**BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY**

A N   I N T E R N A T I O N A L   J O U R N A L

Chemical Composition of *Ulvaria oxysperma* (Kützing) Bliding, *Ulva lactuca* (Linnaeus) and *Ulva fascita* (Delile)

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ABSTRACT

Chemical composition of three species of Chlorophyta, Ulva lactuca, Ulva fasciata and Ulvaria oxysperma, was determined. Ulvaria oxysperma showed humidity (16-20%), ash (17-31% dry-base), proteins (6-10%db), lipids (0.5-3.2%db), fibers (3-12%db) and carbohydrates (46-72%db) which corresponded to 192-270 kcal.100g^{-1} (wet-base). U lactuca (15-18%db) and U fasciata (13-16%db) revealed grades slightly higher for proteins, but with similar energetic contents (250-272 and 225-239 kcal.100g^{-1}), respectively. Natural blades of U lactuca and of U fasciata were more rigid than blades of U oxysperma. Other native algae showed higher protein levels. Ulvaria sp could be cultivated on the cotton-nylon net without having significance competition from other algae or predators degradation. On the other hand, it was essential choose a place without destructive tide action. Cultivated Ulvaria sp showed similar chemical composition as that grown on natural substrates.

Key words: Chemical composition, algae, *Ulvaria, Ulva,* Guaraqueçaba, Ilha do Mel

INTRODUCTION

Oceanic algae are vegetables without vascular system; however, its cells can form agglomerations, as strings or blades. Algae can be applied to obtain several products. It is possible to classify algae in three groups of color: Chlorophyta (green), Phaeophyta (brown) and Rhodophyta (red). Mahasneh et al. (1995) found antibiotic activities in the three groups of algae, which supported the importance of these vegetables. Chlorophyta are commonly used as food due to high contents of vitamins and minerals. Phaeophyta are typical suppliers of alginic acid. Rhodophyta are responsible to produce agar-agar and carragenan. These polymers are used to improve the chemical and physical characteristics of several industrial products (Jarrouj, 1992, Miller and Blunt, 2000, Pal et al., 1998).

Ulvaria oxysperma and *Ulva* spp are Chlorophyta. These species are different by number of cell strata. After a transversal cut*, Ulva* genus shows two stratum of cells, while *Ulvaria* has only one stratum (Joly, 1965). *Ulvaria oxysperma* lives frequently in mangroves over mud or roots, stems of tree and stones. *Ulva fasciata* prefers stones as substratum, while *Ulva lactuca* prefers mangroves and sand (Joly, 1957).

Ulvaria sp and *Ulva* spp grow abundantly in the seaside of the Paraná State. *Ulva lactuca* grows in Guaraqueçaba and *Ulva fasciata* grows in Ilha do Mel, while *Ulvaria oxysperma* grows into the two ecosystems (Shirata, 1993). *U. oxysperma* is harvested by the inhabitants from Guaraqueçaba to improve the domestic budget.

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U. oxysperma is harvested by hand, dried at room temperature, and wrapped into plastic packages commercial purposes. However, there are physical, chemical and biological studies of environment were these algae no exist. It is necessary to preserve this ecosystem (Borges, 1992; Ipardes, 1990; Soares, 1990).

Chemical composition of some species of sea algae from India was studied. *Enteromorpha linza, E. prolifera, Caulerpa taxifolia, Sargassum johnstonii* and *Ulva fasciata* were studied to evaluated in nutritional quality. *Ulva fasciata* showed high concentration of lipids, normal levels of fiber and low proportion of protein (Naidu et al., 1993). Arieli et al. (1993) evaluated the nutritional value of *Ulva lactuca* as food for ruminants. Algae were produced from an integrated marine culture project using six Finn-Merino crossbred ram lambs. *Ulva lactuca* was characterized as low-energy high-nitrogen foodstuff, but it is considered a good food for ruminants.

