only three sample points compared to eight for the other three species, *A. esculenta* was excluded from statistical analysis. Statistically significant interactions were further analysed using a post hoc test (Tukey) to determine sample main effects. For comparison of the association between the ash content and sodium, potassium, magnesium and calcium ions, the Pearson correlation coefficient was determined using Minitab Statistical Software version 15.0.

Results

Moisture, ash and mineral analysis Average moisture content was 84.5±5.7, 87.2±2.2, 84.9±2.9 and 85.5±2.5 % for *L. digitata*, *L. hyperborea*, *S. latissima* and *A. esculenta*, respectively (Fig. 1), whilst the average ash profiles of the same species ranged between 31.6±7.1, 32.0±9.6, 31.7±7.6 and 25.3±5.8 % (Fig. 2). Highest levels of moisture and ash were measured during winter months in all four seaweeds followed by a decline into the autumn months. Two-way ANOVA and pairwise comparison of seasonal ash yields highlighted the

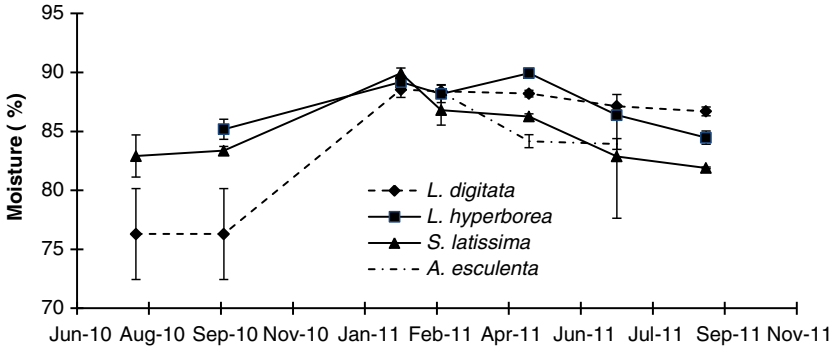

Fig. 1 Moisture content of the kelp species *A. esculenta*, *L. digitata*, *S. latissima and L. hyperborea* between August 2010 and September 2011. *Error bars* are the means±SD of three analyses

significant differences in ash content between all species ($P<0.038$) and between seasons ($P<0.001$).

Micronutrient analysis showed the significant accumulation of a number of metals, especially Na, K, Ca, Mg, Sr and Fe in seaweed biomass as well as the non-metal iodine (I) (Table 1). The major four metal cations in the four seaweeds were in the following order: K>Na>Ca>Mg followed by Sr, Fe, Arsenic (As) and Aluminium (Al), Zinc (Zn) and Titanium (Ti). Iodine was present in similar quantities to Mg in *L. digitata* and *L. hyperborea* and was one of the top five elements measured in all four seaweeds (Table 1). It was found that *S. latissima* and *A. esculenta* contained 80 and 30 % more metal ions, respectively, when compared to *L. hyperborea* and *L. digitata*, of which the latter two were similar. Correlation analysis showed that the ash content in *L. digitata* was strongly associated with Mg, Na, K, Fe, Cu, Rb and Sr (Table 1). In *L. hyperborea*, Mg, Na, K and As were also strongly correlated to the ash content. K and Al were the only two elements that were strongly linked to the ash content in *S. latissima* (Table 1). Two-way ANOVA and pairwise comparison of K, Na, Ca and Mg yields highlighted the significant differences between seasons for Na and K only ($P<0.05$), whilst no significant variation of the K, Na, Ca and Mg content in the species *L. digitata*, *l. hyperborea* and *S. latissima* was shown ($P>0.05$).

