FOOD GRADE ALGINATES EXTRACTED FROM THE GIANT KELP *Macrocystis pyrifera* AT PILOT-PLANT SCALE

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RESUMEN

Se obtuvo el ácido algínico y los alginatos de sodio y potasio a nivel planta piloto a partir de *Macrocystis pyrifera*, empleando nuestra propia tecnología publicada en los artículos que se citan. Los alginatos obtenidos fueron analizados para determinar si cumplían con los estándares internacionales para ser empleados en la industria alimenticia. Los resultados presentaron los siguientes perfiles de calidad: fracción gulurónico F_G 0.38, fracción manurónico F_M 0.62 (también se presentan los valores para las diádas y triádas). La humedad promedio fue de 9.6 a 11.1%; calcio 0.22 a 0.38%; pureza 97.37 a 98.5%. El pH de los alginatos de sodio y potasio varió entre 7.2 y 7.5 y del ácido algínico fue de 2.6. El promedio de grasas fue de 2.11 a 3.85% y fibra cruda de 1.14 a 2.49%. El contenido de cenizas para los alginatos de sodio y potasio vario de 25.4 a 31.6% y para el ácido algínico fue de 1.89%. El contenido de arsénico fue menor a 1.51 mg kg^{-1}; la cuenta total viable de bacterias fue menor a 1956 UFC g^{-1}; la cuenta de hongos y levaduras fue menor a 70 UFC g^{-1}. No fue detectada en ninguna muestra la presencia de proteínas, plomo, coliformes totales o salmonela. Se concluye que el método desarrollado para producir alginatos a partir del alga *Macrocystis pyrifera* a nivel planta piloto puede ser empleado a nivel industrial para producir alginatos de grado alimenticio, ya que cumplen con los parámetros de control de calidad adoptados a nivel internacional.

Palabras clave: macroalgas; productos de algas; alginatos; perfiles de calidad; *Macrocystis pyrifera.*

ABSTRACT

Alginic acid, sodium alginate, and potassium alginate were extracted at the pilot-plant scale from *Macrocystis pyrifera* using our own technology published in previous papers. The alginates obtained were analyzed to determine if they fulfill the international standards to be used in the food industry. The results showed the following quality profiles: guluronic fraction F_G 0.38, mannuronic fraction F_M 0.62 (diads and triads are also showed). Average moisture was 9.6-11.1%; calcium 0.22-0.38%; purity 97.37-98.5%; pH of the sodium and potassium alginate ranged from 7.2-7.5 and it was 2.6 for alginic acid. Raw fat was 2.11–3.85% and raw fiber 1.14–2.49%. The ash content for the sodium and potassium alginates ranged from 25.4 to 31.6%, and was 1.89% for alginic acid. Arsenic was <1.51 mg kg^{-1}; total viable count <1956 UFC g^{-1}; fungi and yeasts count <70 UFC g^{-1}. Protein, lead, total coliforms, and salmonella were not found in any sample. We conclude that the method developed to produce alginates from the algae *Macrocystis pyrifera* at the pilot plant level could be used at an industrial level to produce food grade alginates since they fulfill the international quality control parameters.

Key words: macroalgae; products of algae; alginates; quality profiles; *Macrocystis pyrifera.*

Alginates are present in all brown algae (Phaeophyceae) as the most abundant polysaccharide, representing 18-40% of the dry matter. Alginates are located in the amorphous matrix of the cellular wall of brown algae in a gel state, as the salts of Na^+, Ca^{2+}, Mg^{2+}, Sr^{2+}, and Ba^{2+} of alginic acid (Smidsrød and Draget, 1996).