Specific nutritional evaluation of sea algae about amino acids content has been considered recently. Seventeen of the essential amino acids were detected in hydrolyzed protein in eighteen species of algae, including *Ulva lactuca*. There were significant variations in composition of amino acids for the different genus of algae. On the other hand, low variation was observed in total nitrogen and inorganic contents (Qasim, 1991).

Soluble and insoluble dietary fiber contents of marine algae: wakame (*Undaria pinnatifida*), hijiki (*Hijiki fusiformes*), sea spaghetti (*Himanthalia* elongata), aroma (*Eisenia bicylis*), sea lettuce (*Ulva lactuca*), nori (*Enteromorpha* spp) and nori (*Porphira tenera*) were estimated by the gravimetric method. Dietary fiber contents were between 32.7 and 74.6% (on a dry weigh basis) of which 51.6 to 85.0% were water soluble. These marine algae have are good suppliers in fibers, which shown to have chemical, physicochemical and rheological diversities that may be beneficial in nutrition (Lahaye, 1991). Lahaye and Axelos (1993) suggested that *Ulva* spp is a good source of gelling polysaccharides of potential economic value. Water-soluble polysaccharides were extracted from *Ulva* spp which proliferate along Brittany shores of France. They were composed of the combination of rhamnose, glucose, xylose, mannose, galactose, uronic acid and sulfate group. Mechanisms for gel formation were proposed, which unusually involve both boron and calcium ions.

Ivanova et al. (1994) isolated a polysaccharide with antiviral effect from *Ulva lactuca*. This polysaccharide contains arabinose, xylose, rhamnose, galactose, manose and glucose (1:1:9:5:2:5:16), besides another unidentified sugar. Antiviral activity *in vitro* against human and avian influenza virus was identified. This property was dose-dependent, strain-specific and selective.

Serot et al. (1994) studied the extraction and the characterization of proteins from *Ulva* spp to increase the commercial profit. Extraction was performed according to an experimental design involving extraction time (60 to 300 min), amount of dry algae (1 to 2:100, w:v) and NaOH solution (0.5 to 2 $mol.dm^{-3}$). Optimal extraction condition was obtained after 200 min extraction to 1 g of dry algae in 100 mL of 2 $mol.dm^{-3}$ NaOH solution. 46.6% of crude protein were extracted and a significant amount of non-dialyzable polypeptides too. Molecular weight reveled four major bands (12,400, 18,000, 20,500, 21,700) for the polyacrylamide gel electrophoresis method and three peaks (100,000, 60,000, and 25,000) for the permeation gel chromatography.

Excess nutrients from coastal fish farms produce water pollution. *Ulva* sp was cultivated in the polyculture of seaweed and fish in order to reduce eutrophication in the sea. The growth of Ulva was closely related to the water temperature and the optimum value is between 20 and 28 ^{o}C (Hirata and Kohirata, 1993).

Ulvaria oxysperma is the new name to *Monostroma oxysperma*. This alga is less studied than *Ulva* spp, although it is more used as human food. *U. oxysperma* is more pleasant to sense of taste than *Ulva* spp. Guo and Matthieson (1992) studied the seasonal distribution of *U. oxysperma* in the estuary of Northeast of "New England" (USA). The growth of *U. oxysperma* and the economical viability to large production was studied for Kapraun and Sherman (1989).

Cordeiro-Marino et al. (1993) studied life-history and ontogeny for ulvaceous from State of Espírito Santo, Brazil. Three levels of salinity (1, 20 and 40 $g.dm^{-3}$) and four levels of temperature (15, 20, 25 and 30°C) were tested in factorial design to evaluate the maximum growth rate to one of the population of *Ulvaria* sp. The maximum growth was to 25°C and 40 $g.dm^{-3}$ and the smaller growth

was to 30^oC and 1 $g.dm^{-3}$. Algae died after 54 to 73 days with a combination of 30^oC and 1 $g.dm^{-3}$. *Ulvaria* sp does not grow at 15^oC in any salinity or in 30^oC and 1 $g.dm^{-3}$.