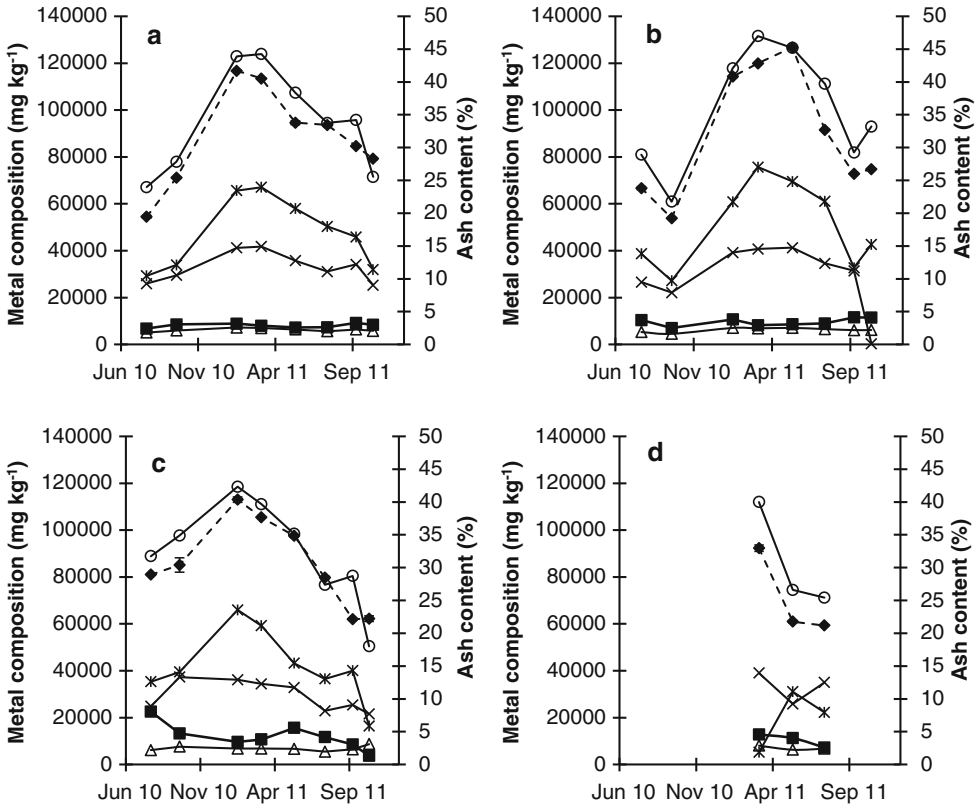

Fig. 2 Content of ash content (*diamond*) and metal composition such as Na (*cross*), K (*star*), Ca (*square*), Mg (*triangle*) and the sum of all four metals (*circle*) in **a** *L. digitata*, **b** *L. hyperborea*, **c** *S. latissima* and **d** *A. esculenta*

Table 1 Micronutrient analysis of *L. digitata*, *L. hyperborea*, *S. latissima* and *A. esculenta* using ICP-OES including Pearson's correlation analysis of each metal identified (mg kg^{-1}) compared to ash content