Alginate is a family of linear copolymers containing 1,4,-linked □-D-mannuronic acid (M) and its 5-epimer □-L-guluronic acid (G) (Smidsrød and Christensen, 1991). Three types of blocks can be found: poly-M, poly-G and alternating blocks of the type M-G-M-G (McHugh, 1987; Skjåk-Bræk and Martinsen, 1991; Smidsrød and Christensen, 1991). The most successful way of describing the composition of alginate is to determine the frequencies of diads and triads by nuclear magnetic resonance (NMR) techniques (Grasdalen *et al.,* 1979) using 400-500 MHz ^{1}H-NMR or 100-125 MHz ^{1}C-NMR (Grasdalen, 1983).

Alginates are produced in three categories in relation to their main application: industrial, food and pharmaceutical grade. Of the total alginate world demand, food grade alginates represent 30%.

The use of food grade alginates is based on their physical properties as a thickening agent, stabilizer, emulsifier, and a moisture retainer. They are used in the production of ice cream, salad dressing, sauces, creams, syrups, bakery goods, vegetables and canned meat. Alginates are also used to stabilize beer foam and in the suspension of solids in fruit drinks. As a gelling agent it is used in instant milk desserts, jellies, fruit desserts, food for animals, and restructured fruit. As a binder agent it is used in reconstructed meat products. In the last case, the binding agent is a mixture of sodium alginate, calcium carbonate, lactic acid and calcium lactate. Alginates are also used to make shrimp and crab analogue products using alginates and proteins such as soy or sodium caseinate. A mixture of the two is extruded into a calcium chloride bath in various shaped molds (Wyliem, 1976; Morimoto, 1985; Means and Schmidt,1986; McHugh, 1987; Indergaard and Ostgaard, 1991). A meat substitute is also derived from a mixture of proteins and alginates (Shenouda, 1983).

The total annual harvestable biomass of *Macrocystis pyrifera* (L.) C. Agardh 1820, in Mexico ranges from 30,000 to 97,800 tons (Hernández-Carmona *et al.,* 1989a, 1989b, 1991). However, all of the kelp harvested in Mexico (29,000 t per year) is exported to USA for alginate production. Although a pilot plant for alginate production has been built, presently Mexico does not have an industrial plant for alginate production. Therefore, alginates are not produced in Mexico and consumers must import them from other countries. This results in a technological dependence, and a waste of part of this renewable resource. The objective of this work was to analyze the alginates obtained at the pilot-plant scale using our own technology, to determine if they fulfill the international standards to be used as food grade alginates.

MATERIAL AND METHODS

Alginate production at the pilot plant level

The giant kelp *Macrocystis pyrifera*, was harvested from Bahía Tortugas, Baja California Sur, Mexico. The kelp was obtained from surface floating fronds to simulate the commercial harvesting. Samples were obtained from an area covered by at least 30 different plants (to reduce individual variability), transported to the boat and dried from the same day during 2-3 days. The dried plants were packed and transported to the laboratory for milling.

The kelp was processed at pilot plant level as follows: 10 kg of dried and milled algae were placed to rehydrate overnight in a tank containing 90 L of a 0.1% formalin solution. The residual solution was drained off and the algae were washed with 100 L of hydrochloric acid solution at pH 4 in the same tank for 15 min with constant agitation (Hernández-Carmona *et al.,* 1998). The material was then transferred to an extraction kettle containing 166 L of water, and the pH was adjusted to 10 with powdered anhydrous sodium carbonate while heated to 80°C for 2 h, with constant stirring. The resultant paste was diluted to 45 mPa s and filtered in a rotary vacuum filter, using diatomaceous earth as a filter aid (Hernández-Carmona *et al.,* 1999a). During filtration, the filtrate was pumped to the precipitation tank and simultaneously a solution of 10% calcium chloride was added to precipitate the alginate as calcium alginate, using a ratio of 2.2 parts of calcium chloride to one part of alginate in the algal raw material. The suspension was filtered on a metal screen; the calcium alginate fibers were suspended in 150 L of water and bleached with 700 mL of sodium hypochlorite solution (5%). After filtration, the fibers were suspended in 150 L of water and washed three times with constant agitation, adjusting the pH with hydrochloric acid to 2, 1.8 and 1.8 respectively (McHugh *et al.,* 2001). The alginic acid was separated on a metal screen and pressed in a hydraulic press to remove as much water as possible. Alginic acid was removed from the screen and broken up, then dried in an oven with a hot air current at 45°C until a constant weight was achieved. To obtain the sodium and potassium alginate, the alginic acid fibers were placed in a double planetary mixer. Enough alcohol was added to reach an ethanol-water proportion of 50:50 in the fibers. Sodium carbonate or potassium carbonate (depending on the salt to be obtained) was added until a pH 8 was reached. The alginate fiber was sampled from the mixer, dissolved in distilled water, and the pH was measured with pH indicator paper. If the pH was lower than 8, more carbonate was added, until a pH 8 was reached. Sodium and potassium alginates were pressed to remove the ethanol-water solution. The resulting mat was loosened and broken up, then dried at 60°C (Hernández-Carmona *et al.,* 2002). All process and analysis were conducted with 3 replicates, and averages and standard deviations were computed as percentage based on the dry weight of the initial algae.