The objective of this work was characterize the chemical composition of *Ulva* spp and *Ulvaria oxysperma* found in the Paraná State. Some of these results were compared to other algae species and cultivated *Ulvaria* sp.

MATERIALS AND METHODS

1 - Harvest and manipulation of algae from natural substrate

Harvest was pre-determined by the Nautical Cards from DRH (Diretoria de Hidrografia e Navegação) to coincide with low tide period, to permit a large area to harvest, which was generally in the morning. The species studied were harvested on the mangrove of the Caetê, in the Bay of Guaraqueçaba (latitude 25°15'-25°20' South and longitude 48°20'-48°25' West) and Saco do Limoeiro, Ilha do Mel, Bay of Paranaguá (latitude 25°33'-25°34'- South - longitude 48°18'-48°19' - West). These two locality belong to Complexo Lagunar de Iguape - Bay of Paranaguá.

The samples were obtained from June 1995 to February 1996. The harvest was manual and sometimes with a stainless knife to untie the algae from soil or stone. Algae were put into plastic packages to carry to the laboratory, and were cleaned under water in the laboratory to remove of particles in suspension and adhered. After that, the algae were dried at room temperature, without direct light of sun.

Dry algae were packaged in paper, identified and stored in cardboard boxes in laboratory to process and analysis. The specie was identified using the Analytical Keys of Joly (Joly 1957 and 1965) and Ugadim (1973). Parts of the material were registered as droughty samples on the HPUC (Herbarium of the Pontificia Universidade Católica do Paraná, PUC-PR).

2 - Cultivation of *Ulvaria oxysperma*

Cotton-nylon nets with 0.7 by 3 m were installed from 1997 to 1999 in the Ilha do Barbado in Guaraqueçaba to evaluate: a) adhesion capacity; b) competition with others algae; c) presence of predators; d) resistance of tide and e) physical (wet and dried algae) and chemical (residual humidity, ash, protein, carbohydrate, fat and fibers) characteristics. Nets were put them above the water levels similar the height found natural *U. oxysperma*. This way, part of time algae on the net was above and other time was below of water.

3 - Analysis

A - Samples Preparation

Samples were dried at room temperature in darkness. Alternatively, some samples were dried in an OLIDEF oven (model CZ) at 50 °C to compare the effects of the industrial technique. The samples were identified by genus and harvest localization and stored in Kraft paper packages. 10 to 15 g of alga was weighed in analytic balance (METTLER H6, 0.1 mg) for assays.

B - Humidity and Ash

Humidity was considered as the losses in mass from a sample (1g) after drying at $100^oC \pm 2^oC$ (ELEKTROHELIOS oven J84234-2, 200^oC). The residual mass in this sample was heated at $600^oC \pm 10^oC$ (FORNITEC 0159, 1200^oC) to obtain ash.

C - Organic Material

It was calculated through the difference between 100% and the sum of humidity and ash values.

D - Nitrogen and Protein

Nitrogen was measured by Kjeldahl method using the distiller-digester PROCION (110V, Brazil) (Yokoyama and Guimarães, 1975).

E - Lipids or Ethereal Extract

2 to 5g of sample were extracted with ethyl ether in Soxhlet for 5 hours. The solvent was evaporated and the residual mass on the balloon was considered lipids.

F - Fibers

2 to 5g of sample were digested with 200 cm^{-3} of 5% HCl for 30 minutes. The mixture was filtrated and washed with hot water. The residue was digested with 200 cm^{-3} of 5% NaOH under reflux to 30 minutes. The mixture was filtered and washed with water until negative alkaline reaction with indicative paper (methyl red). The material was washed with 20 cm^{-3} of ethyl alcohol and with 20 cm^{-3} of ethyl ether. The residue was dried at 100°C by two hours and the residual mass was considered fibers.

