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SEASONAL CHANGES IN THE CONSTITUENTS OF *ULVA LACTUCA*

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Key Word Index—*Ulva lactuca*; Chlorophyceae; sea lettuce; green marine alga; seasonal changes; lipids; sterols; sugars; amino acids; proteolysis.

Abstract—The green marine algal species *Ulva lactuca*, contains appreciable amounts of lipids, but there is no correlation between the lipid content and its unsaponified fraction. The sterol content of this alga depended on the proportion of the unsaponified lipid fraction in total lipids. The presence of cholesterol-like material was demonstrated. The algal material was characterized by a high content of rhamnose (28%) in April and glucuronic acid (15·74%) in February. Variations were also found in the patterns of free amino acids and of those of the isolated algal protein. A milk-clotting factor was detected in the water extract of the algal material.

INTRODUCTION

MARINE algae have been studied intensively because of the industrial importance of their constituents, In the course of studies in our laboratory, the composition of some brown algae has been reported.[1] Although the Alexandrian coast is rich in green algae, knowledge of their constituents is still scanty. One of the dominant algal species is *Ulva lactuca* and the present work was undertaken to investigate the constituents of this algal species as they are affected by seasonal changes.

RESULTS AND DISCUSSION

The data in Table 1 indicate that the ash content of *Ulva lactuca* reaches its maximum in November and minimum in August. However, in February and April the ash contents were nearly identical.

Appreciable amounts of lipids were found in *U. lactuca* and other green marine algae as previously reported.[2–4] Significant variation in lipid content was noticed from one season to another, reaching a maximum in November, which was nearly 10-fold that of the minimum in August. Considerable seasonal variation was found in the composition of the lipid material, but no correlation existed between the content of lipid and that of its unsaponified fraction. Although in August and November the alga contained the least and highest amounts of lipids, yet the unsaponified fraction constituted 71% of the first and 40% of the latter, respectively.

On the other hand, the sterol content reached its maximum in February, but the sterol content was not correlated with the lipid content and depended largely on the proportion of the unsaponified lipid fraction in total lipids. Sterols have been reported by many authors

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in *U. lactuca* and other marine green algae.[2,4–6] Nobuo *et al.*[5] has also found that the sterol content in marine *Chlorophyta* is similar to higher plants.

TLC of the unsaponified lipid fractions revealed the presence of three compounds giving positive reactions with sterol reagents. The chromatographic pattern was the same in all seasons. One was identical with authentic cholesterol in mobility and chromogenic reactions. Thus, on spraying with methanolic sulphuric acid both the algal sterol and authentic cholesterol became red, and then turned to blue, violet and finally brown. The presence of cholesterol in *U. lactuca* and some marine green algae has also been recently reported.[4,5]

TABLE 1. PERCENTAGE COMPOSITION OF *Ulva lactuca* AS INFLUENCED BY SEASONAL CHANGES

Date of collection	April 1971	August 1971	November 1971	February 1972
Total ash	24·03	20·58	35·89	24·42
Total lipids	3·07	0·40	3·94	2·91
Unsaponified lipid fraction	0·70	0·28	1·57	1·90
Total sterols	0·04	0·15	0·70	1·12
Mannitol				
Periodate	1·92	1·65	1·72	2·01
Extraction	0·96	1·89	2·38	2·51
Glucuronic acid	11·68	5·73	2·45	15·74
Glucose	1·67	2·02	0·61	0·98
Arabinose	2·07	0·67	0·25	1·18
Xylose	1·73	0·81	0·39	1·58
Rhamnose	28·00	6·08	1·47	1·96
Rh/G.A.	2·40	1·04	0·91	0·12
Protein	8·70	33·75	21·16	30·13
Total amino nitrogen	2·25	8·13	4·22	6·81

Rh/G.A.—Rhamnose/glucuronic acid.

Although mannitol is not common in green algae, small amounts were found in *U. lactuca*. The isolated mannitol samples were identical in m.p., m.m.p. and R_f with authentic material. Mannitol has previously been found in small amounts in the green seaweeds *Enteromorpha compressa*[7] and *Tetraselmis*[8] species but not in *U. lactuca*.[8] The small amounts of mannitol found in *U. lactuca*, as compared to the higher amounts recorded in our local brown algae,[1] may support the view of Craigie *et al.*[8] that the presence of mannitol in some green algae is due to epiphytic contamination.

PC of the ethanol extracts of the algal material, after removal of mannitol and concentration, indicated the absence of sucrose or any other low MW carbohydrate. On the other hand, mild acid hydrolysis of these extracts and chromatography afforded neither fructose nor glucose. This result provided additional evidence for the absence of sucrose in the algal material of different seasons. The absence of sucrose and low MW carbohydrates in our local *U. lactuca* might be due to their loss during the process of washing the algal material.