Alginate compositions

The alginate compositions were determined by Nuclear Magnetic Resonance (^{1}H-NMR), at 300 MHz, including the frequencies of monads, diads and triads of the mannuronic (M) and guluronic (G) acids (Grasdalen, 1983). Analyses were carried out at the Norwegian Biopolymer Laboratory of the Norwegian University of Science and Technology. The results were compared with the analysis reported by Smidsrød *et al.* (1991) for commercial alginates obtained from *Macrocystis pyrifera.*

Alginate viscosity and pH

The viscosity of the alginates obtained using the methods described above was measured on 300 mL of 1, 2, and 3% (w/v) aqueous solution at 25°C, using a Brookfield viscometer model LVTDV-I, 60 rpm, with the appropriate spindle. A second viscosity measurement was obtained after adding sodium hexametaphosphate (0.5 grams per gram of dry alginate), to sequester any calcium ion present. The differences in viscosities were calculated and the percentage of reduction was used as quality parameter. According to standards regulations, the viscosity reduction should not be greater than 40%. The pH of the alginate solutions were measured using a pH meter (Hernández-Carmona *et al.,* 1999b).

Moisture and ash content

Moisture content was determined by placing 5 g of the alginates obtained in an infrared thermobalance at 110°C for 30 minutes. The ash content was determined by placing 0.5 g of the dried sample in a crucible and calcining in a furnace at 700 ºC for 12 h. The crucibles were cooled in a desiccator and weighed soon after they reached room temperature and the percentage of ash was calculated, based on weight of the initial dry alginate.

Calcium content

The ashes obtained as above were solubilized with hot concentrated hydrochloric acid (37%), and transferred to beaker. The sample was diluted with distilled water to 100 ml, and a 4% solution of ammonium hydroxide was added to obtain a pH between 10-12. The solution was heated close to the boiling point and a solution of ammonium oxalate (10%) was added, while stirring with a glass bar to precipitate all of the calcium as calcium oxalate. The sample was allowed to cool for three hours at room temperature. Calcium oxalate was filtered in a glass fiber funnel, dissolved with hot hydrochloric acid (0.1N), and titrated with potassium permanganate solution (0.1N). The volume of potassium permanganate indicated the quantity of calcium oxalate. One mL of potassium permanganate (0.1N) corresponded to a one miliequivalent, that is, 2.8035 mg of calcium oxide or 2.0035 mg of calcium. The percentage of calcium in the dry alginate was computed as: [(mL of potassium permanganate 0.1N) * (2.0035) * (100) / (mg of alginate in dry basis)] (Hernández-Carmona *et al.,* 1999b).

