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## Decrease in dynamic viscosity and average molecular weight of alginate from *Laminaria digitata* during alkaline extraction

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### Abstract:

Alginates are natural polysaccharides that are extracted from brown seaweeds and widely used for their rheological properties. The central step in the extraction protocol used in the alginate industry is the alkaline extraction, which requires several hours. In this study, a significant decrease in alginate dynamic viscosity was observed after 2 h of alkaline treatment. Intrinsic viscosity and average molecular weight of alginates from alkaline extractions 1–4 h in duration were determined, indicating depolymerization of alginates: average molecular weight decreased significantly during the extraction, falling by a factor of 5 between 1 and 4 h of extraction. These results suggested that reducing extraction time could enable preserving the rheological properties of the extracted alginates.

**Keywords:** alginate; alkaline extraction; average molecular weight; depolymerization; intrinsic viscosity; *Laminaria digitata*.

**Abbreviations:**  $[\eta]$ , intrinsic viscosity (L.g-1); C, alginate content (g.L-1);  $M_w$ , average molecular weight (Da); Na-Alg, sodium alginate; t, average elution time (s);  $t_s$ , average solvent elution time (s);  $\eta_{red}$ , reduced viscosity (L.g-1)

Alginates are naturally present in the cell wall of brown seaweeds (Kloareg and Quatrano 1988). These polysaccharides have interesting rheological properties and are widely used in various fields of industry: textile, food, paper, cosmetics industry, pharmaceuticals and so forth (Pérez et al. 1992). The direct alginate industrial protocol is divided into several steps: acidification, alkaline extraction, solid/liquid separation, precipitation, conversion to the desired salt, drying and milling. Alkaline extraction represents the main step, and, depending on the species, it may require several hours to reach the optimum extraction yield (Pérez 1997). Many works have characterized alginates in terms of rheological and molecular features (Gacesa 1988, Draget et al. 2001, Oberyukhtina et al. 2001, Ballance et al. 2005), but only few from a process point of view. However, alkaline extraction time is supposed to influence the rheological properties of alginates: the reaction conditions are favorable to bacterial development and endogenous alginate lyases activities, which are likely to cause alginate degradation (Moen et al. 1997). Hernández-Carmona et al. (1999) observed a loss of viscosity during alkaline extraction (70°C-90°C) of alginates from *Macrocystis pyrifera*. Their experiments consisted of measuring viscosity to get an indirect measurement of extraction yield. During the first few hours, viscosity increased with sodium alginate concentration, and loss of viscosity was detected only when extraction yield had stabilized. It seemed interesting to eliminate extraction yield influence by working with equal concentration sodium alginate solutions from several alkaline extractions of different durations. In this paper, dynamic viscosity, intrinsic viscosity, and average molecular weight of alginates from *Laminaria digitata* (Huds.) J.V. Lamour. were determined for 1-4 h alkaline extractions at ambient temperature.

All alginate extraction experiments were conducted on *L. digitata* fronds harvested in Portsall, Brittany, France, and stored in a 2% (w/w) formalin solution (for <1 month). Algae discs of 8 mm diameter were punched out of several 2-year-old whole fronds to minimize heterogeneity problems like variation of alginate content in function of the frond and in function of the part of the frond (Pérez 1970). The extraction protocol was a laboratory adaptation of the industrial process described by Pérez et al. (1992). Algal pieces were rinsed and then immersed in a 0.5 M  $H_2SO_4$  solution at 4°C for at least one night. For each extraction experiment, 50 g of algal pieces were rinsed with distilled water and soaked in 1 L of a 4% (w/w)  $Na_2CO_3$  solution under continuous stirring. At the end of the extraction time, algal particles were separated from the solution by means of a nylon filter cloth. Supernatant sodium alginate content was quantified for each sample according to Kennedy and Bradshaw (1984) (polyhexamethylenebiguanidium chloride solution obtained from Bayrol France S.A.S., Dardilly, France, Newcil [15 42 004]; Na-Alg, obtained from Sigma-Aldrich Chemie S.A.R.L., Saint-Quentin, Fallavier, France, alginic acid sodium salt from brown algae – medium viscosity [A2033], was used for the calibration curve). Results are shown in Table 1. A Paar Physica MCR 500 rheometer (Anton Paar S.A.S., Courtaboeuf, France) was used for rheograms (sodium alginate solution concentration: 1 g.L<sup>-1</sup>; shear rate: 0.1 – 3000 s<sup>-1</sup>; temperature: 20°C; distance between the planes: 1mm; diameter of the mobile plane: 50mm; measurement duration: 10 s; 30 measurements, logarithmical distribution). Capillary viscosimetry measurements were conducted with a 0.44 mm diameter Ubbelohde capillary viscosimeter (Ubbelohde viscosimeter [DIN] Schott Instruments [616-1102]; VWR International France, Fontenay-sous-Bois, France), immersed in a heated circulating water bath (Polystat 73 water bath [W85227]; Fisher Bioblock Scientific, Illkirch, France), which maintained temperature at 20°C ( $\pm 0.1^\circ C$ ). The different dilutions of sodium alginate samples were prepared with a 0.1 M NaCl solvent (Mancini et al. 1996 protocol). Elution time of each sample was measured in triplicate for different dilutions. Average elution times and corresponding reduced viscosities were calculated according to

the following equation:  $\eta_{red} = \frac{t-t_s}{C \cdot t_s}$ , where  $\eta_{red}$  is the reduced viscosity, t is the average elution time,  $t_s$  is

the average solvent elution time (NaCl 0.1 M) and C is the alginate content. Reduced viscosity versus concentration curves were then built, and intrinsic viscosity was estimated by extrapolating reduced viscosity when concentration tends toward 0 by means of linear regression. The model proposed by

Mancini et al. (1996) was chosen to estimate the average molecular weight:  $[\eta] = 1.228 \cdot 10^{-4} M_w^{0.963}$ ,

where  $[\eta]$  (L.g<sup>-1</sup>) is the intrinsic viscosity and  $M_w$  (Da) is the average molecular weight.