G - Carbohydrate

Subtraction of the sum of humidity, protein, lipids, fibers and ash values from 100 was the carbohydrate contents in percentage.

H - Calories

Calories was calculated by the following equation:
Calories $(kcal.100g^{-1}) = 4 \cdot$ protein $(\%) + 9 \cdot$ lipids $(\%) + 4 \cdot$ carbohydrate $(\%)$.
Ash, organic material, protein, lipids, fiber, carbohydrate grades were expressed as dry base (db), but calories was expressed as wet base.

RESULTS AND DISCUSSION

1 - Physical aspects

Ulva spp and *Ulvaria* sp were found at several habitats. Their zoospore could be fixed in organic and inorganic materials and they generated blades more than 20 cm. The natural blades of *U. lactuca* and *U. fasciata* were more rigid than *Ulvaria oxysperma* blades. This characteristic was attributed to the number of strata of cells in transversal section. *Ulva* spp had two while *Ulvaria* sp had only one. The blade color was modified during dry operation from light green to dark-green. The dried blade of algae produced rigid texture, as it was a fiber material. Final physical aspects, color and texture, did not change for more than 12 months. The color of dried blade of algae changed brown or light brown on heating above $60^{o}C$.

2 - Cultivation of *Ulvaria oxysperma*

Cotton-nylon net was a good substrate to *U. oxysperma*. *Enteromorpha* sp growth in little quantity in the extreme of net. The density of algae was as high as compared to natural substrate. There were not significant predators. Alternate exposition or immersion of algae was important for the development of *U. oxysperma*. On the other hand, impetuous movement resulted breaking and loosing of algae. Texture of cultivated algae was similar to the natural algae, although the colour was dark green.

3 - Chemical Composition

The main nutritional components to different algae and *U. oxysperma* from two regions of harvest were determined. The content of humidity, ash, proteins, lipids, fibers and carbohydrate were determinated for *Ulvaria oxysperma*, *Ulva lactuca* and *Ulva fasciata* (Tables 1 and 2). These algae dried in darkness remained with a residual humidity between 15.6 and 20.4%, which was sufficient to avoid the development of microorganisms. Ash levels of *U. oxysperma* from Guaraqueçaba were from 16.8% (February, 1996) to 18.9% (November, 1995), slightly lower than Ilha do Mel, from 22.1% (February, 1996) to 31.2% (November, 1995).
The average contents of organic material of *U. oxysperma* from Guaraqueçaba was higher (82.1%) than Ilha do Mel (73.3%). Protein was 6.3%, except in the sample harvested in February 1996 from Ilha do Mel (10.0%). Lipids were between 0.5 and 3.3%. Fibers contents from Guaraqueçaba was lower (3.8%) than from Ilha do Mel (10.3%).

Table 1 - Chemical[*1] composition of *Ulvaria oxysperma* (*U.o.*) harvested in November (**n**), 1995 and in February (**f**), 1996 from Guaraqueçaba (**G**) and from Ilha do Mel (**IM**) to 100 g in sample.

Contents	*U.o.*Gn	*U.o.*Gf	*U.o.*IMn	*U.o.*IMf
humidity, g [*2]	15.58	17.53	20.42	16.79
ash, g [#]	18.91	16.83	22.14	31.22
organic material, g [#]	81.09	83.17	77.86	68.78
protein, g [#]	6.03	6.39	6.51	10.02
lipids, g [#]	0.89	1.44	3.28	0.53
fibers, g [#]	4.60	3.06	8.95	11.73
carbohydrates, g [#]	69.58	72.28	59.12	46.50
calories levels, kcal [*2]	262.07	270.23	232.41	192.08

[*1] Average of two repetitions.
[*2] wet base.
[#] dry base.

Algae from Ilha do Mel showed lower carbohydrate contents (52.8%) than from Guaraqueçaba (70.9%). *Ulvaria oxysperma* from Guaraqueçaba showed higher nutritional energy level (266.2 kcal.100g^{-1}) than from Ilha do Mel (212.2 kcal.100g^{-1}).