Alginate content (purity)

One gram of calcium chloride was dissolved in 100 mL of methanol-water solution (40-60%). The resulting solution was added to 100 mL of 0.5% alginate solution, while gently stirring. The precipitate was removed using a fine filter, and washed with methanol-water solution (20-80%). A second washing was carried out with methanol-water solution (40-60%). The precipitated alginate was dried in an oven at 105°C for 2 h. The alginate was maintained in a desiccator for one hour and weighed. The alginate content (purity) was computed as: (weight of the precipitated / weight of the initial alginate on dry basis * 100) (Hernández-Carmona *et al.,* 1999b)

Protein, fat and fiber

Analyses of raw protein, fat and fiber of the alginates obtained were carried out according to the methods described by the Association of Official Analytical Chemists, using methods (960.52), (930.39) and (962.09) respectively (AOAC, 1990). Results are given as percentage of the initial weight of the dry algae.

Lead and arsenic

Lead contents in the extracted alginates were analyzed using out by atomic absorption spectrophotometry. Arsenic concentrations were determined in a hydride generator, coupled to the atomic absorption spectrophotometer. The instrument operation specifications were as recommended by the manufacturer (Perkin Elmer, 1990). Results are given as mg Kg^{-1} of dry alginate.

Microbiological analysis

Alginate samples obtained were tested for the following microbiological analysis: total viable count, fungi, yeasts, total coliforms, and salmonella, in accordance with the methods described by the American Public Health

Association (APHA, 1992). Results are given as UFC g^{-1} of dry alginate.

RESULTS

The average alginate yield obtained at the pilot-plant scale was 20% for sodium alginate; 21% for potassium alginate, and 16% for alginic acid. The alginate compositions were F_G 0.38, and F_M 0.62 (diads and triads are also presented on Table 1). Fig. 1 shows the ^{1}H-NMR spectra of the sodium alginate obtained at the pilot plant level. Table 2 shows the viscosity of sodium alginate solutions at three different concentrations. The average viscosity after sequestering the calcium ions was 103 mPa s for 1% solution, 1261 mPa s for 2%, and 4842 mPa s for 3%. The average percent reduction of the alginate viscosity was never higher than 13%. Table 2 shows the viscosity of potassium alginate solutions at the same concentrations. The average viscosity after sequestering the calcium ions was 84 mPa s for 1% solution, 849 mPa s for 2% solution, and 3676 mPa s for 3% solution. The percent reduction of the potassium alginate viscosity was 10%.

Table 3 shows the quality profiles of the polysaccharides obtained at the pilot plant level from *M. pyrifera*. The average moisture ranged from 9.6 to 11.1%. The calcium content ranged from 0.22 to 0.38%. The alginate content or purity was from 97.37 to 98.5%, and the pH in the 1% solution ranged from 7.26 to 7.5 for potassium and sodium alginate, and was 2.6 for alginic acid.

Table 4 shows the nutritional profiles for the same products. Protein content was zero. The fat content ranged from 2.11 to 3.85%, and the fiber content ranged from 1.14 to 2.49%. The ash content of the potassium and sodium alginate ranged from 25.44 to 31.6%, and was 1.89% for the alginic acid. The average chemical content for the same components in the algae raw material (*M. pyrifera*) was: 7.39% protein; 4.27% fat; 7.27% fiber and 39.07% ash.

Antinutritional profiles for the polysaccharides obtained were as follows: average arsenic content (mg Kg^{-1}) was 0.51 $\pm$ 0.04 for sodium alginate; 0.62 $\pm$ 0.03 for potassium alginate, and 1.51 $\pm$ 0.01 for alginic acid. Lead was no detected in any sample.

The results of microbiological analyses were as follows: total viable count (UFC g^{-1}) was 1144 $\pm$ 151 for sodium alginate, 1956 $\pm$ 209 for potassium alginate, and zero for alginic acid. Fungi and yeasts (UFC g^{-1}) were 70 $\pm$ 7 for sodium alginate, and zero for potassium alginate and alginic acid. Total coliforms and salmonella were not detected in any of the products obtained.