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The acid hydrolysates of the algal material collected in different seasons showed the presence of glucuronic acid, glucose, arabinose, xylose and rhamnose. The absence of fructose among the hydrolysis products of the algal material confirmed the absence of sucrose in the algal ethanolic extracts. The content of each sugar component varied according to the season. Generally, the total carbohydrate content was maximal in April when maximal contents of arabinose, xylose, and rhamnose components were also noted. However, in November, the algal material contained the least amounts of the sugars. Furthermore, in April and February the algal species was characterized by high contents of rhamnose and glucuronic acid, respectively.

The presence of all the above mentioned sugars, except arabinose, in the acid hydrolysates of *U. lactuca* has been demonstrated previously.[9–11] The presence of arabinose in our local *U. lactuca* may be due to environmental or climatic effect. Similarly, although mannose and galactose were not found in *U. lactuca*, confirming the results of Graigie *et al.*[8] Percival and McDowell[12] reported the presence of trace quantities of these sugars in the hydrolysates of this alga. However, arabinose was found in other green seaweeds like *Cladophora rupestris*,[13] *Chaetomorpha linum* and *Chaetomorpha capillaris*.[14]

Starch was detected in *U. lactuca* collected in different seasons. Thus, on extraction of the algal material with hot water, the extract gave a bluish purple colour with iodine. Percival and Wold[10] also found starch in *U. lactuca*. However, on extraction of the algal material by grinding with cold water, the resulting extract gave no colour with iodine. Furthermore, after dialysis of the latter extract followed by acid hydrolysis and chromatography, the hydrolysate afforded glucuronic acid, glucose, arabinose, xylose, and rhamnose. The presence of glucose in the hydrolysate indicated that part of the glucose found in the acid hydrolysates of the algal material was derived from starch.

Protein comprised the major component of *U. lactuca*, reaching its maximum in August and minimum in April. The total amino nitrogen in the algal species showed the same aspect of seasonal variation like protein. A more or less constant ratio between the protein and amino nitrogen contents was shown in all the seasons.

The qualitative amino acid composition of the isolated algal protein and algal free amino acids varied seasonally (Table 2). In this respect, some similarity was shown between the amino acid pattern of the algal material in August and February. On the other hand, the algal material was characterized by the absence of cysteic acid, threonine and proline in April, valine and proline in August, and cysteic acid in November.

The presence of free amino acids in the algal material might be due to enzymic digestion of the algal proteins. This is supported by the fact that, in most cases, the presence of a free amino acid seemed to depend on its presence in the corresponding protein. Conversely, the absence of a free amino acid was correlated to its absence in the protein of the same algal material. This led to a preliminary investigation of the ability of the dialysed algal water extract to clot reconstituted skim milk, as in proteolysis.[15] Water extracts of the algal

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material, collected in different seasons, was able to clot milk within about 5 hr, indicating weak proteolytic action. On using previously boiled algal water extract as a control, clotting of milk did not occur for a period lasting for 3 days.

TABLE 2. THE AMINO ACID PATTERN OF THE ALGAL WATER EXTRACT IN DIFFERENT SEASONS

Type of amino acid	Date of collection: April 1971 A.A.P.	April 1971 FA	August 1971 A.A.P.	August 1971 FA	November 1971 A.A.P.	November 1971 FA	February 1972 A.A.P.	February 1972 FA
Cysteic acid	−	−	+	+	−	−	+	+
Aspartic acid	+	+	+	+	+	+	+	+
Glutamic acid	+	+	+	+	+	+	+	+
Serine	+	−	+	+	+	+	+	+
Glycine	+	−	+	+	+	+	+	+
Taurine	+	−	+	+	−	−	+	+
Threonine	−	−	+	+	+	−	+	+
Alanine	+	+	+	+	−	+	+	+
Histidine	+	−	+	+	+	−	+	+
Lysine	+	−	+	−	+	−	+	−
Arginine	+	−	+	−	+	−	+	−
Tyrosine	+	+	+	+	−	+	+	+
Proline	−	−	−	−	+	−	+	+
Valine	+	+	−	−	+	+	+	−
Leucine	+	+	+	+	+	+	+	+
Isoleucine	+	+	+	+	+	+	+	+
Phenylalanine	+	+	+	+	+	+	+	+

+ Present. − Absent. A.A.P. Amino acid in protein. FA: Free amino acid.