Harvest	Al	Ti	Cr	Mn	Fe	Ni	Cu	Zn	As	Rb	Sr	Mo	Ba	Pb	I
L. digitata															
10/08/2010	19	39	0.7	7	83	0.3	2	35	72	17	580	0.1	7	0.1	5,204
07/10/2010	60	46	0.9	5	97	0.4	3	33	104	19	634	0.2	8	0.1	8,122
01/02/2011	105	52	0.8	6	199	0.5	16	38	114	35	834	0.3	10	0.4	5,525
21/03/2011	24	47	0.7	5	166	0.5	17	45	96	39	799	0.3	8	0.6	9,014
26/05/2011	121	45	0.8	5	165	0.4	3	38	86	35	661	0.3	8	0.2	5,120
27/07/2011	60	50	0.8	12	175	0.5	3	45	114	29	684	0.2	8	0.2	7,450
21/09/2011	63	53	1.1	6	185	0.6	4	49	106	25	747	0.4	11	0.6	7,058
24/10/2011	8	47	1.3	4	41	0.6	4	35	78	18	752	0.4	9	0.5	4,761
r	0.4	0.7	0.2	0.1	0.7	0.5	0.8	0.5	0.5	0.9	0.8	0.4	0.5	0.5	0.2
P	0.3	0.1	0.6	0.9	0.04	0.2	0.02	0.2	0.1	0.01	0.01	0.3	0.1	0.1	0.5
L. hyperborea															
10/08/2010	31	36	0.6	4	62	0.4	3	23	83	14	486	0.2	7	0.1	–
07/10/2010	2	0	0.5	0	51	0.0	1	1	0	0	–	0.0	0	0.0	10,239
01/02/2011	123	60	0.9	7	250	0.7	4	38	108	33	844	0.3	10	0.3	7,125
21/03/2011	53	62	0.9	21	112	0.6	2	30	85	21	679	0.2	7	0.2	3,289
26/05/2011	85	65	1.4	9	258	0.9	5	42	110	24	718	0.3	9	0.6	6,685
27/07/2011	158	53	1.0	7	297	0.7	7	54	106	45	828	0.4	10	0.3	5,004
21/09/2011	789	106	2.8	38	702	4.3	4	19	5	26	76	0.5	8	0.5	277
24/10/2011	37	52	0.6	6	128	0.4	3	40	88	33	678	0.3	7	0.1	11,096
r	−0.1	0.4	0.1	0.1	0.1	−0.1	0.4	0.6	0.7	0.5	0.2	0.2	0.7	0.6	−0.3
P	0.8	0.3	0.8	0.8	0.9	0.9	0.3	0.1	0.049	0.3	0.6	0.6	0.07	0.2	0.5
S. latissima															
10/08/2010	884	160	2.4	27	623	1.1	2	20	83	18	649	0.4	14	1.1	4,855
07/10/2010	13	3	2.1	21	16	0.9	2	30	74	42	14	0.9	22	2.2	2,877
01/02/2011	336	64	1.3	13	381	0.6	2	31	70	37	629	0.6	10	0.7	3,665
21/03/2011	687	123	2.7	27	720	1.2	2	23	64	23	733	0.4	19	1.3	3,985
26/05/2011	1,877	135	4.5	35	1,159	2.2	4	29	73	33	645	0.6	22	1.9	3,193
27/07/2011	200	63	1.1	7	180	0.6	2	18	88	21	661	0.4	10	0.2	3,499
21/09/2011	1,129	127	4.0	45	1,280	1.9	3	22	83	21	657	0.4	15	1.6	143
24/10/2011	497	45	5.0	15	328	3.4	5	8	3	8	57	0.2	5	0.3	39
r	0.7	0.1	0.1	−0.3	−0.3	−0.1	−0.3	0.4	−0.1	0.3	0.3	0.1	−0.1	−0.2	0.5
P	0.04	0.9	0.9	0.5	0.4	0.9	0.5	0.3	0.9	0.4	0.4	0.9	0.8	0.6	0.2
A. esculenta															
21/03/2011	687	123	2.7	27	720	1.2	2	23	64	23	733	0.4	19	1.3	398
26/05/2011	1,877	135	4.5	35	1,159	2.2	4	29	73	33	645	0.6	22	1.9	809
27/07/2011	200	63	1.1	7	180	0.6	2	18	88	21	661	0.4	10	0.2	1,238

Carbohydrate analysis The seasonal variation of the storage carbohydrates mannitol and laminarin and structural components cellulose and alginate are profiled in Fig. 1 Average mannitol content was 19.4±6.6, 17.5±7.4, 18.6±4.7 and 12.1 ±3.5 % in *L. digitata*, *L. hyperborea*, *S. latissima* and *A. esculenta*, respectively. The average laminarin content for the same species accounted for 6.7±6.0, 7.4±8.0, 8.2±5.3 and 11.1±7.2 % of the dry weight, respectively. In those months covering the autumn period, the highest mannitol yields of 24–27 % were observed and with the lowest levels of 6–8 % in early spring. Laminarin followed a similar trend rising to its highest levels during the summer and autumn months (25 % max. in *L. hyperborea*) and dropping to its lowest levels (1–3 %) in the winter. Pairwise comparison of the seasonal glucose yields of *L. hyperborea*, *L. digitata* and *S. latissima* demonstrated that all but one result (February vs March $P=1.000$) were significantly different ($P<0.001$), whilst pairwise comparison of the means of the seasonal mannitol yields of

the same species have also showed that all but one result (Oct. 2010 vs July 2011 $P=0.846$) was significantly different ($P<0.001$). In addition, pairwise comparisons of mannitol and laminarin yields amongst the three kelp species *L. digitata*, *S. latissima* and *L. hyperborea* highlighted the significant differences in yields between the species ($P<0.001$). Pearson moment correlation analysis highlighted the similarity in profiles for both mannitol and laminarin for each of the three species ($r=0.528$–0.872 with $P<0.008$). Taken together, mannitol and laminarin yields were not only influenced by seasonal variations but also differed between species.