Table 2 showed that *Ulva* spp maintained a residual humidity from 15.1 to 20.8%, similar to *Ulvaria oxysperma* (15.6 to 20.4). The development of microorganisms was not observed to storage in this grade and the coloration was also similar to *U. oxysperma*. Algae harvested from the Ilha do Mel demonstrated higher residual humidity than from Guaraqueçaba. Ash contents from *Ulva lactuca* (12.9%) was lower than *Ulva fasciata* (19.8%). Algae from Ilha do Mel showed ash level higher than algae from Guaraqueçaba.

Table 2 - Chemical composition from *Ulva lactuca* (*U.l.*) collected in Guaraqueçaba (**G**) in October (o), 1995 and in February (**f**), 1996 and from *Ulva fasciata* (*U.f.*) collected in the Ilha do Mel (**IM**) in December (**d**), 1995 and in February (**f**), 1996[*1] to 100 g sample.

Contents	*U.l.*Go	*U.l.*Gf	*U.f.*IMd	*U.f.*IMf
humidity, g [*2]	18.08	15.09	20.80	18.64
ash, g [#]	13.23	12.54	20.61	17.75
organic material, g [#]	86.77	87.46	79.39	82.25
protein, g [#]	15.23	18.35	13.30	16.13
lipids, g [#]	1.22	1.79	1.94	0.34
fibers, g [#]	11.91	9.65	10.85	9.32
Carbohydrates, g [#]	58.40	57.67	53.31	56.47
calories levels, kcal [*2]	250.28	271.88	224.86	238.76

[*1] Average of two repetitions.
[*2] wet base.
[#] dry base.

Organic matter in *Ulva lactuca* from Guaraqueçaba was higher (87.1%) than Ilha do Mel (80.9%). Protein from *Ulva lactuca* was slightly higher (16.8%) than *Ulva fasciata* (14.7%). Lipids were between 0.3 and 1.9%, but without a characteristic behavior. Fibers from *Ulva* spp was between 9.3% and 11.9%, which was similar to *Ulvaria oxysperma* from Ilha do Mel (10.3%), but higher than *U. oxysperma* from Guaraqueçaba (3.8%). Carbohydrate in *Ulva lactuca* (58.0%) and *Ulva fasciata* (54.9%) were similar to *U. oxysperma* from Ilha do Mel (52.8%) and lower than *U. oxysperma* from Guaraqueçaba (70.9%). *Ulva lactuca* showed energy content (261.1 kcal.100g^{-1}) higher than *Ulva fasciata* (231.8 kcal.100g^{-1}). This was similar to *U. oxysperma* from Guaraqueçaba (266.2) in comparison to Ilha do Mel (212.2 kcal.100g^{-1}).

Table 3 shows chemical composition of commercial algae, called Urashima, sold in Brazil in 1998 and cultivated *U. oxysperma*. The blades of commercial algae show format almost square (18x20 cm). Algae are packaged in plastic bags and stamped, with a drain agent to maintain a low residual humidity (9.2%). Ash content was low and organic was high. Protein was very low (3.3%). Carbohydrate was high (88.2%) and fibers was low (0.8%). However, energy content was very high (348.7 kcal.100g^{-1}).

Cultivated *U. oxysperma* showed similar values to chemical composition as compared with algae grown on natural substrate. Nutritional energy content is between 251 and 276 kcal.100g^{-1}. The high ash content suggested a good source of mineral and low calories product to reduce ingestion of high levels of energy; compared to commercial product.

Contents of protein, humidity and ash of other algae frequently found in mangroves, in mud or in trunks from coast of the Paraná were also measured (see Table 4). Protein contents (16.6 to 24.8%) of Rhodophyta and Phaeophyta were frequently higher than Chlorophyta (6.0 to 18.4%). On the other hand, humidity contents (14.2 to 20.8%) and (4.3 to 18.7%) were similar. Carbohydrate and protein ratio were different.