DISCUSSION

The overall composition of the alginates produced at the pilot-plant scale was very similar to the values reported for the same species (*M. pyrifera*) by Smidsrød and Christensen (1991). Frequencies of mannuronic monads (F_M 0.62) were higher than guluronic (F_G 0.38). The mannuronic diads (F_{MM} 0.42), were also higher than guluronic (F_{GG} 0.18) diads. This molecular structure indicates that these alginates with high M blocks will form softer but more elastic gels than alginates with high G blocks (McHugh, 1987). The viscosities of the alginates produced were in the range of products that can be used in the food industry. Kelco (1986) reported that sodium alginates in 1 and 2% solution with viscosities of 80 and 500 mPa s, respectively, can be used in foods such as: cakes, sauces, frozen foods, syrups and nutritious drinks, where alginate is used as a gelling, emulsifying, thickening and agglutinating agent. King (1983) also notes that potassium alginates in a 1 and 2% solution with viscosities of 270 and 3200 mPa s, respectively, can be used in dietary foods with low sodium content; they also can be used as additives with properties such as: jellification, suspension, thickening and agglutination.

All the alginates produced showed viscosity reductions lower than 13% after adding the calcium sequestrant. Commercial alginates show viscosity reductions from 10 to 40% maximum. This percentage is related to the calcium content in the alginate, and corresponds to a concentration of 0.3 to 1.2%. The calcium content in the alginates produced was very low (0.22-0.38%) suggesting that the conversion of calcium alginate to alginic acid was efficient and resulted in a low viscosity reduction in the alginates (McHugh *et al.,* 2001). Calcium content is important, because alginates produced with high calcium content (more than 1.2%) are difficult to dissolve, and may form gels during their use (McHugh, 1987).

The nutritional and antinutritional profiles of the alginic acid and sodium and potassium alginates fulfill the regulations of the Food and Chemicals Codex (1981). The standard values accepted for sodium alginate are: purity 90.8-100%; moisture < 15%, ash 18-27%; lead < 10 ppm and arsenic < 3 ppm. For potassium alginate the standard values are: purity 89.2 -100%; moisture < 15 %; ash 22-33%; lead < 10 ppm, and arsenic < 3 ppm. For alginic acid the values are: purity 91-100%;