To study the effect of alkaline extraction duration on the rheological properties of the extracted alginate, 1-4 h alkaline extractions were conducted. The minimum duration needed to extract a sufficiently concentrated solution (1 g.L<sup>-1</sup>) is 1 h, and 4 h is the extraction duration required to reach

the maximum sodium alginate content. Supernatant sodium alginate content values are displayed in Table 1, and rheograms of 1 g.L<sup>-1</sup> sodium alginate solutions are presented in Figure 1. At high and medium shear rate, viscosity is independent of the extraction time (~1 mPa.s). But at very low shear rate (<1 s<sup>-1</sup>), dynamic viscosity depends on extraction time: a significant decrease appears after a 2 h extraction, and a minimum is reached after 3.5-4 h extraction. Dynamic viscosity decreases from ~0.1 Pa.s to ~0.01 Pa.s between 1 and 4 h extraction at 0.1 s<sup>-1</sup> shear rate. These results highlight the influence of extraction time on alginate intrinsic viscosity and, consequently, on its average molecular weight present in solution, which is important since alginates being polydisperse polysaccharides. Intrinsic viscosity of the different samples was assessed by means of capillary viscosimetry. Elution time of each sample was measured in triplicate for different dilutions. Average elution time, intrinsic viscosity and average molecular weight results are given in Table 1. Average molecular weight decreases significantly during the extraction, falling by a factor 5 between 1 and 4 h of extraction. These results confirm the decline in rheological quality during the alkaline extraction of alginates. According to Moen et al. (1997) and Smidsrød et al. (1963), depolymerization of alginate molecules is linked to the activity of endogenous alginate lyases, catalyzed by polyphenols that are naturally present in seaweeds. As elimination of these compounds is impractical from an industrial point of view, the most evident solution to avoid these effects would be to reduce the extraction time. Future investigations on process parameters influencing the extraction kinetics would be of interest.

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## Figure

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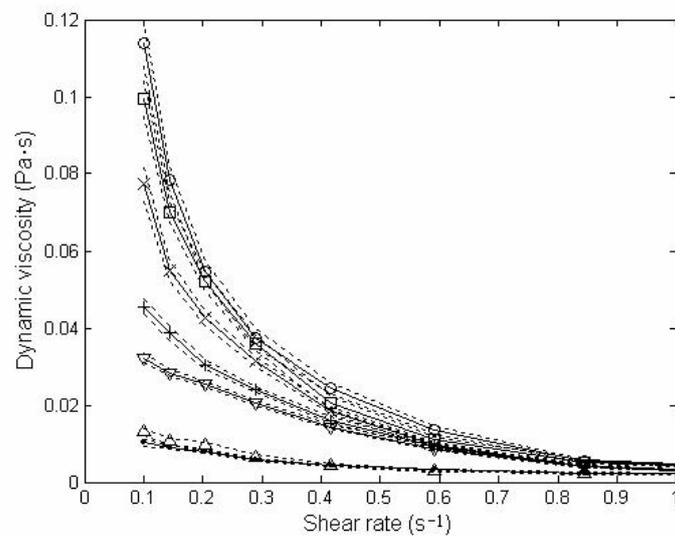


Fig. 1. Dynamic viscosity vs. shear rate graph of  $1\text{g.L}^{-1}$  sodium alginate solutions from extractions of different durations. O : 1h ; □ : 1,5h ; × : 2h ; + : 2,5h ; ∇ : 3h ; Δ : 3,5h ; • : 4h. Dotted lines correspond to 95% confidence bounds.

## Table

Table 1. Sodium alginate content, average elution time, intrinsic viscosity and average molecular weight of sodium alginate solutions from extractions of different durations.

Extraction duration (h)	Sodium alginate content (g.L <sup>-1</sup> )	Average elution time for different sodium alginate solution concentrations (min)						Intrinsic viscosity (L.g <sup>-1</sup> ) (with 95% confidence bounds)	Average molecular weight (Da) (with 95% confidence bounds)
		0.5 g.L <sup>-1</sup>	1 g.L <sup>-1</sup>	2 g.L <sup>-1</sup>	3 g.L <sup>-1</sup>	4 g.L <sup>-1</sup>	5 g.L <sup>-1</sup>		
1	1.32	11.35	11.85	12.86	13.91	14.93	15.98	0.7394 (0.7292;0.7457)	91902 (90586;92715)
1.5	3.67	11.03	11.56	12.44	13.40	14.28	15.25	0.6790 (0.6622;0.6958)	84119 (81958;86281)
2	4.07	10.37	10.72	11.38	11.91	12.48	13.02	0.5643 (0.5369;0.5927)	69414 (65917;73045)
2.5	4.19	9.71	9.90	10.37	10.78	11.19	11.74	0.3941 (0.3719;0.4164)	47814 (45020;50626)
3	4.30	9.23	9.39	9.71	10.02	10.28	10.64	0.2976 (0.2869;0.3084)	35718 (34386;37065)
3.5	4.28	8.72	8.83	9.03	9.22	9.44	9.66	0.1799 (0.1728;0.1869)	21178 (20311;22035)
4	4.29	8.57	8.71	8.98	9.31	9.51	9.75	0.1386 (0.1188;0.1584)	16154 (13764;18556)