Alginate formed the majority of the carbohydrate content in brown seaweeds accounting for 34.6±3.1, 33.2±3.8, 28.5±3.9 and 37.4±4.0 % of the dry weight in *L. digitata*, *L. hyperborea*, *S. latissima* and *A. esculenta*, respectively (Fig. 3). In comparison to the storage carbohydrates laminarin and mannitol, seasonal variations of alginate were less pronounced in all four seaweeds. Alginate profiles of *L. digitata* and *L. hyperborea* were similar with lows of alginate content seen in March, whilst the alginate content in *S. latissima* and *A. esculenta* was lowest in July. ANOVA and pairwise post hoc analysis showed that there were significant differences in the alginate content between *S. latissima*, *L. digitata* and *L. hyperborea* ($P<0.001$) as well as between the harvest dates (08/10 and 02/11, 10/10 and 02/11, 02/11 and 09/11) ($P<0.01$).

Average total glucan levels, which are a combination of laminarin and cellulose, were similar across the four species, spanning from 18.7±5.2, 18.5±7.6, 19.2±6.2, to 22.5±7.6 % of the dry matter in *L. digitata*, *L. hyperborea*, *S. latissima* and *A. esculenta*, respectively. Average cellulose content was less variable across seasons accounting for 11.5±1.0, 11.1±1.0, 11.0 ±1.4 and 11.3±1.0 % of the dry weight of the same species (Fig. 3). Seasonal variations in the total glucan content were dominated by the high degree of variation in laminarin content, whilst cellulose contents remained stable throughout the season.

Combined carbohydrates (alginate, laminarin, cellulose and mannitol) represented up to 84 % of the seaweed biomass dry weight. The average total carbohydrate yields were similar for the four seaweeds and represented 70.7±11.6, 65.5±11.6, 63.1±11.4 and 72.1±6.7 % of the biomass in *L. digitata*, *L. hyperborea*, *S. latissima* and *A. esculenta*, respectively.

Polyphenol analysis The polyphenol content of *L. digitata* and *L. hyperborea* were similar (0.15±0.04 %) and lower than *A. esculenta* (0.87±0.52 %) and *S. latissima* (0.41±0.15 %). Seasonal and species-related differences varied greatly (see Table 2). Accumulation of polyphenols started with the on-set of the growth phase and declined towards the autumn months. Highest polyphenol levels in all seaweeds were found between May and July and lowest levels in October for the *Laminaria* spp. and March for the *A. esculenta* and *S. latissima* (Table 2). Statistical analysis showed that differences in polyphenol content between all species and seasons were highly significant ($P<0.001$). In addition, Pearson correlation analysis comparing polyphenol content of *L. digitata* with *L. hyperborea* ($r=0.75$, $P=0.001$) and *L. hyperborea* with *S. latissima* ($r=0.61$, $P=0.002$) showed that they followed a similar trend to one another.