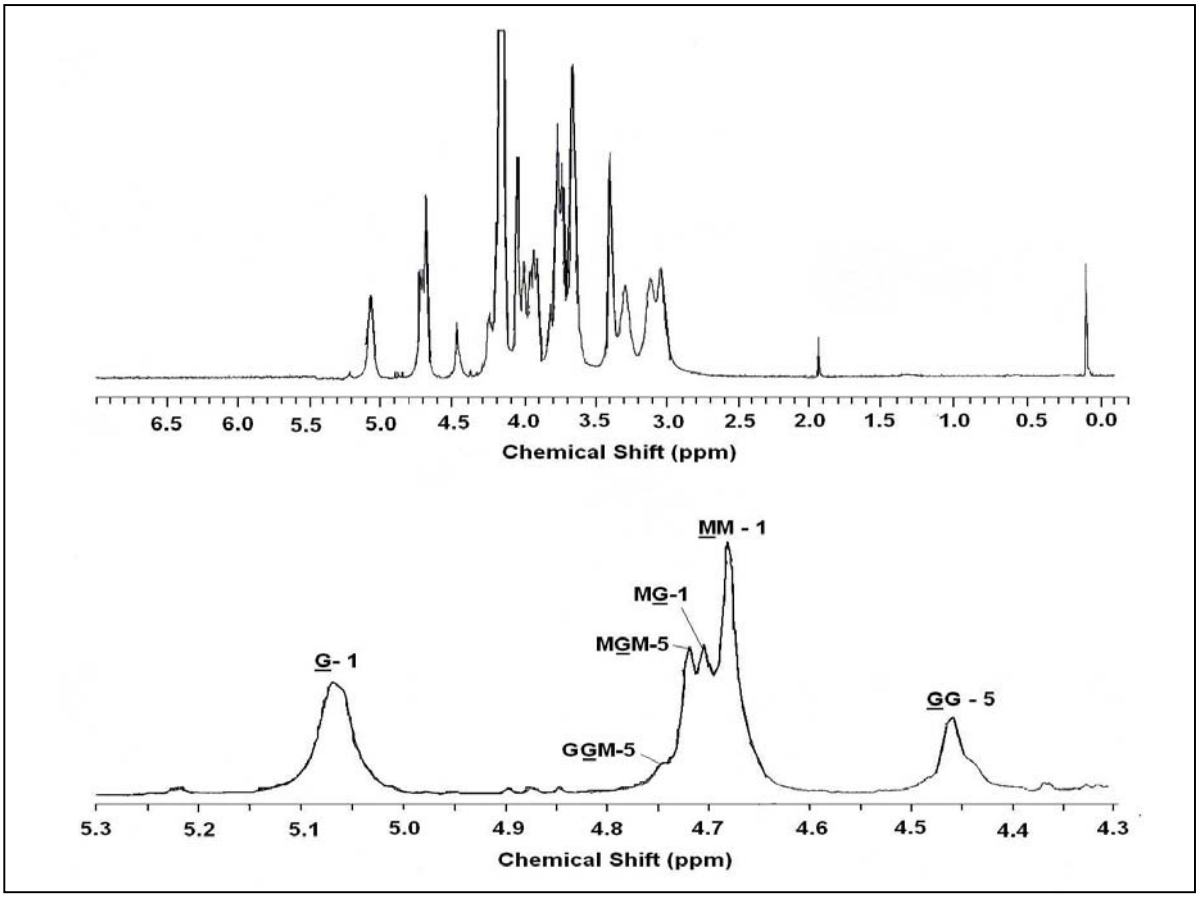

Fig. 1. ^{1}H-NMR spectra of the sodium alginate obtained at pilot plant level from *Macrocystis pyrifera*, collected from Bahía Tortugas, Baja California Sur, Mexico. Chemical shift (ppm) and peak identity: 5.05 (G-1), 4.75 (GGM-5), 4.72 MGM-5, 4.70 (MG-1), 4.67 (MM-1), 4.45 (GG-5). G-1 refers to H1 of residue G, G-5 refers to H5 of residue G, and so on.

Table 1. Comparison between alginates compositions from *Macrocystis pyrifera* analyzed from Mexican samples and commercial samples. F_M, F_G, etc., are frequencies of monads, diads, and triads. $N_{G>1}$ is the average length of G-blocks.

Composition	Alginate analyzed from Mexican samples	Alginate analyzed from commercial samples (Smidsrød *et al.*, 1991)
F_G	0.38	0.39
F_M	0.62	0.61
F_{GG}	0.18	0.16
F_{MM}	0.42	0.38
$F_{MG} = F_{GM}$	0.20	0.23
F_{GGG}	0.13	0.12
$F_{GGM} = F_{MGG}$	0.05	0.03
F_{MGM}	0.18	0.20
$N_{G>1}$	4	6.3

moisture < 15%; ash < 4%; lead < 10 ppm, and arsenic < 3 ppm. All of the alginates produced at the pilot plant level fulfilled the microbiological limits for commercial alginates as recommended by Kelco (1986). Standard maximum values for food grade alginates are: total viable count < 10,000 UFC g^{-1}; fungi and yeast < 200 UFC g^{-1}; total coliforms (negative test) and salmonella (0 UFC g^{-1}).

Table 2. Viscosity of sodium and potassium alginates produced at pilot plant level from *Macrocystis pyrifera* at three different concentrations. Measurements were obtained before and after adding sodium hexametaphosphate (SHMP).