EXPERIMENTAL

Collection and pretreatment of algae. Ulva lactuca was collected periodically in 1971–1972 from the same place at Roushdy, Alexandria. The number of plants included in each sample was about 500, all being at the same stage of development. The algae were thoroughly washed with running H_2O, dried in the sun for several days and then ground. Ashing was carried out at 800°.

Total lipids. Lipids were isolated by subjecting the algal material to Soxhlet extraction with EtOAc for 12 hr.[16] Thereafter, the filtered extract was evaporated under vacuum at 40° to dryness and the dry residue represented the total lipid content.

Total sterols. The lipid material was refluxed with 1 N ethanolic KOH (5 ml/g lipid) and benzene (50 ml) for 2 hr. H_2O (2 vol.) was then added and the unsaponified fraction was extracted by shaking with Et_2O (4 vol.). The ethereal extract was evaporated to dryness and the residue obtained represented the unsaponified fraction. The total sterol content was then determined on the residue according to the Liebermann–Burchard colour reaction method.[17] A standard curve was constructed using an authentic sample of cholesterol.

Chromatographic examination of the algal sterols. The unsaponified lipid fraction was dissolved in benzene and subjected to TLC using silica gel G. The solvent mixtures used were acetone–benzene (3:17), toluene–$CHCl_3$–MeOH) (10:10:3), and cyclohexane–$CHCl_3$–HOAc (15:5:0·1). Spots were detected with the Liebermann–Burchard reagent and with H_2SO_4–MeOH (1:1). As reference substances, cholesterol, sitosterol, lanosterol, and stigmasterol were used.

Mannitol. Mannitol was determined by two methods; (a) by the direct periodate oxidation method of Cameron *et al.*,[18] and (b) by extraction and weighing. In the second method the alga was extracted with boiling 85% EtOH for 24 hr. After filtration, the extract was concentrated and then treated with excess acetone. On cooling, mannitol crystallized out. After isolation of crystalline mannitol, its m.p. and m.m.p.

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were determined. It was also detected by PC using pyridine–EtOAc–HOAc–H_2O (5:5:1:3)[19] as solvent, and the Dedonder chromogenic reagent.[20]

Quantitative determination of the sugar components in the algal hydrolysate. Each algal material was hydrolysed by boiling under reflux with 85% HCO_2H for 6 hr. The hydrolysate was then diluted with 5 vol. H_2O and the boiling extended for a further 2 hr. Quantitative PC separation of the algal hydrolysates was achieved on Whatman 3 MM paper using the solvent *n*-BuOH–pyridine–H_2O (6:4:3).[21] Spots were detected with aniline phthalate and aniline diphenylamine phosphate reagents.[22] The unstained area corresponding to the position of each sugar was cut off and eluted with H_2O. Glucuronic acid was determined by reaction with carbazole.[23] Reaction with orcinol[24] was used for the determination of arabinose and xylose, while glucose and rhamnose were determined by reaction with L-cysteine–H_2SO_4.[25,26]

Crude protein. Organic N was determined by the micro Kjeldahl method and multiplied by 6·25.

Total amino nitrogen. The algal material was hydrolysed in 6 N HCl in a sealed tube for 16 hr at 105°. After removal of HCl by evaporation at 100° with occasional addition of H_2O, total amino nitrogen was determined in the hydrolysate as glycine according to the method of Müting and Kaiser.[27]

Water extraction of the algal material. The algal material (5 g) was ground 2× with distilled H_2O (50 ml) in a mortar for 20 min at room temp. The filtered extract was then deionised by dialysis against H_2O at 2°.

Examination of milk-clotting activity of the algal water extract. This was done by the method of Berridge;[28] 2·5 ml of buffered (0·02 M acetate buffer, pH 4·0) algal H_2O extract were incubated with 10 ml reconstituted skim milk (12 g dry skim milk/100 ml 0·01 M $CaCl_2$), under toluene, at 40° and clotting was observed. A control was performed in the same manner using boiled algal water extract.

Chromatographic examination of amino acids in the algal water extract. Each algal material was extracted with H_2O as described above and the undialysed extract was treated with 1 vol. acetone with 1% HCl. The protein ppt. was isolated by centrifugation, washed several times with acetone until it was free from acid and dried in vacuum oven at 40°. The supernatant was washed several times with Et_2O and the aq. layer evaporated to dryness. The residue contained the free amino acids in the algal material. Two dimensional PC of the algal free amino acids was performed using the solvent mixture *n*-BuOH–HOAc–H_2O (4:1:5)[29] and phenol–H_2O (4:1, wt: vol.).[30] The amino acid composition of the isolated algal protein was also investigated chromatographically after hydroylsis with 6 N HCl for 16 hr at 105°. Dectection of spots was achieved with ninhydrin.[22]

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