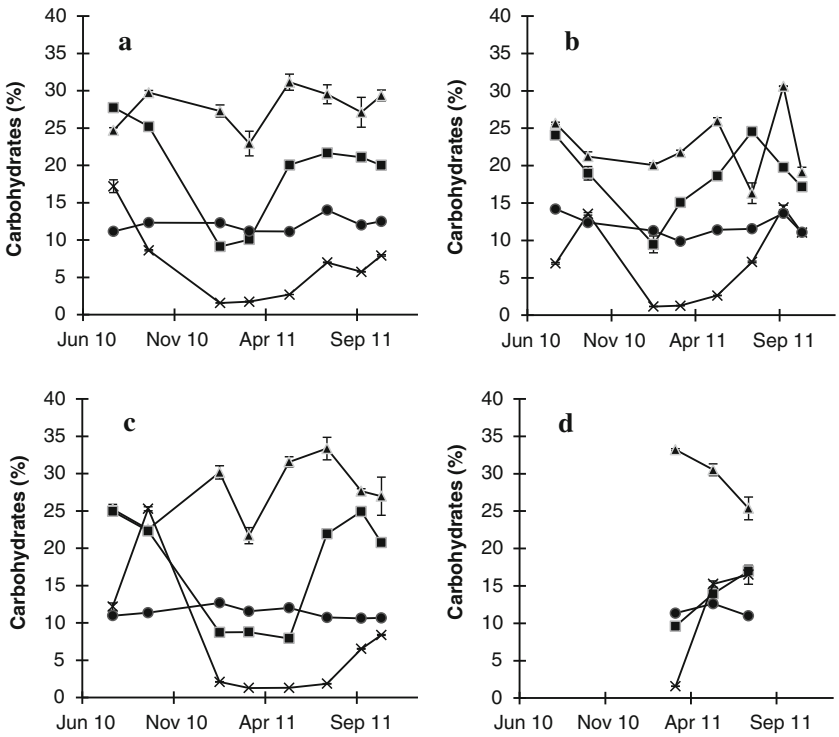

Fig. 3 Carbohydrate content of **a** *L. digitata*, **b** *S. latissima*, **c** *L. hyperborea* and **d** *A. esculenta*. Alginate (*triangle*). Mannitol (*square*). Cellulose (*circle*). Laminarin (*cross*). Carbohydrate yields are expressed as a percentage to the dry weight; *error bars* represent the means± SD of three analyses for alginate, laminarin and mannitol; and cellulose profiles were derived from one sample point

Table 2 Total carbon, nitrogen, protein and polyphenol content and nitrogen to protein conversion factors (Jones' factor) in *L.digitata*, *L. hyperborea*, *S. latissima* and *A. esculenta*. Two-way analysis of variance (ANOVA) considering the proportion of each compound (% biomass) within species and against harvest times. Tukey test highlighting insignificant seasonal associations between harvest dates

Species	Harvest	Carbon (%)	Nitrogen (%)	Protein (%)	Jones' factor	Polyphenols (%)
L. digitata	10/08/2010	36.4±1.7	1.0±0.0	4.9±0.1	4.8	0.15±0.04
	07/10/2010	32.6±0.8	1.2±0.0	5.6±0.3	4.8	0.09±0.01
	01/02/2011	24.5±0.1	2.1±0.0	8.2±0.2	3.9	0.15±0.01
	21/03/2011	26.0±0.4	2.5±0.1	7.7±0.2	3.1	0.16±0.01
	26/05/2011	27.1±0.2	1.6±0.0	7.8±0.1	4.9	0.18±0.01
	27/07/2011	30.4±0.3	1.1±0.0	7.3±0.2	6.6	0.17±0.01
	21/09/2011	29.2±0.3	1.4±0.0	7.0±0.2	5.1	0.11±0.02
	24/10/2011	27.3±2.8	1.4±0.1	7.0±0.2	5.0	0.14±0.01
L. hyperborea	10/08/2010	33.1±0.2	1.0±0.0	5.3±0.1	5.3	0.16±0.02
	07/10/2010	38.6±1.1	1.0±0.0	4.3±0.3	4.4	0.10±0.01
	01/02/2011	25.7±1.6	1.8±0.1	7.7±0.4	4.2	0.17±0.00
	21/03/2011	25.0±0.4	2.1±0.1	8.1±0.2	3.8	0.16±0.01
	26/05/2011	21.8±1.2	2.0±0.1	7.8±0.3	3.9	0.19±0.02
	27/07/2011	27.5±0.3	1.3±0.1	7.7±0.2	5.9	0.23±0.00
	21/09/2011	30.6±0.3	1.4±0.0	6.9±0.3	5.1	0.12±0.01
	24/10/2011	28.8±0.8	1.4±0.1	7.1±0.0	5.1	0.13±0.01
S. latissima	10/08/2010	30.3±0.5	0.8±0.0	5.3±0.1	6.5	0.42±0.01
	07/10/2010	21.1±5.1	1.8±0.4	5.1±0.0	2.9	0.39±0.03
	01/02/2011	23.2±0.1	2.0±0.0	8.8±0.1	4.4	0.40±0.03
	21/03/2011	26.8±1.8	2.2±0.2	9.9±0.1	4.5	0.23±0.02
	26/05/2011	26.6±0.1	1.6±0.1	7.6±0.0	4.6	0.46±0.01
	27/07/2011	26.5±2.0	0.9±0.1	6.0±0.1	7.1	0.68±0.01
	21/09/2011	30.5±1.2	1.1±0.1	7.0±0.0	6.6	0.43±0.03
	24/10/2011	28.0±0.4	1.2±0.0	7.0±0.5	6.1	0.35±0.01
A. esculenta	21/03/2011	28.5±4.0	2.1±0.3	12.1±0.7	5.7	0.31±0.01
	26/05/2011	31.0±1.0	1.9±0.0	11.6±0.3	6.2	1.49±0.04
	27/07/2011	31.2±0.1	1.5±0.1	9.4±0.1	6.1	0.81±0.02
Two-way ANOVA (statistical significance between species)		$F_{2/48}$=14	$F_{2/48}$=2.6	$F_{2/48}$=14	–	$F_{2/48}$=2,065
		P<0.001	P=0.08	P<0.001	–	P<0.001
Two-way ANOVA (statistical significance between harvest dates)		$F_{7/48}$=30	$F_{7/48}$=98	$F_{7/48}$=331	–	$F_{7/48}$=105
		P<0.001	P<0.001	P<0.001	–	P<0.001
Post hoc analysis, statistical insignificant seasonal associations between harvest dates (month/year) (P>0.05)		08/10–10/10	08/10–07/11	08/10–10/10	–	08/10–10/10
		10/10–07/11	10/10–09/11	02/11–03/11	–	10/10–09/11
		02/11–03/11	10/10–10/11	07/11–09/11	–	02/11–03/11
		02/11–05/11	07/11–09/11	07/11–10/11	–	02/11–05/11
		03/11–05/11	09/11–10/11	09/11–10/11	–	03/11–05/11
		03/11–07/11	–	–	–	03/11–07/11
		03/11–09/11	–	–	–	03/11–10/11
		07/11–09/11	–	–	–	07/11–09/11
		07/11–10/11	–	–	–	07/11–10/11