Table 3 - Chemical composition of a commercial alga sold in Brazil and cultivated algae to 100g sample.

Contents	cultivated *U. oxysperma*	commercial[*1]
humidity, g [*2]	15.4-18.8	9.24
ash, g [#]	15.4-17.8	5.87
organic material, g [#]	77.9-85.0	94.13
protein, g [#]	5.9-11.7	3.27
lipids, g [#]	0.5-1.1	2.15
fibers, g [#]	3.16-6.3	0.76
carbohydrates, g [#]	67.0-59.9	88.24
calories levels, kcal [*2]	251-276	348.7

[*1] Urashima, Chlorophyta algae in blades with 18x20 cm with soybean juice and sugar.
[*2] wet base.
[#] dry base.

Table 4 - Protein level, humidity and ash from several algae Rhodophyta and Phaeophyta from Paraná Coastline harvested in Ilha do Mel, Paranaguá District.

Algae	Date	Protein, % [#]	Humidity, %	Ash, % [#]
Acanthophora spicifera	Apr 95	17.27	15.80	16.58
Hypnea cervicornis	Apr 95	18.72	15.08	21.96
Dictyota cervicornis	Apr 95	18.82	14.10	20.48
Bostrychia montagney	Apr 95	24.77	15.95	16.18
Bostrychia scorpioides	Sep 95	20.29	13.78	17.77
Porphyra spiralis	Jul 95	16.56	14.25	12.17
Gelidium floridanum	Feb 96	20.54	16.13	5.14

[#] dry base.

CONCLUSIONS

Analysis of the protein, ash, humidity and carbohydrate contents was evaluated to estimate the chemical composition of Chlorophyta. *U. oxysperma* and *Ulva* spp showed higher levels minerals and lower levels of calories. Dried product at room temperature and in the shadow could stored by several months without change its aspect. *U. oxysperma* presented lower protein levels than *Ulva* spp, but higher than the commercial alga Urashima, which demonstrated lower fibers. Low level in lipids showed that would be an excellent food to dietary.

Guaraqueçaba was the best option to commercial production of *U. oxysperma*. Residual humidity of algae (16 to 20%) was not modified after 12 months in storage in plastic. Organic material (69% to 87%) and inorganic material (13% to 31%) demonstrated to be a food with low level calories and rich in minerals. *U. oxysperma* show lower protein levels (6 to 10%) than *Ulva lactuca* and *Ulva fasciata* (13 to 18%), but higher than the commercial alga (3%).

ACKNOWLEDGEMENTS

RESUMO

A composição química de três espécies de Chlorophyta foi determinada. *Ulva lactuca* se desenvolve em Guaraqueçaba e *Ulva fasciata* na Ilha do Mel, enquanto que *Ulvaria oxysperma* se desenvolve nos dois ecossistemas. Umidade (16-20%), cinzas (17-31%, base seca), proteínas (6-10%bs), lipídeos (0,5-3.2%bs), fibras (3-12%bs) e carboidratos (46-72%bs) para *U.oxysperma* correspondeu a 192-270 kcal.100g^{-1} (base úmida). *U.lactuca* (15-18%bs) e *U.fasciata* (13-16%bs) mostraram teores levemente maiores para a proteína, mas com conteúdos energéticos similares (250-272 e 225-239 kcal.100g^{-1}), respectivamente. As folhas *in natura* de *U.lactuca* e de *U.fasciata* eram mais rígidas do que as de *U.oxysperma.* Outras algas nativas apresentaram teores de proteínas maiores. *Ulvaria* sp pode ser cultivada sobre redes, sem apresentar competição

com outras algas ou ação significativa de predadores. *Ulvaria* sp cultivada apresentou composição química similar ao da *Ulvaria* sp crescida sobre substratos naturais. É essencial escolher um local protegido da ação destrutiva da maré.

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