	Alginate	Viscosity (mPa s)		Viscosity
	Concentration	Without SHMP	With SHMP	Concentration
Sodium Alginate				
Average	1%	117	103	13
Std Deviation		37	36	8
Average	2%	1442	1261	13
Std Deviation		839	719	8
Average	3%	5511	4842	13
Std Deviation		2714	2457	8
Potassium Alginate				
Average	1%	93	84	10
Std Deviation		54	49	1
Average	2%	944	849	10
Std Deviation		608	549	0
Average	3%	4086	3676	10
Std Deviation		2845	2587	1

Table 3. Quality profiles (dry basis) of sodium alginate, potassium alginate, and alginic acid obtained from *Macrocystis pyrifera* at pilot plant level.

Product	Moisture	Calcium	Purity	pH
	(%)	(%)	(%)	(1%) solution
NaAlg	12.10	0.45	98.50	7.35
NaAlg	10.30	0.11	98.10	7.45
NaAlg	10.90	0.48	98.90	7.71
Average	**11.10**	**0.35**	**98.50**	**7.50**
Std Deviation	0.92	0.21	0.40	0.19
Kalg	8.80	0.34	96.20	7.14
Kalg	8.50	0.40	97.40	7.30
Kalg	11.50	0.41	98.50	7.33
Average	**9.60**	**0.38**	**97.37**	**7.26**
Std Deviation	1.65	0.04	1.15	0.10
Halg	10.40	0.14	97.80	2.61
Halg	10.90	0.25	97.20	2.61
Halg	10.50	0.28	97.70	2.58
Average	**10.60**	**0.22**	**97.57**	**2.60**
Std Deviation	0.26	0.07	0.32	0.02

The crude protein content of all the alginates was zero. This suggests that the method used was efficient in removing all proteins from the final product. Zero is a normal value since alginates are used mainly as additive, without adding any nutritional value to the products. The values for crude fat (2.11-3.85%) in the alginates may correspond to liposoluble pigments that contribute to the brown color of the algae (Lüning, 1990). The crude fiber content of the alginates was low (1.14-2.49%), however, small amounts of cellulose and hemicellulose may have passed through the filter aid retaining a minimum amounts after filtration.

CONCLUSION

The results obtained suggest that alginic acid, sodium alginate and potassium alginate obtained at the pilot plant level from the alga *M. pyrifera* can be produced at the industrial level with the technology developed. Alginate concentration varied seasonally and geographically and yield could increase to 26.5%, and viscosity to 999 mPa s (Rodríguez-Montesinos and Hernández-Carmona, 1991). The alginate products obtained fulfill the quality control parameters and can be used as food grade alginate. The industrial plant should be careful with microbiological sanitation in all steps of the production process, storage and handling of the product, in order to keep the products within the international standards.

Table 4. Nutritional profiles (dry basis) of sodium alginate, potassium alginate, and alginic acid obtained from *Macrocystis pyrifera* at pilot plant level.

Product	Protein	Fat	Fiber	Ash
	(%)	(%)	(%)	(%)
NaAlg	0	2.27	1.66	24.73
NaAlg	0	1.96	1.02	25.16
NaAlg	0	2.10	0.74	26.44
Average	**0**	**2.11**	**1.14**	**25.44**
Std Deviation	0	0.16	0.47	0.89
Kalg	0	3.71	1.88	32.43
Kalg	0	4.41	1.94	31.47
Kalg	0	3.44	1.42	30.90
Average	**0**	**3.85**	**1.75**	**31.60**
Std Deviation	0	0.50	0.28	0.77
Halg	0	2.41	3.26	1.49
Halg	0	2.97	2.26	1.65
Halg	0	2.25	1.94	2.52
Average	**0**	**2.54**	**2.49**	**1.89**
Std Deviation	0	0.38	0.69	0.55

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