Protein analysis The average protein content was 6.9±1.1 % in *L. digitata*, 6.8±1.3 % in *L . hyperborea*, 7.1±1.7 % in *S. latissima* and 11.0±1.4 % in *A. esculenta* (Table 2). Seasonal and species related differences in the protein content of the species *L. digitata*, *L. hyperborea* and *S. latissima* varied significantly (see Table 2). In all four kelps, protein levels were highest in the first quarter and lowest in the third quarter of the year. Statistical analysis showed that differences in protein content between all species were significant (P<0.033).

Total organic carbon and nitrogen content Average total carbon content in *L. digitata* and *L. hyperborea* was 29.2±3.9 and 28.9±5.2 %, which were not significantly different (P= 0.45), whilst in *S. latissima*, total carbon content was 26.6± 3.2 % which was significantly different to the carbon content in *L. digitata* and *L. hyperborea* ($P<0.001$). For the *A. esculenta* samples, total carbon accounted for 30.3±1.5 % of the total weight, which was highest between May and July. The highest levels of carbon for all the other seaweeds were reached in the autumn months. All seaweed biomass contained the least amount of carbon in March. The seasonal variation in total carbon content of *L. digitata* compared to *S. latissima*, and *L. hyperborea* compared to *S. latissima* were highly significant (see Table 2). This reflected largely the seasonal pattern of the storage carbohydrates laminarin and mannitol, which influenced the total carbon content significantly ($r\geq0.80$; $P\leq0.03$) in the species *L. digitata*, *L. hyperborea* and *A. esculenta*. No statistically significant differences between the carbon content and the structural components alginate and cellulose were demonstrated.

The average total nitrogen content accounted for 1.5± 0.5 % of the dry matter in *L. digitata*, *S. latissima* and *L. hyperborea* and 1.9±0.3 % in *A. esculenta*. Seasonal variation between *L. digitata*, *L. hyperborea* and *S. latissima* was insignificant (see Table 2). Highest nitrogen yields for all seaweeds were seen between February and May, whilst in autumn, nitrogen content was lowest. It was found that the nitrogen profiles closely followed the protein content profiles ($r\geq0.70$, $P\leq0.05$) for the seaweeds *L. digitata*, *L. hyperborea* and *S. latissima*.

Average nitrogen-to-protein conversion factors (Jones' factor) of 4.7 for *L. digitata* and *L. hyperborea*, 5.3 for *S. latissima* and 6.0 for *A. esculenta* were calculated. N-conversion values are generally found to be lower than the traditional factor of 6.25, possibly due to the presence of non-protein N-containing compounds (Lourenço et al. 1998). However, lower conversion factors of 5.3±0.5 for four brown seaweeds have also been reported elsewhere (Lourenço et al. 2002).

Discussion

Whilst approximately 66 % of the cultivated macroalgal biomass is used for human consumption, the remaining 33 % is utilised by the phycolloid industry to produce products such as alginate, agar and carrageenan (McHugh 2003; Zemke-White and Ohno 1999; Glicksman 1987). There is growing interest in utilising other seaweed components such as polyphenols and carbohydrates by the nutraceutical and pharmaceutical industries because of their potential beneficial impact on human health. Polyphenols, mainly from the brown seaweeds, have been investigated because of their wide range of antioxidative, antimicrobial and anti-inflammatory properties (Zhang et al. 2006). Macroalgal biomass has also been proven to be a rich source of vitamins, especially vitamin B_{12} (Phaneuf et al. 1999), and within the food industry, it is used as stabilisers and emulsifiers (Kailasapathy and Sellepan 1998) and as encapsulation agents (Zuidam and Shimoni 2010). Fatty acids, sulphated and brominated polysaccharides have been shown to possess antimicrobial activities (Rosell and Srivastava 1987; Kubanek et al. 2003) and have been identified in all three major divisions of macroalgae (Val et al. 2001).

As seaweed biomass is carbohydrate rich, there has been growing interest in using the biomass for third generation biofuels. For this, it is necessary that seaweeds are harvested when the sugar content is highest, making the seasonality an important factor affecting the economics of this process. As there will be seasonal variability in the nutrients available to support the growth of macroalgal species (Sanderson et al. 2008), this will result in variations in the chemical and biochemical content of the biomass. For example, McHugh (2003) demonstrated that the alginate content and composition varied not only between species but also between habitats—with plants growing in more turbulent water usually containing more alginate than the same species grown in calmer water (McHugh 2003) and between seasons—where the highest alginate content in kelp species were measured in summer months (Rosell and Srivastava 1984). In addition to alginate, laminarin, mannitol and fucoidan contents have also been reported to comprise a significant proportion of the carbohydrate pool of brown seaweeds (Black et al. 1951; Usov et al. 2001). In the Laminariales, the storage carbohydrates mannitol and laminarin have been found to accumulate during summer and autumn for a limited number of species (Adams et al. 2011a; Rosell and Srivastava 1984) and decline during winter as they are utilised as an energy source for new tissue growth. Although the age of the seaweed biomass harvested within this study was not determined, by sampling over a 14-month period, it was still possible to determine the fluctuations that occurred within the natural population. Overall, the results presented within this paper confirm that a similar situation can be seen across a range of brown macroalgal species found growing along the western margin of Scotland.

Analysis of the seasonal composition of seaweeds can be used to determine the best time to harvest and be used to target components of most interest commercially. This relationship, for example, has been demonstrated in other kelp species to monitor the calorific value of *L. digitata* (Adams et al. 2011a) for use as a biofuel, investigate the nutritional value of *Macrocystis* for the mass cultivation of abalone (Westermeier et al. 2012) and to identify seasonal patterns of agricultural biostimulants in *Ecklonia maxima* (Papenfus et al. 2012).

Brown seaweed biomass contains approximately 85 % of its mass as water, with highs around winter time to lows

towards summer (Adams et al. 2011b). Compared to 30 % moisture content in terrestrial biomass such as wood, barley and wheat straw (McKendry 2002), the high water content makes seaweed a more suitable candidate for aqueous bioenergy conversion processes such as fermentation (McKendry 2002) rather than for combustion or pyrolysis (Adams et al. 2011b). In addition, the absence of lignin or lignin-like compounds in brown seaweed biomass has been proposed as an advantage over terrestrial biomass. As the content of cellulose was found to be low (~10 %) compared to that in terrestrial biomass (51 %) (Ingram et al. 1999), it is the alginate content in brown seaweed biomass which is of interest for future biofuel production, as it is readily available throughout a season with little variability compared to the storage carbohydrates mannitol and laminarin. This was borne out by the results reported here for the four different species where the measured alginate content demonstrated little variation over the sampling period. The pattern of accumulation and utilisation of laminarin and mannitol was also in line with reports from other kelps species where maximum yields were also seen during autumn months and minimum yields during the winter months (Adams et al. 2011b; Black 1948). It is worth noting that with *A. esculenta*, the carbohydrates seemed to peak earlier (June to July) than within the biomass of the other species. This suggests that by growing more than one species the period of harvesting the biomass, for example, for bioethanol production, could be extended. But, this would need to be confirmed by extending the number of sampling periods available for *A. esculenta*.

The ash content, another important parameter, is also much higher in seaweeds than it is in terrestrial biomass where it only accounts for 1–10 % (Grohmann and Bothast 1994; Lynd 1996), compared to around 30 % in kelps. Metal analysis of the four seaweeds revealed that high ash contents are due to the accumulation of mainly potassium and sodium ions, which more than doubled in concentration between lows in the summer months and highs during the winter months, which has also been reported in *L. digitata* (Adams et al. 2011a). Other members of the Laminariales and Fucales were also found to be rich in the macro-nutrients such as Na, K, Ca, Mg, P and Si along with trace metals such as Fe, Zn, Mn, Al and Cu (Ross et al. 2008). Even though the mineral composition of seaweeds can provide a large amount of the micronutrients required for microbial growth such as that seen within fermentation, the high concentration of the alkali metal sodium can also interfere with this processes (Bautista-Gallego et al. 2008).

Protein made up 7.0±1.3 % of the total weight in the three kelp species and was found to be similar to eight other members of the Laminariales where an average of 7.5±1.9 % was reported (Dawczynski et al. 2007). A positive correlation between protein and nitrogen content has previously been reported in the brown, red and green seaweeds (Kim et al. 2013; Lapointe and Ryther 1979; Robertson-Andersson et al. 2009). As protein levels in brown seaweeds are considerably lower compared to red species, where protein levels can make up to 50 % of the dry weight in *Porphyra* spp. (McHugh 2003), their use as a protein resource is therefore limited. However, as *Saccharomyces* spp. are less nitrogen demanding during fermentation than during their growth (Backhus et al. 2001; McHugh 2003) the lower protein content in brown seaweed does not pose a disadvantage when used for fermentation processes.

It is also worth noting that the biochemical composition of other compounds within the macroalgal biomass can also be highly variable. For example the fatty acid content varies within species (Sánchez-Machado et al. 2004) and with environmental conditions such as light, salinity and temperature (Floreto et al. 1993). There is now interest in countries outside those who have traditional cultivated macroalgae for alginates and food. This includes Europe where interest has centred on cultivating brown macroalgae species for biofuels (Adams et al. 2011b). But, if brown macroalgae is to be utilised as a form of biofuel, there needs to be a more complete understanding of the seasonal variation of the both the biochemical and chemical composition for a range of species. The results on chemical analysis of seaweeds, including this one, are currently limited to the analysis of wild material, and as cultivation becomes more widespread, this should be expanded to include this material.

In conclusion, ethanol production from *L. digitata* has previously been demonstrated over one season (Adams et al. 2011b) where it was found that there was a direct correlation between laminarin yields in seaweed and ethanol yields, with minimum and maximum yields found in March and July, respectively. This research has shown that accumulation of the storage components laminarin and mannitol strongly influenced the total carbon content, which usually was highest in autumn and lowest in winter. As fermentation industries commonly operate with high-strength feedstocks, concentration of seaweed extracts would therefore increase components such as polyphenols and metal ions with negative consequences to overall fermentation performance. In addition, seasonal composition analysis carried out in this study of the four kelp species *L. digitata*, *L. hyperborea*, *S. latissima* and *A. esculenta* demonstrated that maximum values of carbohydrates coincided with reduced concentrations of the components protein, ash, polyphenols and moisture. These relationships, which vary between seasons and species, can be used by industries not only to maximise yields of targeted seaweed components but also to minimise yields of components considered to be undesirable or inhibitory to processes such as ethanol fermentation.

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